

## ASPA 24th Congress Book of Abstract

Roberto Mantovani & Alessio Cecchinato

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# Italian Journal of Animal Science

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# Italian Journal of Animal Science

The *Italian Journal of Animal Science* is an international peer-reviewed open access journal publishing original scientific papers, reviews and short communications.

The journal serves as essential reading for animal scientists, technicians and all those who research animal production.

The journal encourages submissions of international relevance on the following subjects:

- Animal derived food quality and safety
- Animal genetics and breeding
- Aquaculture, poultry, companion and wild game animals
- Livestock systems, management and environment
- Non-ruminant or ruminant nutrition and feeding
- Production physiology and functional biology of farmed, companions and wild game animals.
- Animal behavior
- Animal welfare
- In vitro studies that have an application to farmed livestock

Manuscripts must address topics based on research at molecular, cellular, organ, whole animal and production system levels. Manuscripts discussing milk or meat analysis and compositions must show a direct link to either livestock production system, product quality, animal feeding/nutrition, animal genetics or breeding. Manuscripts describing laboratory animal models will be considered where the study highlights a potential benefit to farmed livestock.

Submissions discussing epidemiology, parasitology, infective diseases, food-borne diseases do not fit with the aims and scope of the journal.

Meeting reviews, book reviews and conference supplements are also published, as well as news and guidelines from the Animal Science and Production Association (ASPA). We welcome submissions from ASPA members and non-members alike.

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# Italian Journal of Animal Science

volume 20, supplement 1, 2021

## ASPA 24<sup>th</sup> Congress

*Padova, September 21-24, 2021*

### Guest Editors

**Roberto Mantovani (Coordinator), Alessio Cecchinato, Giovanni Bittante, Maurizio Ramanzin, Lucia Bailoni, Mauro Penasa, Flaviana Gottardo, Sara Pegolo, Giorgio Marchesini, Rebecca Ricci, Cristina Sartori, Marco Cullere, Marco Birolo, Severino Segato, Valentina Bonfatti, Marta Brscic, Luigi Gallo, Stefano Schiavon, Franco Tagliapietra**

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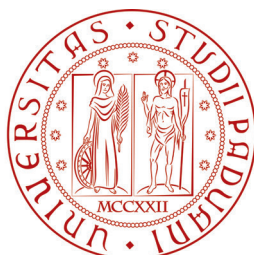


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# **ASPA 24<sup>th</sup> Congress**

## ***Padova, September 21-24, 2021***

**#ASPA2021**

**24<sup>th</sup>**

**Congress of the Animal Science and production Association**

**24° Congresso dell' Associazione per la Scienza e le Produzioni Animali**

**Padova,  
September 21-24, 2021**

**Venue**

**“Fiore di Botta”**

**University of Padova**

**Via del Pescarotto, 8-Padova**

# ASPA 24<sup>th</sup> Congress

## Padova, September 21-24, 2021

### LEGEND

ENVIRONMENT (E)
ANIMALS (A)
FOOD (F)
OTHER (O)
to be defined

Tuesday 21 <sup>st</sup> September 2021	
Fiera di Padova	
12:00	ARRIVAL - REGISTRATION
PLENARY SESSION: Animal Science and Society Concerns	
15:00	Introduction
15:15	Nicolò Pietro Paolo Macciotta
15:15	MAIN LECTURE 1
15:45	<i>Animal production and society</i> Massimiano Bucchi
15:45	MAIN LECTURE 2
16:15	<i>Societal and political demands on farmed animal welfare: an evolving landscape</i> Elena Nalon
16:15	Coffee Break
16:45	MAIN LECTURE 3
17:15	<i>From Padova 1987 to Padova 2021: evolution of the scientific research</i> Giovanni Bittante
17:15	MAIN LECTURE 4
17:45	<i>Challenges of Italian livestock systems towards a full environmental sustainability</i> Bruno Ronchi
18:00	OPENING CEREMONY
	Welcome addresses
18:15	MAIN LECTURE 5
18:45	<i>Eight centuries of the University of Padua (1222-2022): An historical sketch</i> Fabio Zampieri
18:45	MAIN LECTURE 6
19:15	<i>From Pietro Arduino and Giuseppe Orus to the Agropolis Campus: analogies with present times between historical realities and possible fakes</i> Lucia Bailoni
19:30	Welcome Cocktail Dinner

Wednesday 22 <sup>nd</sup> September 2021					
Fiore di Botta					
ROOM GALILEO		ROOM GIOTTO		ROOM PETRARCA	
09:00	SESSION 1 - (E)	08:30	SESSION 2 - (A)	09:00	SESSION 4 - (F)
10:30	Alternative feeds and waste recycling - I (6 oral)	10:15	Management strategies to improve animal health, welfare and resilience - I (1 main lecture + 5 oral)	10:30	Nutritional profile of food - I (1 main lecture + 4 oral)
Coffee Break / Poster Session 1					
11:00	SESSION 5 - (E)	11:00	SESSION 6 - (A)	11:00	SESSION 8 - (F)
12:30	Alternative feeds and waste recycling - II (4 oral)	12:30	Management strategies to improve animal health, welfare and resilience - II (6 oral)	12:30	Nutritional profile of food - II (5 oral)
Lunch					
14:00	SESSION 9 - (E)	14:00	SESSION 10 - (A)	14:00	SESSION 12 - (F)
15:30	Non conventional livestock systems (1 main lecture + 4 oral)	15:30	Management strategies to improve animal health, welfare and resilience - III (6 oral)	15:30	Food production and processing (6 oral)

Thursday 23 <sup>rd</sup> September 2021					
Fiore di Botta					
ROOM GALILEO		ROOM GIOTTO	ROOM PETRARCA		ROOM ELENA CORNARO PISCOPIA
09:00 <b>SESSION 13 - (E)</b> Monitoring and mitigating impacts on environment - I (6 oral)		08:30 <b>SESSION 14 - (A)</b> Breeding strategies to improve animal health, welfare and resilience (1 main lecture + 6 oral)	<b>SESSION 15 - (O)</b> Feed efficiency and growth performance - I (5 oral)	09:00 <b>SESSION 16 - (F)</b> Food sensory quality, labelling and valorization (6 oral)	
10:30 Coffee Break / Poster Session 2					
11:00 <b>SESSION 17 - (E)</b> Monitoring and mitigating impacts on environment - II (1 main lecture + 4 oral)		11:00 <b>SESSION 18 - (A)</b> Behaviour and welfare (6 oral)	<b>SESSION 19 - (O)</b> Feed efficiency and growth performance - II (6 oral)	11:00 <b>SESSION 20 - (F)</b> Feed effects on nutritional profile of animal products - I (6 oral)	
12:30 Lunch					
14:00 <b>SESSION 21 - (A)</b> Precision livestock farming - I (1 main lecture + 4 oral)		14:00 <b>SESSION 22 - (O)</b> Free Communications (6 oral)	<b>SESSION 23 - (A)</b> Genomics of local breeds (1 main lecture + 4 oral)	14:00 <b>SESSION 24 - (F)</b> Feed effects on nutritional profile of animal products - II (6 oral)	
15:30 Coffee Break					
16:00 <b>SESSION 25 - (A)</b> Precision livestock farming - II (3 oral)		16:00 <b>SESSION 26 - (A)</b> Omics technologies - I (3 oral)	<b>SESSION 27 - (A)</b> Quantitative approaches, inbreeding and crossbreeding - I (4 oral)	16:00 <b>SESSION 28</b> to be defined	
17:00 Oral presentations & Posters Awards					
17:15 Meeting of ASPA Members					
19:30					
20:00 Transfer by bus from Fiore di Botta to Abano Terme for Social Dinner					

Friday 24 <sup>th</sup> September 2021					
Fiore di Botta					
ROOM GALILEO		ROOM GIOTTO	ROOM PETRARCA		ROOM ELENA CORNARO PISCOPIA
09:00 <b>SESSION 29 - (A)</b> Animal efficiency - I (6 oral)		09:00 <b>SESSION 30 - (A)</b> Omics technologies - II (6 oral)	<b>SESSION 31 - (A)</b> Quantitative approaches, inbreeding and crossbreeding - II (6 oral)	09:00 <b>SESSION 32 - (A)</b> Companion animals and society - I (1 main lecture + 4 oral)	
10:30 Coffee Break					
11:00 <b>SESSION 33 - (A)</b> Animal efficiency - II (6 oral)		11:00 <b>SESSION 34 - (A)</b> Omics technologies - III (6 oral)	<b>SESSION 35 - (A)</b> Genetic diversity (6 oral)	11:00 <b>SESSION 36 - (A)</b> Companion animals and society - II (5 oral)	
12:30					
End of the congress					

# ASPA 24<sup>th</sup> Congress

## Padova, September 21-24, 2021

### Italian Journal of Animal Science 2021; volume 20, supplement S1

#### Agenda Invited Speakers

September, 21<sup>st</sup>

##### *Fiera di Padova, PLENARY SESSION "Animal Science and Society Concerns"*

Chair:	Nicolò Pietro Paolo Macciotta	
15.15-15.45	Massimiano Bucchi	
	<i>Animal production and society</i> .....	IS001
15.45-16.15	Elena Nalon	
	<i>Societal and political demands on farmed animal welfare: an evolving landscape</i> .....	IS002
16.45-17.15	Giovanni Bittante	
	<i>From Padova 1987 to Padova 2021: evolution of the scientific research</i> .....	IS003
17.15-17.45	Bruno Ronchi	
	<i>Challenges of Italian livestock systems towards a full environmental sustainability</i> .....	IS004

##### *Fiera di Padova - Opening Ceremony*

Chair:	Giulio Cozzi	
18.15-18.45	Fabio Zampieri	
	<i>Eight centuries of the University of Padua (1222-2022): An historical sketch</i> .....	S005
18.45-19.15	Lucia Bailoni	
	<i>From Pietro Arduino and Giuseppe Orus to the Agripolis Campus: analogies with present times between historical realities and possible fakes</i> .....	IS006

September, 22<sup>nd</sup>

##### *Fiore di Botta, Room Giotto - Management strategies to improve animal health, welfare and resilience - I*

Chairs:	Giulio Cozzi, Giovanna Martelli	
8.30-09.00	Annalisa Scollo	
	<i>Mutilations in pigs: searching for a new state-of-mind of the farmer</i> .....	IS007

##### *Fiore di Botta, Room Elena Cornaro Piscopia - Nutritional profile of food - I*

Chairs:	Massimiliano Petracci, Angela Trocino	
09.00-09.30	Frédéric Leroy	
	<i>Animal source foods in the 21st century - obsolete or essential?</i> .....	IS008

##### *Fiore di Botta, Room Petrarca - Genomic and phenomic tools to improve livestock animals - I*

Chairs:	Alessio Cecchinato, Martina Zappaterra	
11.00-11.30	Joao Reboucas Dorea	
	<i>Harnessing the Power of Computer Vision System to Improve Management Decisions in Livestock Operations</i> .....	IS009



**Fiore di Botta, Room Galileo - Non-conventional  
livestock systems**

Chairs: Maurizio Ramanzin, Luca Maria Battaglini  
14.00-14.30 *Daniel Martin Collado*  
*Best practices for targeted policies to enhance ecosystem services in European  
livestock agroecosystems* ..... IS010

**Fiore di Botta, Room Petrarca - Genomic and phenomic  
tools to improve livestock - II**

Chairs: Roberto Mantovani, Riccardo Bozzi  
14.00-14.30 *Ignacy Misztal*  
*Genomic tools to improve livestock* ..... IS011

**September, 23<sup>rd</sup>**

**Fiore di Botta, Room Giotto - Breeding strategies to improve  
animal health, welfare and resilience**

Chairs: Fabio Pilla, Valentina Bonfatti  
08.30-09.00 *Augustin Blasco*  
*New challenges: Global warming, welfare, resilience. How can genetics help?* ..... IS012

**Fiore di Botta, Room Galileo - Monitoring and mitigating  
impacts on environment**

Chairs: Mauro Spanghero, Stefano Schiavon  
11.00-11.30 *Maurizio Ramanzin*  
*Environmental sustainability of livestock systems: going beyond impacts?* ..... IS013

**Fiore di Botta, Room Galileo - Precision livestock farming - I**

Chairs: Giorgio Marchesini, Fabio Abeni  
14.00-14.30 *Fabio Abeni*  
*PLF applications for Italian animal productions: critical aspects and opportunities* ..... IS014

**Fiore di Botta, Room Petrarca - Genomics of local breeds**

Chairs: Baldassare Portolano, Emiliano Lasagna  
14.00-14.30 *Paolo Ajmone Marsan*  
*Livestock biodiversity in the genomic era* ..... IS015

**September, 24<sup>th</sup>**

**Fiore di Botta, Room Elena Cornaro Piscopia - Companion  
animals and society**

Chairs: Rebecca Ricci, Paola Crepaldi  
09.00-09.30 *Marianne Diez*  
*The evolution of proteins in the nutrition of dogs and cats* ..... IS016

# ASPA 24<sup>th</sup> Congress

## Padova, September 21-24, 2021

### Italian Journal of Animal Science 2021; volume 20, supplement S1

#### Agenda for oral presentations

#### Wednesday, September 22<sup>nd</sup> - Room Galileo

##### SESSION 1 - Alternative feeds and waste recycling - I

Chairs:	Antonella Dalle Zotte, Laura Gasco	
09.00-09.15	Erika Pellattiero, Marco Cullere, Federica Zanetti, Barbara Alberghini, Sara Berzuini, Silvia Tavarini, Incoronata Galasso, <u>Antonella Dalle Zotte</u> <i>Effect of Camelina (Camelina sativa L. Crantz) dietary inclusion on quails growth performance and carcass traits</i> .....	O001
09.15-09.30	<u>Nicola Francesco Addeo</u> , Alessandra Roncarati, Giovanni Piccolo, Antonino Nizza, Fulvia Bovera <i>Potential use of a queen bee larvae meal (Apis mellifera ligustica Spin.) in animal nutrition: a nutritional and chemical-toxicological evaluation</i> .....	O002
09.30-09.45	<u>Yazavinder Singh</u> , Eszter Zsedely, Georgina Takacs, Zsolt Herman, Klaudia Szalai, Marco Cullere, Silvia Cappelozza, Antonella Dalle Zotte <i>Effect of silkworm (Bombyx mori) dietary inclusion on chickens' growth performance and carcass traits</i> .....	O003
09.45-10.00	<u>Giulia Pascon</u> , Maria Messina, Lisa Petit, Luísa Maria Pinheiro Valente, Beatriz Monteiro Oliveira, Cyrille Przybyla, Gilbert Dutto, Francesca Tulli <i>Marine microalgal biomass from fish farming effluents: potential application in aquafeeds</i> .....	O004
10.00-10.15	<u>Sara Bellezza Oddon</u> , Ilaria Biasato, Laura Gasco <i>Isoenergetic-practical and semi-purified diets for protein requirement determination in Hermetia illucens larvae: consequences on life history traits</i> .....	O005
10.15-10.30	<u>Francesco Bordignon</u> , Laura Gasco, Marco Birolo, Christian Caimi, Gerolamo Xiccato <i>Effects of the inclusion of Hermetia illucens meal in diets for rainbow trout (Oncorhynchus mykiss) reared in a low-tech aquaponic system</i> .....	O006

#### Wednesday, September 22<sup>nd</sup> - Room Giotto

##### SESSION 2 - Management strategies to improve animal health, welfare and resilience - I

Chairs:	Giulio Cozzi, Giovanna Martelli	
09.00-09.15	<u>Luciana Bava</u> , Alice Comparelli, Maria Cecilia Bianchi, Alberto Tamburini, Maddalena Zucali <i>Dry period management practices in 130 dairy farms</i> .....	O007
09.15-09.30	<u>Luca Turini</u> , Alice Cappucci, Fabrizio Bertelloni, Elisa Gasparoni, Francesca Vichi, Marcello Mele <i>Mutual relationship between technological, structural and productive features in dairy sheep farms: a multivariate approach</i> .....	O008
09.30-09.45	<u>Diana Giannuzzi</u> , Rossella Tessari, Enrico Fiore, Matteo Ganesella, Vittoria Bisutti, Sara Pegolo <i>Association between liver ultrasound measurements and hematochemical indicators for early prediction of metabolic alterations in Holstein cows</i> .....	O009
09.45-10.00	<u>Luca Cattaneo</u> , Andrea Minuti, Fiorenzo Piccioli Cappelli, Erminio Trevisi <i>Blood biomarkers before dry-off are related to better adaptation to transition period in dairy cows</i> .....	O010
10.00-10.15	<u>Isabella Lora</u> , Giovanna Ranzato, Luisa Magrin, Giulio Cozzi <i>Comparison between cow milk and a high-energy whey-based milk replacer for the weaning of Holstein dairy heifers</i> .....	O011

## Wednesday, September 22<sup>nd</sup> - Room Petrarca

### SESSION 3 - Role and characterization of local breeds

Chairs:	Luca Fontanesi, Cristina Sartori	
08.30-08.45	<u>Elena Benedetti del Rio</u> , Marta Teston, Edi Defrancesco, Paola Gatto <i>Sustainable use of local sheep breeds in Italian Eastern Alps</i> .....	O012
08.45-09.00	<u>Enrico Mancin</u> , Cristina Sartori, Nadia Guzzo, Beniamino Tuliozzi, Roberto Mantovani <i>Change of accuracy in EBVs due to genomic information in the small Rendena cattle populations</i> ....	O013
09.00-09.15	<u>Giulia Foggi</u> , Francesca Ciucci, Laura Casarosa, Elisabetta Giannessi, Carla Lenzi, Maria Conte, Marcello Mele <i>Histochemical muscle fibre features and gene expression in Maremmana and Aubrac steers</i> .....	O014
09.15-09.30	<u>Mario Barbato</u> , Elia Vajana, Elisa Somenzi, Oliver Selmoni, Marcello Del Corvo, Michèle Tixier-Boichard, Stéphane Joost, Barbara Lazzari, Alessandra Stella, Paolo Ajmone Marsan <i>Deciphering climate-mediated adaptation in European sheep</i> .....	O015
09.30-09.45	<u>Rosalina Di Gerlando</u> , Ilaria Rizzuto, Marco Tolone, Angelo Moscarelli, Maria Teresa Sardina <i>Prion protein (PRNP) gene polymorphisms in Valle del Belice sheep breed</i> .....	O016
09.45-10.00	<u>Piero Franceschi</u> , Paolo Formaggioni, Francesca Vighi, Elena Bortolazzo, Massimo Malacarne <i>Physico-chemical characteristics of milk from autochthonous cattle breeds of the Emilia Romagna region</i> .....	O017

## Wednesday, September 22<sup>nd</sup> - Room Elena Cornaro Piscopia

### SESSION 4 - Nutritional profile of food - I

Chairs:	Massimiliano Petracci, Angela Trocino	
09.30-09.45	<u>Alice Cartoni Mancinelli</u> , Alessandro Dal Bosco, Elisa Angelucci, Claudia Ciarelli, Castellini Cesare <i>Lipid metabolism in different Italian poultry breed and crossbreed</i> .....	O018
09.45-10.00	<u>Nicolo' Amalfitano</u> , Mohamed-Laid Haddi, Hamida Benabid, Franco Tagliapietra <i>Detailed mineral profile of milk from cows, buffaloes, goats, sheep, dromedary camels, and donkeys and minerals recovery in cheese</i> .....	O019
10.00-10.15	<u>Elena Mariani</u> , Anna Lante, Claudio Cipolat-Gotet, Giovanna Lomolino, Federica Tinello, Massimo Malacarne, Andrea Summer <i>Animal and environmental effects on protease and lipase activity of bovine milk</i> .....	O020
10.15-10.30	<u>Vittoria Bisutti</u> , Alice Vanzin, Alessandro Toscano, Lucio Flavio Macedo Mota <i>Associations among somatic cell count, differential somatic cell count and milk protein profile in Holstein cattle</i> .....	O021

## Wednesday, September 22<sup>nd</sup> - Room Galileo

### SESSION 5 - Alternative feeds and waste recycling - II

Chairs:	G. Matteo Crovetto, Biagina Chiofalo	
11.00-11.15	<u>Francesco Serrapica</u> , Felicia Masucci, Maura Sannino, Salvatore Faugno, Claudia Lambiase, Antonio Di Francia <i>Peanut (<i>Arachis hypogaea</i>) crop within a circular economy perspective: potential use of biomass as forage</i> .....	O022
11.15-11.30	<u>Sheyla Arango</u> , Elisabetta Bacchin, Federico Fontana, Massimo Montanari, Lucia Bailoni <i>Agronomical traits and chemical characterization of whole plant and botanical fractions of six varieties of hemp cultivated in the Veneto region</i> .....	O023
11.30-11.45	<u>Andrea Rota Graziosi</u> , Stefania Colombini, Gianni Matteo Crovetto, Gianluca Galassi, Maria Chiaravalli, Marco Battelli, Luca Rapetti <i>Partial replacement of soybean meal with self-produced whole-plant soybean silage in lactating dairy cows diet</i> .....	O024
11.45-12.00	<u>Doriana E.A. Tedesco</u> , Sveva Scarioni, Aldo Tava <i>Biorefinery approach to valorise vegetable waste from the wholesale market</i> .....	O025

## Wednesday, September 22<sup>nd</sup> - Room Giotto

### SESSION 6 - Management strategies to improve animal health, welfare and resilience - II

Chairs: Flaviana Gottardo, Martina Tarantola

- 11.00-11.15 Sara Pegolo, Rossella Tessari, Enrico Fiore, Matteo Ganesella, Diana Giannuzzi, Riccardo Negrini, Alessio Cecchinato  
*Quarter-level analysis of mastitis infection and relationships with milk traits in Holstein cattle* ..... **O026**
- 11.15-11.30 Basilio Randazzo, Matteo Zarantonello, Roberto Cerri, Giorgia Gioacchini, Maria Messina, Alessia Belloni, Enrico Daniso, Emilio Tibaldi, Ike Olivotto  
*Effects of microalgae and insect meal supplementation to diets high in soybean meal, deprived of fish meal, on gut health of seabass (*D. labrax*) and sea bream (*S. aurata*)* ..... **O027**
- 11.30-11.45 Aristide Maggolino, Nicola Bartolomeo, Umberto Bernabucci, Attilio Rossoni, Vincenzo Landi, Pasquale De Palo  
*Heat waves effects on Italian Brown Swiss milk production* ..... **O028**
- 11.45-12.00 Francesca Rita Dinardo, Elisabetta Casalino, Grazia Maria Liuzzi, Giovanna Calzaretto, Pasquale De Palo  
*Dietary supplementation with nucleotides in calves: effects on oxidative balance and immune response* ..... **O029**
- 12.00-12.15 Silvia Carta, Eleni Tsiplakou, Christina Mitsiopolou, Giuseppe Pulina  
*The effect of cocoa husks supplementation on fatty acids profile and blood and milk antioxidant status of dairy ewes* ..... **O030**
- 12.15-12.30 Paolo Trevisi, Diana Luise, Federico Correa, Marika Vitali, Elisa Spinelli, Roberta Ruggeri, Kristian Juul Volshøj, Jørn Munch Madsen, Mai Anh Ton Nu, Paolo Bosi  
*Effect of two different ways for milk replacer administration on the growth performance and welfare of piglets* ..... **O031**

## Wednesday, September 22<sup>nd</sup> - Room Petrarca

### SESSION 7 - Genomic and phenomic tools to improve livestock animals - I

Chairs: Alessio Cecchinato, Martina Zappaterra

- 11.30-11.45 Giacomo Rovelli, Maria Gracia Luigi Sierra, Simone Ceccobelli, Dailu Guan, Francesco Perini, Fiorella Sbarra, Andrea Quaglia, Marcel Amills Eras, Emiliano Lasagna  
*A genome wide association study for beef production traits in five Italian cattle breeds* ..... **O032**
- 11.45-12.00 Vincenzo Landi, Gabriele Senczuk, Marco Ragni, Simona Taricone, Francesco Giannico, Francesca Maria Sarti, Emiliano Lasagna, Luigina Rillo, Giuseppe Marsico, Christian Persichilli, Samira Giovannini, Domato Matassino, Elena Ciani, Fabio Pilla  
*Complex genetic pattern supported by genome wide SNPs data in Mediterranean goat breeds* ..... **O033**
- 12.00-12.15 Marco Milanesi, Cinzia Marchitelli, Michela Contò, Simona Rinaldi, Danilo Pignotti, Giovanni Chillemi, Alessandra Crisà  
*Preliminary results of Genome Wide Association Study with milk functional molecules in different cow breeds* ..... **O034**
- 12.15-12.30 Daniele Bigi, Nicola Rambaldi Migliore, Riccardo Negrini, Andrea Verini-Supplizi, Luigi Liotta, Antonio Torroni, Alessandro Achilli, Licia Colli  
*Donkey worldwide diversity based on control-region data and entire mitogenomes* ..... **O035**

## Wednesday, September 22<sup>nd</sup> - Room Elena Cornaro Piscopia

### SESSION 8 - Nutritional profile of food - II

Chairs: Andrea Formigoni, Gianluca Neglia

- 11.00-11.15 Chiara Ceccotti, Simona Rimoldi, Federica Iannini, Genciana Terova  
*Evaluation of oil from *Camelina sativa* in feeds for gilthead sea bream (*Sparus aurata*): effects on fish growth, fillet fatty acid profile, and gut microbiota composition* ..... **O036**
- 11.15-11.30 Cinzia Marchitelli, Daniele Pietrucci, Marco Milanesi, Luca Casadei, Fernando Porcelli, Giovanni Chillemi, Alessandra Crisà  
*Evaluation of milk quality in different cow breeds by biological markers* ..... **O037**

11.30-11.45	<u>Amalia Simonetti</u> , Giulia Grassi, Emilio Gambacorta, Annamaria Perna <i>Antioxidant capacity and polyphenol content of goat milk: comparison among autochthonous breeds</i> .....	<b>O038</b>
11.45-12.00	<u>Martina Di Corcia</u> , Marzia Albenzio, Antonella della Malva, Mariangela Caroprese, Maria Giovanna Ciliberti, Antonella Santillo, Agostino Sevi, Rosaria Marino <i>Fatty acid profile and endogenous bioactive compounds of beef as affect by duration of linseed supplementation</i> .....	<b>O039</b>
12.00-12.15	<u>Maria Francesca Guiso</u> , Giorgia Serra, Antonio Fenu, Anna Nudda <i>Colostrum composition of Sarda Dairy Ewes</i> .....	<b>O040</b>

## Wednesday, September 22<sup>nd</sup> - Room Galileo

### SESSION 9 – Non-conventional livestock systems

Chairs:	Maurizio Ramanzin, Luca Maria Battaglini	
14.30-14.45	<u>Ginevra Nota</u> , Roberta Berretti, Marco Pittarello, Davide Barberis, Davide Ascoli, Simone Ravetto Enri, Dino Genovese, Paolo Cornale, Giampiero Lombardi, Michele Lonati, Renzo Motta, Luca Maria Battaglini <i>Food For Forest: an integrative silvi-pastoral system with semi-free-ranging pigs</i> .....	<b>O041</b>
14.45-15.00	<u>Amanda McPherson</u> , Enrico Sturaro, Maurizio Ramanzin, Andrea Squartini, Salvatore Raniolo, Dorcas H. Franklin, Giuseppe Concheri <i>Investigating dairy grazing patterns and soil microbial trends in the nitrogen cycle across traditional mountain landscapes</i> .....	<b>O042</b>
15.00-15.15	<u>Salvatore Raniolo</u> , Maurizio Ramanzin <i>Human choices, slope, and vegetation productivity determine pattern of traditional Alpine summer grazing</i> .....	<b>O043</b>
15.15-15.30	<u>Andrea Confessore</u> , Chiara Aquilani, Lapo Nannucci, Edoardo Bellini, Camilla Dibari, Giovanni Argenti, Nicolina Staglianò, Marco Moriondo, Paola Semenzato, Sandro Nicoloso, Carolina Pugliese <i>Application of Virtual Fencing for the management of Limousine cows at pasture during spring-summer season: preliminary results</i> .....	<b>O044</b>

## Wednesday, September 22<sup>nd</sup> - Room Giotto

### SESSION 10 - Management strategies to improve animal health, welfare and resilience - III

Chairs:	Leonardo Nanni Costa, Silvana Mattiello	
14.00-14.15	<u>Alessio Cotticelli</u> , Angela Salzano, Anna Balestrieri, Pasquale Scala, Giacomo Bertolini, Oscar Tamburis, Gianluca Neglia <i>Relationship among production traits, milk conductivity, somatic cell score and THI in the Italian Mediterranean Buffalo</i> .....	<b>O045</b>
14.15-14.30	<u>Alessandra Nicolao</u> , Claire Mosnier, Bruno Martin, Dominique Pomies, Enrico Sturaro <i>Suckling of dairy calves by their dams: trade-off between ethical and economic sustainability</i> .....	<b>O046</b>
14.30-14.45	<u>Giovanna Ranzato</u> , Maria Costanza Galli, Giulio Cozzi <i>Modeling of milk fatty acids profiles in Holstein dairy cows during early and mid lactation</i> .....	<b>O047</b>
14.45-15.00	Patrizia Di Marco, Donatella Volpatti, Valeria Donadelli, Alberta Mandich, Alessandro Longobardi, Tommaso Petochi, Maria Grazia Finoia, Gloriana Cardinaletti, <u>Giovanna Marino</u> <i>Growth performance and welfare status of gilthead sea bream (<i>Sparus aurata</i>) fed low fish meal diets</i> .....	<b>O048</b>
15.00-15.15	<u>Diana Luise</u> , Paolo Trevisi, Tristan Chalvon-Demersay, Paolo Bosi <i>Description of the effects of reducing protein content diet on piglets post-weaning intestinal health: a meta-analysis</i> .....	<b>O049</b>
15.15-15.30	<u>Mayra Gómez Carpio</u> , Dario Rossi, Gianluigi Zullo, Damiano Altieri, Roberta Cimmino <i>Preliminary investigations on functional longevity in Italian Mediterranean Buffaloes (<i>Bubalus bubalis</i>)</i> .....	<b>O050</b>

## Wednesday, September 22<sup>nd</sup> - Room Petrarca

### SESSION 11 - Genomic and phenomic tools to improve livestock animals - II

Chairs:	Roberto Mantovani, Riccardo Bozzi	
14.30-14.45	<u>Samuele Fontanesi</u> , Giuseppina Schiavo, Mohamad Ballan, Matteo Bolner, Luca Fontanesi <i>Mining livestock genomes for a One Health perspective in the management of animal genetic resources</i> .....	O051
14.45-15.00	<u>Lucio Flavio Macedo Mota</u> , Gota Morota, Francisco Peñagaricano, Alessio Cecchinato <i>Machine learning and variable selection methods for Fourier transform infrared prediction in Holstein cattle</i> .....	O052
15.00-15.15	<u>Fabio Correddu</u> , Giustino Gaspa, Nicolò P.P. Macchiotta <i>Non coagulating sheep milk characterization through Fourier-Transform IR spectroscopy</i> .....	O053
15.15-15.30	<u>Sara Faggion</u> , Massimiliano Babbucci, Giulia Dalla Rovere, Rafaella Franch, Matteo Freguglia, Luca Bargelloni, Daniela Bertotto <i>Genomic prediction of VNN resistance, body weight, cortisol concentration and antibody titer in European sea bass (<i>Dicentrarchus labrax</i> L.)</i> .....	O054
15.30-15.45	<u>Valentina Bonfatti</u> , Elena Boschi, Paolo Carnier <i>Genomic prediction of dry-cured ham weight loss in Italian heavy pigs</i> .....	O055

## Wednesday, September 22<sup>nd</sup> - Room Elena Cornaro Piscopia

### SESSION 12 - Food production and processing

Chairs:	Marcello Mele, Andrea Summer	
14.00-14.15	<u>Giulia Baldi</u> , Fabio D'Elia, Urszula Tylewicz, Massimiliano Petracci <i>Innovative approach for the tenderization of Wooden Breast meat in broilers</i> .....	O056
14.15-14.30	<u>Simone Mancini</u> , Simona Mattioli, Alice Cartoni Mancinelli, Roberta Nuvoloni, Gisella Paci <i>Effects of rosemary powder and salt additions in goose meat burgers</i> .....	O057
14.30-14.45	<u>Roxana Elena Amarie</u> , Sara Tinagli, Laura Casarosa, Giulia Foggi, Monica Tognocchi, Andrea Serra <i>Effects of cooking with Superheated Steam on organic Maremmana breed beef</i> .....	O058
14.45-15.00	<u>Isaac Hyeladi Malgwi</u> , Paolo Carnier, Celio Paolo Sasso, Veronika Halas, Stefano Schiavon <i>Innovative feeding strategies for the heavy pig: effects on technological and quality traits of dry-cured hams</i> .....	O059
15.00-15.15	<u>Giorgia Stocco</u> , Michele Pazzola, Maria Luisa Dettori, Andrea Summer, Giuseppe Massimo Vacca <i>Goat milk-based predictive formulas for different measures of cheese yield</i> .....	O060
15.15-15.30	<u>Giulia Grassi</u> , Amalia Simonetti, Emilio Gambacorta, Annamaria Perna <i>Effect of species on oxidative stability of ozone treated milk cream</i> .....	O061

## Thursday, September 23<sup>rd</sup> - Room Galileo

### SESSION 13 - Monitoring and mitigating impacts on environment - I

Chairs:	Umberto Bernabucci, Anna Sandrucci	
09.00-09.15	<u>Sara Carè</u> , Giacomo Pirlo, Luciano Migliorati <i>Environmental impact of Italian beef suckler to finish system</i> .....	O062
09.15-09.30	<u>Marco Berton</u> , Stefano Schiavon, Luigi Gallo <i>Polluting compounds in Po Valley dairy system: amount, variation and some determinants in Veneto region farms</i> .....	O063
09.30-09.45	<u>Gustavo Javier Martínez Marín</u> , Giovanni Bittante, Hugo Toledo-Alvarado <i>Effect of breed, farm intensiveness and cow productivity on predicted enteric methane emissions predicted at the population level</i> .....	O064
09.45-10.00	<u>Andrea Vitali</u> , Giampiero Grossi, Nicola Lacetera <i>Effect of summer cooling of dairy cows on carbon and water footprint intensity of milk</i> .....	O065



10.00-10.15	<u>Giulia Gislon</u> , Ernesto Tabacco, Maria Teresa Pacchioli, Alberto Tamburini, Anna Sandrucci <i>Farm level mitigation strategies to improve sustainability of milk production: effects on GHG emissions.....</i>	<b>O066</b>
10.15-10.30	<u>Valentina Ferrari</u> , Maurizio Marusi, Martino Cassandro <i>A tool to efficiently replace dairy heifers using genomic information .....</i>	<b>O067</b>

## Thursday, September 23<sup>rd</sup> - Room Giotto

### SESSION 14 - Breeding strategies to improve animal health, welfare and resilience

Chairs:	Fabio Pilla, Valentina Bonfatti	
09.00-09.15	<u>Giustino Gaspa</u> , Roberto Steri, Lorenzo Degano, Daniele Vicario <i>Lifetime profitability assessed through multivariate health index measured on primiparous Italian Simmental cows .....</i>	<b>O068</b>
09.15-09.30	<u>Angela Costa</u> , Marco Franzoi, Arianna Goi, Giulio Visentin, Massimo De Marchi <i>Genetic aspects of immunoglobulins G concentration measured with gold standard and predicted from infrared spectra in bovine colostrum.....</i>	<b>O069</b>
09.30-09.45	<u>Raffaella Finocchiaro</u> , Jan-Thijs van Kaam, Valentina Ferrari <i>Genetic Modelling of Heat Stress in Italian Holstein cows .....</i>	<b>O070</b>
09.45-10.00	<u>Anna Fabris</u> , Mauro Penasa, Tania Bobbo <i>Implementation of SCC breeding values in Italian Jersey breed .....</i>	<b>O071</b>
10.00-10.15	<u>Giulio Visentin</u> , Giovanni Buonaiuto, Angela Costa, Yari Vecchio, Ferdinando Galluzzo, Maurizio Marusi <i>Development of a selection index for resistance to subclinical ketosis in Holstein Friesian dairy cows .....</i>	<b>O072</b>
10.15-10.30	<u>Matilde Maria Passamonti</u> , Mario Barbato, Johanna Ramirez-Diaz, Michele Premi, Alessandra Stella, John Lewis Williams, Paolo Ajmone-Marsan <i>Can SNPs associated to the variation of stress biomarkers be used for the selection of stress resilient dairy cows? .....</i>	<b>O073</b>

## Thursday, September 23<sup>rd</sup> - Room Petrarca

### SESSION 15 - Feed efficiency and growth performance - I

Chairs:	Erminio Trevisi, Severino Segato	
08.30-08.45	<u>Vincenzo Lopreiato</u> , Giulia Ferronato, Giorgia Lovotti, Erminio Trevisi <i>Residual feed intake and its association with growth performance and blood metabolic profile in preweaning Simmental calves .....</i>	<b>O074</b>
08.45-09.00	<u>Alberto Romanzin</u> , Lorenzo Degano, Daniele Vicario, Mauro Spanghero <i>Residual feed intake of Italian Simmental bulls in performance test .....</i>	<b>O075</b>
09.00-09.15	<u>Ilaria Biasato</u> , Giulia Chemello, Ilario Ferrocino, Christian Caimi, Aman Paul, Monika Dalim, Michel van Spankeren, Maria Teresa Capucchio, Elena Colombino, Luca Cocolin, Francesco Gai, Achille Schiavone, Laura Gasco <i>Growth performance and gut health of rainbow trout fed commercial diets containing black soldier fly larva meal .....</i>	<b>O076</b>
09.15-09.30	<u>Matteo Daghigh</u> , Francesca Ciucci, Carlo Viti, Breanne M. McAmmond, Jonathan D. Van Hamme, Federica Mannelli, Federica Scicutella, Arianna Buccioni <i>Rumen microbiota and growth performance in Aubrac and Maremmana steers reared in pasture and in feedlot .....</i>	<b>O077</b>
09.30-09.45	<u>Alice Luciano</u> , Marco Tretola, Michele Manoni, Nicoletta Rovere, Sharon Mazzoleni, Francesca Fumagalli, Luciano Pinotti <i>Effect of salty or sweet food leftover-based diets on growth performance and digestibility in piglets .....</i>	<b>O078</b>

## Thursday, September 23<sup>rd</sup> - Room Elena Cornaro Piscopia

### SESSION 16 - Food sensory quality, labelling and valorization

- Chairs: Massimo Malacarne, Adriana Bonanno
- 09.00-09.15 Ambra Rita Di Rosa, Marianna Oteri, Vittorio Lo Presti, Chiofalo Biagina  
*Feed Insects For Aquaculture. Use of Hermetia illucens L. meal for Sparus aurata L.: Emerging trends of advanced sensor-based instruments (E-eye, E-nose, E-tongue) for fish quality.....* **O079**
- 09.15-09.30 Federico Nassivera, Emilio Tibaldi, Rodolfo Ballestrazzi, Luca Iseppi  
*To eat or not to eat: consumer behavior towards sustainable fish.....* **O080**
- 09.30-09.45 Pier Paolo Danieli, Lorenzo Romagnoli, Andrea Amici, Bruno Ronchi, Giuseppe Russo, Riccardo Primi, Marco Lauteri  
*Effects of diets with different protein sources on carbon and nitrogen isotopic signatures in Hermetia illucens: potential zootecnical implications and traceability of insect meals.....* **O081**
- 09.45-10.00 Aristide Maggiolino, Giovanna Calzaretto, Maria Federica Sgarro, Gerardo Centoducati, Francesco Giannico, Andrea Bragaglio  
*Dry vs Vacuum aging in horse meat: oxidative stability and volatolomic profile.....* **O082**
- 10.00-10.15 Silvia Parrini, Francesco Sirtori, Alessandro Crovetto, Maria Chiara Fabbri, Riccardo Bozzi  
*Potential use of near infrared spectra from meat and fat samples for the identification of autochthonous pig breeds.....* **O083**
- 10.15-10.30 Ilaria Lanza, Andrea Massaro, Alessandra Tata, Alessandro Negro, Marco Bragolusi, Roberto Piro, Lorenzo Serva, Barbara Contiero, Severino Segato  
*High-alpine forage milk characterization by DART-HRMS signatures.....* **O084**

## Thursday, September 23<sup>rd</sup> - Room Galileo

### SESSION 17 - Monitoring and mitigating impacts on environment - II

- Chairs: Mauro Spanghero, Stefano Schiavon
- 11.30-11.45 Anna Simonetto, Davide Gibin, Barbara Zanini, Valentina Caprarulo, Gianni Gilioli  
*A general framework for the assessment of environmental impact of food: an application to six healthy dietary patterns.....* **O085**
- 11.45-12.00 Franco Tagliapietra, Sara Pontini  
*Comparison of different models to predict methane emission from milk fatty acids in dairy cows.....* **O086**
- 12.00-12.15 Matteo Braidot, Alessandro Lotto, Nicola Martinelli, Chiara Sarnataro, Carla Fabro, Mauro Spanghero  
*Kinetics of rumen methane production in in vitro system.....* **O087**
- 12.15-12.30 Mondina Francesca Lunesu, Paola Sau, Fabio Correddu  
*Carbon footprint of fully and partially indoor dairy goat farming systems in Sardinia: a comparison between IPCC 2006 and IPCC 2019 prediction methods.....* **O088**

## Thursday, September 23<sup>rd</sup> - Room Giotto

### SESSION 18 - Behaviour and welfare

- Chairs: Marta Brscic, Fabio Napolitano
- 11.00-11.15 Laura Menchetti, Martina Zappaterra, Dabide Monaco, Leonardo Nanni Costa, Barbara Padalino  
*An innovative tool for assessing welfare of camels.....* **O089**
- 11.15-11.30 Alberto Cesarani, Giuseppe Pulina  
*Farm animals are long away from natural behavior: genetic and technical perspectives to improve welfare.....* **O090**
- 11.30-11.45 Stefania Celozzi, Monica Battini, Federica Manila Soli, Silvana Mattiello  
*Measuring feeding synchronization for welfare assessment in dairy farms.....* **O091**
- 11.45-12.00 Mauro Giammarino, Silvana Mattiello, Monica Battini, Piero Quatto, Luca Maria Battaglini, Ana Lopes Vieira, George Stilwell, Manuela Renna  
*Evaluation of inter-observer reliability of animal welfare indicators: which is the best index to use?.....* **O092**



12.00-12.15	Maria Costanza Galli, Annalisa Scollo, Barbara Contiero, Isabella Lora, <u>Flaviana Gottardo</u> <i>Alternative farrowing systems for sows can affect litters performance?</i> ..... <b>O093</b>
12.15-12.30	<u>Fabrizio Pirrone</u> , Cristina Zomeño, Francesco Bordignon, Angela Trocino <i>Hopping displacements of rabbit does kept in a collective housing system</i> ..... <b>O094</b>

## Thursday, September 23<sup>rd</sup> - Room Petrarca

### SESSION 19 - Feed efficiency and growth performance - II

Chairs:	Paolo Boni, Antonello Cannas
11.00-11.15	<u>Matteo Dell'Anno</u> , Carlotta Giromini, Serena Reggi, Tamil Selvi Sundaram, Simona Coranelli, Ambra Spalletta, Luciana Rossi <i>Evaluation of Lactobacillus plantarum and Lactobacillus reuteri as feed additives for swine</i> ..... <b>O095</b>
11.15-11.30	<u>Marco Zampiga</u> , Giorgio Brugaletta, Stefano Pignata, Federico Sirri <i>Effects of dietary metabolizable energy content on productive performance of turkeys</i> ..... <b>O096</b>
11.30-11.45	<u>Marco Birolo</u> , Anton Pascual Guzman, Giulio Pillan, Fabrizio Pirrone, Andrea Zuffellato, Gerolamo Xiccato <i>Effect of genotype and feeding plan on growth performance, slaughter results and meat quality of growing rabbits</i> ..... <b>O097</b>
11.45-12.00	<u>Damiano Cavallini</u> , Alessandro Concolino, Emanuela Valle, Luca Campidonico, Isa Fusaro, Ludovica Mammi, Andrea Formigoni <i>Effects of different lipid sources and tannins on performances of high producing dairy cows</i> ..... <b>O098</b>
12.00-12.15	<u>Federico Moroni</u> , Simona Rimoldi, Fabio Brambilla, Genciana Terova <i>Evaluation of lactic acid bacteria as probiotics for gilthead sea bream (Sparus aurata): effects on growth and intestinal morphology, transcriptional response, and microbiota</i> ..... <b>O099</b>
12.15-12.30	<u>Paolo Bani</u> , Andrea Fiorini, Fiorenzo Piccioli-Cappelli, Olga Gachiuta <i>Effects of ensiling treatments on the fermentation profile of pulp and stem fractions after green biorefining of alfalfa</i> ..... <b>O100</b>

## Thursday, September 23<sup>rd</sup> - Room Elena Cornaro Piscopia

### SESSION 20 - Feed effects on nutritional profile animal products - I

Chairs:	Serena Calabrò, Marco Cullere
11.00-11.15	<u>Camilla Pomete</u> , Ruggero Menci, Bernardo Valenti, Gianluca Veneziani, Mariano Pauselli, Luciano Morbidini <i>Dietary olive pomace: effects on meat and back-fat quality traits in heavy pigs</i> ..... <b>O101</b>
11.15-11.30	<u>Giuseppe Conte</u> , Eleonora Longo, Sara Tinagli, Roxana Elena Amarie, Monica Tognocchi <i>Linseed supplementation in the diet of fattening pigs: effect on the fatty acid profile of different meat cuts</i> ..... <b>O102</b>
11.30-11.45	<u>Lina Fernanda Pulido Rodriguez</u> , Giulia Secci, Francesco Gai, Giulia Maricchiolo, Giuliana Parisi <i>Effect of dietary Hermetia illucens larvae meal on triglyceride composition of sea bream fillets</i> ..... <b>O103</b>
11.45-12.00	<u>Giulia Secci</u> , Lina Fernanda Pulido Rodriguez, Adja Cristina Lira de Medeiros, Giuliana Parisi <i>Alternative protein sources in aquafeeds for rainbow trout: overview on fillet quality modifications</i> .. <b>O104</b>
12.00-12.15	<u>Nadia Piscopo</u> , Domenico Rufrano, Roberta Matera, Carmelina Lovallo, Salvatore Claps, Lucia Sepe, Angela Salzano <i>Productive performance and meat characteristics of kids fed anthocyanes</i> ..... <b>O105</b>
12.15-12.30	<u>Simona Mattioli</u> , Elisa Angelucci, Claudia Ciarelli, Alessandro Dal Bosco, Cesare Castellini <i>Effect of dietary alpha-linolenic acid on meat quality of Italian poultry breed and crossbreed</i> ..... <b>O106</b>

## Thursday, September 23<sup>rd</sup> - Room Galileo

### SESSION 21 - Precision livestock farming - I

Chairs:	Giorgio Marchesini, Fabio Abeni
14.30-14.45	<u>Maddalena Zucali</u> , Giulia Gislou, Milena Brasca, Serena Bonizzi, Luciana Bava, Stefano Morandi, Anna Sandrucci <i>Pre-weaned dairy calf housing: preliminary results on microbial air contamination</i> ..... <b>O107</b>

14.45-15.00	<u>Giorgio Marchesini</u> , Paolo Balasso, Nicola Ughelini <i>The left side flank as source of information for animal behaviour and welfare in dairy cow</i> ..... <b>O108</b>
15.00-15.15	<u>Colomba Sermoneta</u> , Andrea Serra, Gianluca Brunori <i>The livestock sector digitalization: indicators on livestock's farming digital performance</i> ..... <b>O109</b>
15.15-15.30	<u>Giulia Ferronato</u> , Michele Premi, Andrea Minuti, Olga Gachiuta, Paolo Bani <i>Near-Infrared Spectroscopy for the prediction of plasma parameters for Liver Functionality Index in dairy cows</i> ..... <b>O110</b>

## Thursday, September 23<sup>rd</sup> - Room Giotto

### SESSION 22 - Free Communications

Chairs:	Massimo De Marchi, Gianni Battacone
14.00-14.15	<u>Carmine Versace</u> , Jiang-Hua Shang, Ling-Yu Li, Angela Perucatti, Liliana Di Stasio, Alfredo Pauciuolo <i>Aneuploidy detection in Murrah buffalo MII oocytes by tri-colour FISH</i> ..... <b>O111</b>
14.15-14.30	<u>Luca Ferrari</u> , Matteo Ottoboni, Francesco Defilippo, Paolo Bonilauri, Robin Ørnsrud, Luciano Pinotti <i>Hermetia illucens reared on selenium enriched substrates for designing fortified insect meal</i> ..... <b>O112</b>
14.30-14.45	Matteo Santinello, <u>Alessia Diana</u> , Federico Scali, Giovanni Loris Alborali, Luigi Bertocchi, Valentina Lorenzi, Massimo De Marchi <i>Management strategies reduce antimicrobial use in Italian beef farms</i> ..... <b>O113</b>
14.45-15.00	<u>Matteo Santinello</u> , Alessia Diana, Federico Scali, Giovanni Loris Alborali, Luigi Bertocchi, Mauro Penasa <i>Effect of mixing animals in France on the use of antimicrobials in Italian beef fattening farms</i> ..... <b>O114</b>
15.00-15.15	<u>Maria Rita Mellino</u> , Federico Correa, Catherine Ollagnier, Giuseppe Bee, Gianni Battacone <i>Response of weaned piglet's gut microbiota to the dietary inclusion of different doses of extracted olive oil wastewater</i> ..... <b>O115</b>
15.15-15.30	<u>Riccardo Negrini</u> <i>LEO project: developing a national open data repository to support livestock biodiversity, health, sustainability, and welfare</i> ..... <b>O116</b>

## Thursday, September 23<sup>rd</sup> - Room Petrarca

### SESSION 23 - Genomics of local breeds

Chairs:	Baldassare Portolano, Emiliano Lasagna
14.30-14.45	<u>Gabriele Senczuk</u> , Slim Ben-Jemaa, Fabio Pilla, Johannes A. Lenstra, Meng-Hua Li, Elena Ciani <i>Genome-wide diversity of African and Eurasian fat-tailed sheep</i> ..... <b>O117</b>
14.45-15.00	<u>Beniamino Tuliozi</u> , Enrico Mancin, Roberto Mantovani, Alessandro Bagnato, Maria Giuseppina Strillacci, Mario Vevey, Cristina Sartori <i>GWAS of fitness and productive traits in the local Valdostana breeds</i> ..... <b>O118</b>
15.00-15.15	Slim Ben-Jemaa, Roberta Ciampolini, Gennaro Catillo, Mekki Boussaha, <u>Salvatore Mastrangelo</u> <i>Genome-wide search of distinctiveness selection signatures in Maremmana cattle compared to Italian Podolian-derived breeds</i> ..... <b>O119</b>
15.15-15.30	<u>Marco Tolone</u> , Domenico Giosa, Rosalia Di Gerlando, Anna Maria Suter, Angelo Moscarelli, Ilaria Rizzuto, Baldassare Portolano, Salvatore Mastrangelo <i>Whole-genome resequencing reveals specific genomic variants in Italian insular sheep breeds</i> ..... <b>O120</b>

## Thursday, September 23<sup>rd</sup> - Room Elena Cornaro Piscopia

### SESSION 24 - Feed effects on nutritional profile of animal products - II

Chairs:	Antonella Baldi, Ludovica Mammi
14.00-14.15	<u>Federica Mannelli</u> , Matteo Daghigh, Roberta Pastorelli, Carlo Viti, Maria Alexandra Cucu, Luca Turini, Federica Scicutella, Arianna Buccioni <i>Milk nutritional quality and rumen microbial community of Holstein-Friesian cows fed a diet supplemented with olive oil pomace</i> ..... <b>O121</b>

14.15-14.30	<u>Giorgia Secchi</u> , Nicolò Amalfitano, Ilaria Carafa, Kieran Michael Tuohy, Elena Franciosi, Giovanni Bittante <i>Metagenomics of milk before, during and after summer transhumance to highland pasture in relation to human health and cheese making properties</i> .....	<b>O122</b>
14.30-14.45	Antonino Di Grigoli, Marialetizia Ponte, Riccardo Gannuscio, Margherita Addis, Marco Alabiso, Giuseppe Maniaci, Monica Bacchi, Massimo Todaro, <u>Adriana Bonanno</u> <i>Dehydrated sulla forage in dairy ewes feeding: effects on cheese properties</i> .....	<b>O123</b>
14.45-15.00	<u>Elisabetta Bacchin</u> , Nadia Guzzo, Lucia Bailoni <i>Use of different levels of hemp meal supplementation in diets of veal calves: effects on in vivo performances and metabolic profile</i> .....	<b>O124</b>
15.00-15.15	<u>Mengjun Wu</u> , Siria Tavaniello, Sanije Zejnelhoxha, Antonella Fatica, Marisa Palazzo, Elisabetta Salimei, Giuseppe Maiorano <i>Effect of genotype and diet on carcass and meat quality traits of broiler chickens</i> .....	<b>O125</b>
15.15-15.30	Silvia Carta, Eleni Tsiplakou, <u>Anna Nudda</u> <i>Novel by-products in goat nutrition: effect on milk yield and composition</i> .....	<b>O126</b>

### Thursday, September 23<sup>rd</sup> - Room Galileo

#### SESSION 25 - Precision livestock farming - II

Chairs:	Paolo Berzaghi, Pasquale De Palo	
16.00-16.15	<u>Lorenzo Serva</u> , Giacomo Bison, Mattia Zago, Giorgio Marchesini, Igino Andrighetto <i>Use of a portable NIR instrument as a rapid tool for cattle feeding control</i> .....	<b>O127</b>
16.15-16.30	<u>David Meo Zilio</u> , Miriam Iacurto, Francesco Cenci, Roberto Steri <i>Precision Buffalo Farming: preliminary results from the AGRIDIGIT project</i> .....	<b>O128</b>
16.30-16.45	<u>Pier Paolo Danieli</u> , Giuseppe Bianchi, Marco Maerini, Gabriele Locci, Filippo Lazzari, Gianluca Piovesan <i>Precision livestock farming and beekeeping: development and testing of a prototypal smart hive</i> .....	<b>O129</b>

### Thursday, September 23<sup>rd</sup> - Room Giotto

#### SESSION 26 - Omics technologies - I

Chairs:	Giuseppe Conte, Sara Pegolo	
16.00-16.15	Barbara Lazzari, Gianluigi Paolillo, Maria Grazia De Iorio, Rita Rizzi, Gustavo Gandini, <u>Giulietta Minozzi</u> <i>An alternative method to identify Csd alleles of the honey bees based on NGS data</i> .....	<b>O130</b>
16.15-16.30	<u>Martina Zappaterra</u> , Paolo Zambonelli, Anna Maria Belmonte, Giovanna Minelli, Domenico Pietro Lo Fiego <i>Investigating the effects of diets enriched in PUFA and antioxidants on the gene expression networks and intramuscular fatty acid composition in porcine Longissimus thoracis et lumborum muscle</i> .....	<b>O131</b>
16.30-16.45	<u>Martina Bordini</u> , Francesca Soglia, Adele Meluzzi, Roberta Davoli <i>Weighted Gene Co-Expression Network Analysis identifies the main gene networks and biological pathways associated to chicken Pectoralis major myopathies</i> .....	<b>O132</b>

### Thursday, September 23<sup>rd</sup> - Room Petrarca

#### SESSION 27 - Quantitative approaches, inbreeding and crossbreeding - I

Chairs:	Mauro Penasa, Giustino Gaspa	
16.00-16.15	<u>Ferdinando Galluzzo</u> , Raffaella Finocchiaro <i>Variance components estimation for milkability in Italian Holstein breed</i> .....	<b>O133</b>
16.15-16.30	<u>Roberta Cimmino</u> , Dario Rossi, Gianluigi Zullo, Damiano Altieri, Yuri Gombia, Rossella Di Palo, Mayra Gomez Carpio <i>The relationship between somatic cell score and udder type traits in Italian Mediterranean Buffaloes (Bubalus bubalis)</i> .....	<b>O134</b>

16.30-16.45	<u>Martina Piazza</u> , Luigi Gallo <i>Carcass value of purebred Holstein and crossbred cull cows from a 3 breed rotational scheme involving Viking Red, Montbéliarde, and Holstein bulls</i> .....	O135
16.45-17.00	<u>Francesca Soglia</u> , Martina Bordini, Mattia Di Nunzio, Giulia Lattanzio, Adele Meluzzi, Maurizio Mazzoni, Massimiliano Petracchi <i>Implication of collagen type IV in the onset of breast muscle disorders in broilers</i> .....	O136

## Thursday, September 23<sup>rd</sup> - Room Elena Cornaro Piscopia

### SESSION 28 –

*This is a free session dedicated to short meetings of the ASPA councils, study commissions' encounters, and free meetings left for ASPA members.*

## Friday, September 24<sup>th</sup> - Room Galileo

### SESSION 29 - Animal efficiency - I

Chairs:	Franco Tagliapietra, Antonio Gallo	
09.00-09.15	<u>Luisa Magrin</u> , Flaviana Gottardo, Chiara Mondin, Paola Prevedello, Samuele Trestini <i>Technical-economic analysis of ventilation systems used to mitigate heat stress in dairy cows</i> .....	O137
09.15-09.30	Marica Simoni, Rosario Pitimo, Elisa Battaglia, Annamaria Paolino, Afro Quarantelli, <u>Federico Righi</u> <i>Assessment of ruminal and fecal microbial biomass and protein content and their relationship with the digestion indicators</i> .....	O138
09.30-09.45	<u>Dimitrios Sioutas</u> , Antonio Mazza, Beatrice Pileri, Alessandra Marzano, Antonello Ledda, Antonello Cannas <i>Growth curve characteristics of dairy ewe-lambs of the Sarda breed from birth until start of mating age</i> .....	O139
09.45-10.00	<u>Valentina Caprarulo</u> , Monika Hejna, Serena Reggi, Lauretta Turin, Antonella Baldi, Luciana Rossi <i>Evaluation of phytochemical dietary supplements on O138 E. coli challenged piglets</i> .....	O140
10.00-10.15	<u>Chiara Aquilani</u> , Lapo Nannucci, Martina Benedetti, Andrea Confessore, Silvia Parrini, Francesco Sirtori, Oreste Franci, Rosamaria Smaldone, Ilaria Paladini, Carolina Pugliese <i>Use of zeolite as zinc oxide replacement to control diarrhea in weaned pigs (from 8 to 20 kg): preliminary results on growth and microbiological profile of feces</i> .....	O141
10.15-10.30	<u>Alessia Tondo</u> , Israel Flamenbaum, Mauro Fioretti, Lorenzo Pascarella <i>Summer-Winter index: a visual tool to monitor dairy cow response to heat stress at farm level</i> .....	O142

## Friday, September 24<sup>th</sup> - Room Giotto

### SESSION 30 - Omics technologies - II

Chairs:	Alessia Cecchinato, Francesca Ciotola	
09.00-09.15	Katia Cappelli, Gianpiero Marconi, Samanta Mecocci, Giorgia Mazza, Andrea Giontella, Emidio Albertini, <u>Stefano Capomaccio</u> <i>Epigenetic modifications in sports horses during training: analysis of genome-wide methylation as an adaptation phenomenon</i> .....	O143
09.15-09.30	<u>Samanta Mecocci</u> , Stefano Capomaccio, Daniele Pietrucci, Katia Cappelli <i>OMIC characterization of cow, donkey and goat milk extracellular vesicles reveals their anti-inflammatory and immune-modulatory potential</i> .....	O144
09.30-09.45	<u>Paolo Zambonelli</u> , Silvia Gioiosa, Marco Milanese, Tiziana Castrignanò, Roberta Davoli <i>SNPs and genes identification from muscle and backfat transcriptome of Italian Large White pigs with extreme and divergent fat deposition</i> .....	O145
09.45-10.00	<u>Daniele Pietrucci</u> , Daniele Alberoni, Loredana Baffoni, Diana Di Gioia, Giovanni Chillemi <i>Genomic and functional diversity of honey bee gut microbiota, after exposure to veterinary drugs or natural feed additives</i> .....	O146

10.00-10.15	Fabio Omodei Zorini, Matteo Dell'Anno, Georgia Pennarossa, Paola Cremonesi, Bianca Castiglioni, Filippo Biscarini, <u>Guido Invernizzi</u> <i>Rumen and hindgut microbiomes of dairy Italian Holstein Friesian heifers fed with camelina sativa cake</i> .....	O147
10.15-10.30	<u>Valentino Palombo</u> , Abdulrahman S. Alharthi, Fernanda Batistel, Mariasilvia D'Andrea, Claudia Parys, Jessie Guyader, Juan J. Loor <i>Adaptations in neonatal calf hepatic transcriptome in response to enhanced post-ruminal maternal supply of methionine</i> .....	O148

## Friday, September 24<sup>th</sup> - Room Petrarca

### SESSION 31 - Quantitative approaches, inbreeding and crossbreeding - II

Chairs:	Paolo Carnier, Giuseppe Campanile	
09.00-09.15	<u>Simone Ceccobelli</u> , Francesco Perini, Lorenzo Rosa, Maria Federica Trombetta, Marina Pasquini <i>Myostatin gene affects carcass and meat quality in Marchigiana beef cattle</i> .....	O149
09.15-09.30	Nicolaia Iaffaldano, <u>Giusy Rusco</u> , Roberta Iampietro, Pier Paolo Gibertoni, Alessandra Roncarati, Michele Di Iorio <i>How post-collection storage time can affect the semen freezability of Mediterranean trout?</i> .....	O150
09.30-09.45	<u>Hugo Toledo-Alvarado</u> , Gustavo Javier Martínez Marín <i>Genetic parameters of predicted enteric methane emissions of Brown Swiss cattle at the population level in Italy</i> .....	O151
09.45-10.00	<u>Stefano Biffani</u> , Giulietta Minozzi, Maria Grazia Di Iorio, Giulio Pagnacco <i>Genetic Evaluation and Variance component estimation in an Italian honey bee population</i> .....	O152
10.00-10.15	<u>Maria Chiara Fabbri</u> , Francesco Tiezzi, Alessandro Crovetto, Riccardo Bozzi <i>Genetic parameters for carcass weight at different age-classes in Italian Limousine beef cattle</i> .....	O153
10.15-10.30	<u>Francesca Maria Sarti</u> , Samira Giovannini, Camillo Pieramati, Pancrazio Fresi, Silverio Grande <i>Merinizzata Italiana sheep breed: genetic gain after 20 years of Performance test</i> .....	O154

## Friday, September 24<sup>th</sup> - Room Elena Cornaro Piscopia

### SESSION 32 - Companion animals and society - I

Chairs:	Rebecca Ricci, Paola Crepaldi	
09.30-09.45	<u>Carla Giuditta Vecchiato</u> , Carlo Pinna, Alba Gaspardo, Monica Grandi, Costanza Delsante, Paola Parazza, Pietra Marco, Giacomo Biagi <i>Dietary supplementation with coconut oil in dogs with chronic enteropathies</i> .....	O155
09.45-10.00	<u>Gerardo Siani</u> , Giada Morelli, Davide Stefanutti, Rebecca Ricci <i>Evaluation of body weight and body condition score in 64 European Shorthair adult cats</i> .....	O156
10.00-10.15	<u>Giada Morelli</u> , Roberta Di Maggio, Rebecca Ricci <i>Copper-associated liver disease in a Bergamasco Sheepdog: a case report</i> .....	O157
10.15-10.30	<u>Sara Albarella</u> , Emanuele D'Anza, Giacomo Galdiero, Alessandra Iannuzzi, Pietro Parma, Vincenzo Peretti, Francesca Ciotola <i>Disorders of sexual development in 9 dogs: clinical, cytogenetics and molecular findings</i> .....	O158

## Friday, September 24<sup>th</sup> - Room Galileo

### SESSION 33 - Animal efficiency - II

Chairs:	Gerolamo Xiccato, Rodolfo Ballestrazzi	
11.00-11.15	<u>Giulio Pillan</u> , Anton Pascual, Luca Carraro, Anna Concollato, Angela Trocino <i>Production and egg quality in brown hens kept in a cage-free system: effects of hen age and nest lighting</i> .....	O159



11.15-11.30	<u>Roberto Cerri</u> , Fabio Mina, Rodolfo Ballestrazzi, Antonio Jesus Vizcaino Torres, Francesca Tulli <i>In vitro protein degradability in fish: a tool to evaluate novel protein sources</i> .....	<b>O160</b>
11.30-11.45	<u>Alberto Stanislao Atzori</u> , Antonio Gallo <i>System dynamics applications in animal science and dairy farm management</i> .....	<b>O161</b>
11.45-12.00	<u>Antonio Gallo</u> , Claudio Valsecchi, Elisabetta Manca, Francesco Masoero, Alberto Stanislao Atzori <i>A Survey on feeding economics in intensive dairy farms</i> .....	<b>O162</b>
12.00-12.15	<u>Castro Ndong Ncogo Nchama</u> , Mario Baldini, Carla Fabro, Elena Saccà, Vinicius Foletto, Silvia Pianezze, Mirco Corazzin, Edi Piasentier <i>Performance of cull cows fed diets supplemented with hempseed cake</i> .....	<b>O163</b>
12.15-12.30	<u>Marica Simoni</u> , Claudia De Lorenzi, Marta Pozza, Daniele Giaccone, Andrea Revello Chion, Luciano Comino, Federico Righi <i>Bovine milk quality and apparent digestibility of diets as affected by diet characteristics and milking system</i> .....	<b>O164</b>

## Friday, September 24<sup>th</sup> - Room Giotto

### SESSION 34 - Omics technologies - III

Chairs:	Roberto Antoveni, Maria Silvia D'Andrea	
11.00-11.15	<u>Giovanna Salvatore</u> , Valentino Palombo, Mario Vailati Riboni, Mariasilvia D'Andrea, Adam J. Geiger, Michael R. Akers, Juan J. Looor <i>Estrogen levels effect on mammary parenchyma explants from Holstein heifer calves fed an enhanced preweaning diet evaluated via RNA-sequencing</i> .....	<b>O165</b>
11.15-11.30	<u>Isaac Hyeledi Malgwi</u> , Perta Grünvald, Ildikó Jócsák, Veronika Halas <i>Genes related to fat metabolism in pigs and intramuscular fat content of pork</i> .....	<b>O166</b>
11.30-11.45	<u>Antonella della Malva</u> , Marzia Albenzio, Mariangela Caroprese, Maria Giovanna Ciliberti, Lucia Figliola, Antonella Santillo, Agostino Sevi, Rosaria Marino <i>Proteomics to investigate tenderness biomarkers in Martina Franca donkey meat</i> .....	<b>O167</b>
11.45-12.00	<u>Alberto Palmonari</u> , Damiano Cavallini, Silvia Turrone, Francesca Ghiaccio, Giovanni Buonaiuto, Giorgia Canestrari, Ludovica Mammi <i>Impact of acidogenic diets on rumen microbial community</i> .....	<b>O168</b>
12.00-12.15	<u>Bruno Stefanon</u> , Michela Cintio, Sandy Sgorlon, Alfonso Zecconi, Monica Colitti, Elisa Scarsella <i>MiRNAs cargo of milk exosomes are related to somatic cell count in dairy cows</i> .....	<b>O169</b>
12.15-12.30	<u>Edo D'Agaro</u> , Andrea Favaro, Stefano Mattiussi <i>First assembled reference genome of white-clawed crayfish (Austropotamobius pallipes italicus)</i> .....	<b>O170</b>

## Friday, September 24<sup>th</sup> - Room Petrarca

### SESSION 35 - Genetic diversity

Chairs:	Alfredo Pauciullo, Stefano Capomaccio	
11.00-11.15	<u>Alberto Cesarani</u> , Andre Garcia, Giacomo Bertolini, Daniela Lourenco, Nicolò Macciotta <i>Using runs of homozygosity within single-step genomic BLUP</i> .....	<b>O171</b>
11.15-11.30	<u>Christos Dadousis</u> , Michela Ablondi, Jan-Thijs van Kaam, Alberto Sabbioni <i>Genomic inbreeding estimates with imputed genotypes in Italian Holstein dairy cattle</i> .....	<b>O172</b>
11.30-11.45	<u>Matteo Cortellari</u> , Alessio Negro, Arianna Bionda, Stefano Frattini, Silverio Grande, Paola Crepaldi <i>Inbreeding depression in small ruminants: from pedigree to genomic estimation</i> .....	<b>O173</b>
11.45-12.00	Elisa Somenzi, Ettore Randi, Gwenola Tosser-Klop, Roberta Ciampolini, <u>Licia Colli</u> <i>SNP-based characterization of Montecristo feral goats in a Mediterranean perspective</i> .....	<b>O174</b>
12.00-12.15	<u>Giuseppina Schiavo</u> , Samuele Bovo, Silvia Tinarelli, Manolo Cappelloni, Maurizio Gallo, Luca Fontanesi <i>A comparative analysis of pedigree- and genomic-derived inbreeding parameters in Italian heavy pig breeds over the last decades</i> .....	<b>O175</b>
12.15-12.30	<u>Michela Ablondi</u> , Claudio Cipolat-Gotet, Christos Dadousis, Giorgia Stocco, Anna, Alberto Sabbioni <i>The impact of inbreeding in the Italian Holstein breed</i> .....	<b>O176</b>

## Friday, September 24<sup>th</sup> - Room Elena Cornaro Piscopia

### SESSION 36 - Companion animals and society - II

Chairs:	Luigi Liotta, Monica Cutrignelli	
11.00-11.15	<u>Arianna Bionda</u> , Matteo Cortellari, Alessio Negro, Stefano Frattini, Andrea Talenti, Luigi Liotta, Paola Crepaldi <i>Genomic evaluation of the Italian shepherd dogs</i> .....	<b>O177</b>
11.15-11.30	<u>Marco Isidori</u> , Fabrizio Rueca, Francesca Romana Massacci, Manuela Diaferia, Andrea Giontella, Marco Caldin, Tommaso Furlanello, Ronald J. Corbee, Gabriele Mannucci, Giovanni Pezzotti, Massimo Tralbalza-Marinucci <i>Dietary Ascophyllum nodosum and Bacillus subtilis C-3102 influence faecal fermentation patterns in dogs with chronic enteropathies</i> .....	<b>O178</b>
11.30-11.45	Alessandro Vastolo, Serena Calabrò, <u>Monica Isabella Cutrignelli</u> <i>Effect of different diets on metabolic profile of healthy adult dogs</i> .....	<b>O179</b>
11.45-12.00	<u>Elisa Scarsella</u> , Aashish Jha, Bruno Stefanon <i>Network-based gut microbiome analysis in dogs</i> .....	<b>O180</b>
12.00-12.15	<u>Eleonora Fusi</u> , Annalaura, Laura Rua, Raffaella Rossi <i>Renal petfood for dogs and cats: fatty acids profile</i> .....	<b>O181</b>

# ASPA 24th Congress

## Padova, September 21-24, 2021

### Italian Journal of Animal Science 2021; volume 20, supplement S1

#### Agenda for poster presentations

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- Fiorella Sbarra, Andrea Quaglia, Giovanni Bittante, Roberto Mantovani  
*Genetic parameters of temperament traits in Marchigiana, Chianina and Romagnola young bulls* ..... **P001**
- Silvana Mattiello, Renata Piccinini, Stefano Biffani, Giovanni Bailo, Monica Battini, Giulietta Minozzi  
*Does the presence of weeping teats affect behaviour, health and productivity of dairy goats?* ..... **P002**
- Michele Premi, Giulia Ferronato, Matteo Mezzetti, Mario Barbato, Fiorenzo Piccioli-Cappelli, Andrea Minuti, Erminio Trevisi  
*Reference intervals for plasma parameters in high-welfare multiparous dairy cows in different physiological phases* ..... **P003**
- Alice Vanzin, Alessandro Toscano, Vittoria Bisutti, Sara Pegolo, Diana Giannuzzi, Alessio Cecchinato  
*Associations between udder health traits and milk mineral profile in Holstein Friesian cattle* ..... **P004**
- Andrea Minuti, Luca Cattaneo, Vincenzo Lopreiato, Silvia Zini, Fiorenzo Piccioli Cappelli, Erminio Trevisi  
*Effects of a selective dry-cow therapy approach on milk, inflammation, and metabolic profile during the dry-period* ..... **P005**
- Francesco Ciampi, Lorraine Sordillo, Jeffrey Gandy, Marzia Albenzio, Mariangela Caroprese, Maria Giovanna Ciliberti, Antonella della Malva, Martina Di Corcia, Rosaria Marino, Agostino Sevi, Antonella Santillo  
*Use of pomegranate extracts in bovine oxidative stress mitigation* ..... **P006**
- Giorgia Riuzzi, Iliana Lanza, Barbara Contiero, Severino Segato, Flaviana Gottardo  
*Influence of the main dietary roughage sources on the health status of Italian Friesian lactating dairy cows* ..... **P007**
- Vincenzo Landi, Alessio Cecchinato, Aristide Maggiolino, Umberto Bernabucci, Pasquale De Palo  
*Heat stress in Italian Brown Swiss: effect of the interaction of genotype by environment using reaction norm analysis* ..... **P008**
- Matteo Braidot, Chiara Sarnataro, Carla Fabro, Alberto Romanzin, Francesco Fancello, Severino Zara, Mauro Spanghero  
*Effect of lactic acid bacteria addition or protozoa modulation on the in vitro rumen aflatoxin B1 decontamination* ..... **P009**
- Riccardo Moretti, Stefania Chessa, Stefano Sartore, Dominga Soglia, Daniele Giaccone, Francesca Tiziana Canizzo, Paola Sacchi  
*Application of a genomic tool for heifers selection in Italian Holstein cattle* ..... **P010**
- Maria Costanza Galli, Claudio Mazzoni, Federica Mereghetti, Barbara Contiero, Flaviana Gottardo  
*How the stage of gestation for moving at the group housing can affect sow's reproductive performance?* ..... **P011**
- Mariangela Caroprese, Maria Giovanna Ciliberti, Rosaria Marino, Fabio Napolitano, Ada Braghieri, Agostino Sevi, Antonella Santillo, Martina di Corcia, Antonella della Malva, Marzia Albenzio  
*What consumers perceive from geographical information and welfare of lambs?* ..... **P012**
- Maria Francesca Sgadari, Alessio Cotticelli, Brunella Restucci, Angela Salzano, Rossella Di Palo, Gianluca Neglia  
*Differential somatic cell count in buffalo species: preliminary results* ..... **P013**



Damiano Cavallini, Alberto Palmonari, Emanuela Valle, Francesca Ghiaccio, Ludovica Mammi, Andrea Formigoni <i>Health and immunological parameters of susceptible and resistant cows exposed to a rich grain diet.....</i>	<b>P014</b>
Filippo Lazzari, Tiziana Vetturini, Pierpaolo Danieli <i>Preliminary laboratory trials on the efficacy of hop alpha iso-acids on the control of the honey bee (Apis mellifera L.) ectoparasite Varroa destructor.....</i>	<b>P015</b>
Pier Paolo Danieli, Davide Bassignana, Francesco Barbarese, Remigio Ercolani, Filippo Lazzari <i>Practical perspectives of using the Bee Varroa Scanner (BeeVS), as an automated tool for the Varroa destructor parasite load monitoring in honey bee (Apis mellifera) colonies.....</i>	<b>P016</b>
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## IS001

### Animal production and society

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The paper will look at social perceptions and attitudes towards animal production and its environmental, ethical and healthy implications.

The most recent data will be presented regarding the key dimension of citizens and consumer perceptions, including key drivers of consumer choices about food in Europe (e.g. safety, sustainability, taste).

## IS002

### Societal and political demands on farmed animal welfare: an evolving landscape

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Public perceptions about farmed animal welfare are rapidly evolving in the European Union (EU) and, albeit at different speeds, worldwide. Already in 2016, the special Eurobarometer on animal welfare had shown that 82% of EU citizens want better protection for farmed animals, with over 50% stating that they look for labels certifying higher welfare when buying animal products. In 2018 and 2019, two surveys commissioned by Eurogroup for Animals showed that EU citizens are also concerned about the welfare of fish in aquaculture and the treatment of broiler chickens. Very recently, a public consultation by the European Commission revealed that citizens do not agree with public spending in promotional campaigns to stimulate the consumption of meat and dairy. Last, but not least, the overwhelming success of the European Citizens' Initiative (ECI) 'End The Cage Age' made it clear that the use of cages in livestock farming is no longer considered acceptable by civil society.

Until recently, there has been limited concrete action at the EU level to address a situation that is objectively dire for farmed animals. To give but one example, 25 Member States are in breach of the Pigs Directive and not a single infringement procedure has been launched. However, after the latest European elections, there is a new political wind blowing and this opens up tremendous opportunities to drive real change. Among its deliverables, the EU Farm to Fork Strategy includes a revision of farmed animal welfare legislation as well as the possibility to introduce harmonised front-of-pack labelling to inform consumers on sustainability and animal welfare.

The enormous success and widespread support at all political levels (European Parliament, Council and Commission) of the ECI 'End The Cage Age' should be seen as highly representative of the scale of the changes that potentially await animal productions in the EU. As the prospective revision of EU legislation on farmed animal welfare will have to be based on scientific evidence, the field of the animal sciences at large has a crucial role in supporting this process. The solutions envisaged by different stakeholders to the challenges that such a profound transformation poses are diverse. Some propose to maintain or increase the current levels of animal production using new genetic techniques, new feeding strategies and precision livestock farming to offset environmental externalities and improve animal health and welfare. According to others, to respect planetary boundaries and protect public health while at the same time ensuring adequate nutrition for all, our food environment will have to look completely different from what it is now: cell-based and plant-based protein sources should become mainstream and livestock farming should play a complementary role.

## IS003

### The Italian research in animal science: from ASPA-Padova-1987 to ASPA-Padova-2021 and beyond

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In 1987, the national ASPA Congress was held for the first time in Padova. Prof. Mario Bonsembiante, President of the Congress, presented the main lecture on 'The structures and the role of research in animal science in Italy' describing the size, organization, funding, and the major research topics of those years. In 2021, 34 years later, the ASPA Congress is held again in Padova. The objective of this presentation is to describe the changes and to postulate some perspectives for the national animal science in the next future. About the Institutions, the Faculties of Agriculture and Veterinary Medicine have been replaced by Schools, with some downsizing in the authority and responsibilities. On the other side, the old, small, and very specialized Institutes of Zootechnics have been replaced by much broader and interdisciplinary Departments that have taken charge of research, teaching activities and dissemination, and technical assistance. In the Italian universities, the number of professors and permanent researchers working in the animal science sector did not change with time (304 in 1987, 309 in 2021), but new contract researcher positions have been recently introduced (66 in 2021). The PhD courses, not yet completed in 1987, are now a powerful engine of research activities. The scientific articles published yearly have increased enormously in number and moved from being almost exclusively

published in Italian on national paper journals to electronic international journals in English. The leading 'Zootecnica e Nutrizione Animale' was replaced by the 'Italian Journal of Animal Science', which is continually increasing its Impact Factor and CiteScore. From the bibliometric point of view, the Italian research in animal science is currently in the leading four positions in Europe in terms of articles published and journals ranking. Moreover, international collaborations are now widespread. The number of abstracts of ASPA 2021 congress (353) is more than doubled compared to ASPA 1987 (147). The general disciplines are the same, but the main topics are profoundly changed: 'omics' sciences, environmental impact, insects, animal welfare, and animal foods for human health are now leading research topics.

## IS004

### Challenges of Italian livestock systems towards a full environmental sustainability

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Italy is characterized by the presence of many livestock production systems, from the traditional ones in the mountain areas to the specialised in the most favourable conditions. Livestock farming plays important roles at the national and local levels, both from an economic point of view and for the provision of ecosystem services. In the last four decades, the livestock sector in Italy has experienced a decrease in the numbers of farms, particularly the small ones, together with a decrease in the numbers of animals (from 1961 to 2019: -21.3% Large Animal Unit) and enlargement of farms. If we consider the carbon footprint of the Italian livestock sector, in the same period a reduction of global emissions have been calculated (e.g.: -40% of methane). This can be attributed to the combination of the reduction of animal units, improvements of the management and structural characteristics, and the application of new technologies. The horizon point of carbon-neutrality of Italian livestock farming systems can be fixed in a medium-term perspective. Different options are available according to the nature of the system. Precision farming represents one of the most important approaches for the management of all components, especially for an efficient feed formulation (e.g. protein feeding), but also to cope with the foreseeable changes induced at various levels by climatic changes on agro-ecosystems. The use of by-products of the food chain can be enhanced, both for animal nutrition, or other uses in the context of a circular economy. The increasing number of multidimensional datasets, e.g. deriving from quality control protocols, and biosensors, will require sophisticated

techniques of machine learning and expertise. A comprehensive approach for research and innovation is needed to optimize sustainable agriculture and livestock farming systems, with advances in animal science, technology, and social sciences. There is also a need for significant funding and action plans for basic and applied research in the fields of animal science, and for improving the cultural dimension of farmers and technicians. The process of a full transition towards the EU 'green deal' should be sustained by a regulatory framework and new governance/business models, with a good connection between actions and impacts, and better integration of environmental sustainability with social and economic sustainability.

## IS005

### Eight centuries of the University of Padua (1222–2022): a historical sketch

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The University of Padua is the third oldest university in the world, being founded in 1222. The next year 2022, will mark eight centuries of existence for this extraordinary cultural institution. The University of Padua hosted many eminent figures in all fields of science and humanities, such as Pietro Bembo (poet and linguists), Giacomo Casanova (humanist), Pietro Arduino (botanist), Nicolaus Copernicus (astronomer and physician), Elena Cornaro Piscopia (philosopher and the first woman to have a doctorate in the western world), Galileo Galilei (mathematician and astronomer), Andrea Vesalius (father of modern anatomy), and Giovanni Battista Morgagni (father of pathology).

The history of the Padua University saw its 'Golden Age' from the XVI to the XVIII century, when the Serenissima Republic of Venice, ruling the city of Padua, made its university the unique cultural institution of the whole Republic. Venetian administrators called the best professors from all around Europe and gave the University great freedom and tolerance of researching and teaching. However, after the fall of the Serenissima, the University of Padua maintained a prominent role in several fields of science and humanities. Still, now, it is one of the best universities at the international level, continuing an uninterrupted tradition of liberty and independence of thought. Not by chance, our motto remains 'universa universis patavina libertas', meaning 'the freedom of Padua is universal and for everyone'.



## IS006

### From Pietro Arduino and Giuseppe Orus to the Agripolis Campus: analogies with present times between historical realities and possible fakes

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The 800-year-old University of Padua boasts ancient roots also in the field of Agricultural Sciences and Veterinary Medicine. The first Italian chair of Agronomy was established in Padua in 1765 and it was assigned to Pietro Arduino, professor of Botany. Arduino, the historic custodian of the Botanical Garden, was asked to identify the causes of the low production of cattle, one of the most significant items in the deficit budget of the Serenissima. In 1773 Giuseppe Orus, a young man from Parma who had completed his studies at the Veterinary School of Alfort in France was called to direct the 'Collegium Zooiatricum' in Padua. Orus began by visiting various places in Dalmatia struck by epizootic diseases. On September 27, 1792, he suddenly died, being only 42, leaving handwritten documentation of his theoretical and practical teaching. The causes of his death can now most likely be associated with a contagious disease he might have contracted while practicing his veterinary profession. The most interesting evidence of the activity of the Veterinary School is the recent discovery of the first Veterinary Graduate diploma, handwritten by Orus and dated 30 October 1779; although a debate could open up on which university can rightfully boast the first graduate in Veterinary Medicine, the contenders being Padua and Turin. Even the portrait depicting Orus, presently located in the study of the Director of the University Library of Padua, could lead to new intriguing conclusions if carefully studied. Two steps allowed the prosecution of research and teaching in the field of Agricultural Sciences and Veterinary Medicine in Padua. First of all, the birth of the Faculty of Agricultural Science, which was authorized to hold courses since 1946 and was legally established in 1951 with the arrival of Elvio Borgioli from Florence. Afterwards, in 1992, the establishment of the Faculty of Veterinary Medicine in Legnaro, thanks to the great intuition of Mario Bonsembiante. The Agripolis Campus was to become a Scientific and Technological Pole where the 2 Faculties, together with the Experimental Farm, the Istituto Zooprofilattico Sperimentale delle Venezie and the Regional Agency of Veneto Agricoltura can cooperate in an interdisciplinary way, in close Contact with the territory but with an open and international vision. The heart of the Campus is the library located at the Pentagon building, named after Pietro Arduino. And here the story begins again from its origins.

## IS007

### Mutilations in pigs: searching for a new state-of-mind of the farmer

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The public opinion considers mutilations in pigs to be a violation of the animal's wellbeing and integrity. Whereas tail docking and teeth clipping are banned by the EU regulation in force (Council Directive 2008/120/EC) but still performed in a large part of the farms due to a derogation of the law in case of evidence that injuries to sows' teats or other pigs' tails have occurred., Surgical castration is permitted without pain relief when the animal is less than 7-days-old. In spite of the negative perception of consumers regarding mutilation, effective incorporation of mutilation-free farming as a normal rearing attribute is hampered, partly because of diverging perceptions and the lack of consensus among the different stakeholders with respect to the topic.

The challenge to achieve a more welfare-friendly approach regarding mutilation in pig farms is to reach a new and proactive state of mind of the farmer. To this purpose, a crucial aspect is the training of people who care for animals as stock-keepers, stockmen, farmers and producers, that are placed in a position where they can greatly influence the quality of life of the animals they manage. Sometimes the capacity to care is subsumed by commercial production pressures, lack of time, lack of motivation, perceived lack of resources, perceived lack of 'value' for individual animals, lack of perception of animal issues, or sometimes through a lack of knowledge or exposure to concepts of animal care and welfare. For these reasons, the introduction of new skills in managing pigs mutilations should be supported: for tail biting, new management of the environmental enrichments, principles of ethology to learn to identify behavioural premonitory signs of aggressiveness, early recognition of hierarchical interactions among animals leading to a biter-victim and their management in the daily work are increasingly required. As well as for tail docking, pig castration needs a new attitude of the farmers towards the management of intact males or the adoption of pain relief even when surgical castration is performed in piglets less than 7-days-old.

## IS008

### Animal source foods in the 21st century – obsolete or essential?

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Animal source foods are evolutionary foods that have shaped human physiology and metabolism. Their consumption in substantial amounts goes back at least 2 million years with the emergence of the genus *Homo* and the appearance of tools and carnivory in the archeological record. Even if the human diet is an evolving concept rather than a static one, and should be considered as such by policy makers, animal source foods still provide key nutrients in contemporary diets that are less easily obtained from plants. This not only relates to the intake of high-quality protein but, even more importantly so, also to the role of some already limiting minerals (e.g. iron, zinc, calcium, and iodine) and vitamins (e.g. B12, A, and D), and various other nutrients of concern (e.g. DHA and choline). Despite controversies, which are driven by social anxieties, vested interests, and/or ideologies, it is argued that there is no good reason to eliminate the consumption of animal-source foods from a human health perspective, well on the contrary. People who nonetheless decide to do so on ethical or environmental grounds should keep in mind that the robustness of restrictive diets depends on knowledge, resources, and careful supplementation. Although current omnivore diets are often not well-formulated either, discouraging the consumption of some of the most nutrient-rich and species-adapted foods would constitute an additional barrier to achieving adequate essential nutrition in an already problematic foodscape. Such intervention risks causing damage in the more vulnerable parts of the population, in particular among the young, elderly, and metabolically challenged. Whereas planetary challenges indeed require urgent dietary transformation, both for reasons of human health and the environment, nutritional needs and food security should always be at the heart of decision making. This is also valid within the broader sustainability debate, where environmental impact should never be uncoupled from food security and other ways through which animal source foods contribute to the food system and societal wellbeing. Doing otherwise would result in harmful reductionism, not in the least because animal source foods and livestock farming are intimately intertwined with crop agriculture, the valorization of marginal lands and waste, the provision of ecosystem services and non-edible products (e.g. clothing and manure), food culture and heritage, regional identities, and livelihoods.

## IS009

### **Harnessing the power of computer vision system to improve management decisions in livestock operations**

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In livestock operations, systematically monitoring the animal body weight, biometric body measurements, animal behavior, feed bunk, and other difficult-to-measure phenotypes is manually unfeasible due to labor, costs, and animal stress. Applications of computer vision are growing in importance in livestock systems due to their ability to generate real-time, non-invasive, and accurate animal-level information. However, the development of a computer vision system requires sophisticated statistical and computational approaches for efficient data management and appropriate data mining, as it involves massive datasets. In this talk, we will discuss some of the challenges, applications, and potentials of computer vision systems in livestock operations and some examples to be presented include (1) monitoring animal growth and behavior; (2) automated feed bunk management; (3) body tissue mobilization in dairy cows; and (4) individual animal recognition. The development of computer vision technologies will potentially have a major impact in the livestock industry by predicting real-time and accurate phenotypes, which in the future could be used to improve farm management decisions, breeding programs, and to build optimal data-driven interventions.

## IS010

### **Best practices for targeted policies to enhance ecosystem services in European livestock agroecosystems**

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Agri-environmental policies in European livestock agroecosystems are failing to address ongoing landscape change, biodiversity decline, and societal demands for sustainability due to the lack of clear and objective targets. In this context, payments for ecosystem services (PES) arises as a promising instrument to reconcile agriculture development and nature conservation objectives, encouraging the maintenance and recovery of sustainable livestock farming systems. However, the value of nonmarket functions depends deeply on societal perception. How do farmers (as providers) and citizens (as main beneficiaries and consumers) value the ecosystem services delivered from livestock agroecosystems and the agricultural practices that provide them? Moreover, given the great heterogeneity of livestock agroecosystems in Europe and their traditional practices, are all ecosystem services equally important everywhere? Do common agricultural practices have the same effect on all agroecosystems?

We present the results of long-term research, based on sociocultural valuation methods, on-mountain livestock agroecosystems in the Mediterranean and Nordic regions of Europe. We used focus groups to analyze the perception of farmers and other citizens regarding the relationship between agricultural practices and the provision of key ecosystem services. We also analyzed the best agricultural practices to reach the targeted environmental outcomes under three plausible policy scenarios.

Our results highlight the intuitive recognition of the ecosystem services derived from mountain livestock agroecosystems by farmers and citizens. Farmers showed a large capacity to recognize the complexity of ecological processes in agroecosystems and identified more clearly the cause-and-effect relationship between agricultural practices and ecosystem services. On the one hand, some agricultural practices did not have the same effect on ecosystems services in different agroecosystems. This suggests the need for regionalizing research priorities and the design of agri-environmental policies. On the other hand, a number of practices were found to be relevant for ecosystem service delivery across policy scenarios and agroecosystems. Especially, grazing practices such as extending the grazing period, grazing in semi-natural habitats, grazing in abandoned areas, adapting stocking rate to the carrying capacity, and moving flocks seasonally stand out for their relevance in all policy scenarios.

## IS011

### Genomic tools to improve livestock

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Genomic selection is now applied widely in the industry for all the major species. The number of genotyped animals exceeds 3 million for US Holsteins, is almost a million for the US Angus, and is over 100k per line in major pig and broiler companies.

The initial application of genomic selection was based on SNP estimation with phenotypes or de-regressed proofs (DRP). Chips of 50k SNP seemed sufficient as higher-density chips only marginally improved predictions. The estimated breeding value was an index with parent average and correction to eliminate double counting. Use of SNP selection or weighting increased accuracy with small data sets but less or none with large data sets. Use of DRP with female information required *ad-hoc* modifications. As BLUP is biased by the genomic selection, the use of DRP under genomic selection required adjustments. Efforts to include potentially causative SNP derived from sequence analysis so far showed limited or no gain. The genomic selection was greatly simplified using single-step GBLUP (ssGBLUP) because the procedure automatically creates the index, can use any combination of male and female genotypes and accounts for preselection. ssGBLUP requires careful scaling for compatibility between pedigree and genomic relationships to avoid biases, especially under strong selection. Large data computations in ssGBLUP were solved by exploiting the limited dimensionality of SNP due to the limited effective population size. With such dimensionality ranging from 4k in chicken to about 15k in Holsteins, the inverse of GRM can be created directly (e.g. by the APY algorithm) in linear cost. Population-wide accuracy can be calculated for any model and data size by Method LR. Due to its simplicity and accuracy ssGBLUP is routinely used for genomic selection by major companies in chicken, pigs, and beef, and soon in dairy. ssGBLUP can be used to derive SNP effects for indirect prediction, and GWAS, including computations of the *p*-values. Recent work at UGA includes a comparison of BLUP and GBLUP trends to evaluate the efficiency of genomic selection, reduction of additive variance due to the intensive genomic selection, deciphering the contents of Manhattan plots into contributions from relationships and QTN, approximation of individual accuracies for arbitrarily large data sets, and efficiency of various genomic models for analyses of multibreed and crossbred data.

## IS012

### New challenges: global warming, welfare, resilience. How can genetics help?

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Animal production is facing new challenges. Global warming is one of the most important concerns nowadays. Livestock is responsible for around 15% of total human-induced Greenhouse gas (GHG) emissions. Most of the GHG emissions are produced by beef and dairy cattle, with significant but much lower contributions of pigs, poultry, buffalo and small ruminants. Increasing production efficiency reduces GHG emissions and other



contaminants for the unit of product; e.g. intensive poultry production is less contaminant per kg of poultry than organic low growing poultry. Genetics can help by increasing the efficiency of animal production. Animals having high growth rates are sent to the slaughterhouse before animals with low growth rates, producing less emissions, an animal with higher residual feed intake or better food conversion rate produces less emissions per kg of product, and the same can be applied to milk or egg production. However, intensive production can lead to poorer welfare, health or resilience. Welfare and health can be better in intensive production than in extensive or organic production, depending on the facilities and management, as can be seen in many examples in both ruminants and monogastrics. Moreover, welfare, health and resilience are under genetic control and can be genetically improved without compromising the performance of economically relevant traits. For example, reducing the intra-doe litter size variability leads to healthier and more resilient rabbits without deteriorating litter size. Most main health problems derived from the growing selection of broilers are now included in selection programs in order to reduce the impact of selection for growth in health and welfare. Finally, it should be considered the impact of artificial meat and milk derived from cell cultures if they are produced at a competitive cost in the future. Artificial meat and milk can solve a great part of the challenges we have considered, having the advantage of being more appropriate for human consumption, since we can include beneficial additives and remove harmful factors in the process. The pressure of vegetarians and vegans will presumably increase and the stock of farm animals may dramatically decrease. Farm animals will not disappear but may dramatically decrease in number, and perhaps they will be limited to local breeds. Here genetics can also help, not only improving the cell cultures but developing efficient conservation programs.

### IS013

#### **Environmental sustainability of livestock systems: going beyond impacts?**

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The Farm to Fork strategy, as part of the EU Green Deal, aims at promoting the transition towards a sustainable European food system. In this perspective, livestock production systems will be asked to reduce their emissions of greenhouse gases and other pollutants, while contributing to the conservation of agro-ecosystems and their biodiversity and ecosystem services. This calls for a comprehensive understanding of the environmental sustainability of livestock production, considering the impacts but also the benefits associated with the great variability of livestock farming systems and integrating their synergies and trade-offs,

which are often context-specific. Reference tools for impact evaluations are based on Life Cycle Assessment (LCA) approaches, which still need to be further standardized and improved to better implement carbon sequestration/loss in soils in relation to management practices; better differentiate between use of renewable and non-renewable resources; include and compare different functional units, which might be more relevant than the unit of product in relation with specific impact categories and their positive/negative correlations. So far, mostly unresolved problems are those of assessing impacts on biodiversity and other ecosystem services and of considering in the allocation of impacts not only the marketable co-products but also the non-marketable public benefits which can be supplied by livestock farming, especially for grassland-based and extensive systems. In addition, also in view of implementing payments for 'carbon farming', tools for evaluating the environmental footprint at the farm level need to be able to consider the cascading effects that farm management choices aimed to reduce emissions may have on other impacts or components of the agri-food system at different scales (farm, territorial, global). Applications of consequential LCA or recent territorial and socio-economic expansions of LCA are promising but so far very seldom tested on livestock farming systems. Finally, the need for identifying and measuring public benefits associated with livestock systems is relevant not only to allow their implementation in impacts assessment but also to inform effective policies for remunerating livestock systems for their supply. Animal Science scientists may play a key role in the interdisciplinary approach necessary for informing the transition towards environmentally sustainable livestock systems.

### IS014

#### **PLF applications for Italian animal productions: critical aspects and opportunities**

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The growing international interest for the application of precision livestock farming (PLF) is reported by a short bibliometric analysis of the available peer reviewed literature, followed by a summary on the PLF adoption on the basis of international surveys. The Italian situation is described by the published results of the national institute of statistics (ISTAT) on the Italian farmers' attitude for digitalization. The state of the art of the different sectors characterizing Italian animal production (dairy and beef cattle; buffalo; small ruminants; pigs; poultry; rabbits; aquaculture) is analyzed focusing on the main adopted tools, with special emphasis on their advantages for the typical Italian production systems. The ongoing diffusion of PLF in our country needs also to match some critical constraints/problems, both at research and

at-farm level. Data ownership is an important aspect for the freedom of the farmers to use raw data from each tool to generate customized reports. The tools present on the market differ in their performance (i.e., sensitivity, specificity) to detect animal's conditions; the role of a "third part" evaluation (by transparent peer reviewed research) is of paramount importance, namely for a successful transfer at on-farm level adoption. The process of digitalization in animal farming is basic for a full application of PLF principles and to gain its opportunities. Structural (at farm level) and infrastructural constraints must be considered, because they can affect technical choices (i.e., edge vs. cloud computing). The ability of farmers, as well as that of their consultants, to manage and interpret data (not always and necessary "big") will be crucial: do they will depend on external big companies? This is closely related to the farmers' skill and awareness about this "technological revolution" that can give them several opportunities. Among them, we can remember the following: new on-field phenotyping; on-farm(s) applied research; improved management also in extensive systems; improved animal feeding and nutrition; enhanced reproductive efficiency; better animal health and welfare (also supported by improved imaging techniques such as thermography); improved facing with climate change and environmental impact; certifications for animal welfare, product quality, and environmental related issues

## ISO15

### Livestock biodiversity in the genomic era

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Livestock species were domesticated in defined regions around the world. One of the main domestication centers is located in the Fertile Crescent, in South West Asia. Here some 10,000 years ago the wild ancestors of cattle, pigs, sheep and goats have progressively adapted to cope with humans. Then, domestic livestock spread across the world, along with the Neolithic expansion of agriculture and, later following human migrations, conquers and trade. They adapted to live and produce in a variety of different environments, in terms of altitude, temperature, humidity, fodder availability, disease challenges and farming systems. During population expansion, colonization and adaptation, the genomes of livestock species were molded by natural selection and selection for production traits, genetic drift due to isolation, admixture with domestic and wild populations, and occasionally by new mutations. In 1700 in the UK the concept of breeds was established, followed by the development of selective breeding that

applied methods of increasing efficiency to identify genetically superior animals based on the recording of phenotypes and pedigrees and recently also DNA variants. The process of selection has created many populations with different levels of diversity around the world. While in developed countries breeds and cross-bred are well characterized and monitored for their risk of extinction, in the developing world, populations are highly admixed and generally poorly or not characterized at all. The diversity in these populations is presently at risk, as a limited number of highly productive improved breeds are replacing local populations that are not economically competitive. While the future of local breeds remains uncertain, the scientific community has started to use genomic tools to investigate their DNA, assess their diversity, reconstruct their history and seek the genes controlling the unique traits they possess. This research may add value to their products and promote their sustainable conservation.

#### Acknowledgements

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## ISO16

### The evolution of proteins in the nutrition of dogs and cats

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Carnivorous domestic animals such as dogs and cats have 'relatively' high protein requirements, less in dogs than in cats, which are strict carnivores (McDonald et al. 1984). From a quantitative point of view, although it is recommended to use diets providing at least 20% and 30–35% of metabolizable energy of protein origin in dogs and cats respectively, the actual requirements of the adult animal are lower (McDonald et al. 1984). The current trend, marked by the recent interest of owners for cereal-free diets, is to offer foods that far exceed the above figures. From a qualitative point of view, proteins of animal origin are better provided with essential amino acids (EAAs) than vegetable proteins and they are also more digestible in their native form (without hydrolysis).

The availability of protein for pet food depends on human consumption of protein; in fact, it is the co- or by-products not used for human consumption that constitutes the stock of protein available on the world market, in fresh form or in the form of the meal (or processed animal protein) of the meats most commonly consumed for human consumption: chicken, pork or beef. Specific fishing for animal feed is also organized, but these sources are very expensive and not sustainable. It, therefore, appears that animal protein sources are not unlimited. The notion of sustainable protein implies finding new sources of protein but also

testing and validating them before using them on a large scale. Over the last decade, insect proteins have come onto the market but have not yet been studied. The arrival on the market of vegetarian foods has also brought new plant sources (peas, potatoes, ...), which are not yet validated.

The proteins of the future will have to be safe, of good quality (in terms of essential amino acids and digestibility) and also sustainable and ethical. To meet the needs of petfood in the future, the following protein sources are mentioned: algae proteins, plant proteins (in mixtures to cover amino acid requirements or supplemented with purified EAAs), hydrolyzed proteins. It seems equally ethical to ensure that there is no competition between human and animal feed. In conclusion, at the end of a year marked by deficits in animal protein sources on the market, and consequently, disruption in the supply of certain products, there is an urgent need to develop research on proteins that can be used in the long term.

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### SESSION 1 ALTERNATIVE FEEDS AND WASTE RECYCLING – I

#### O001

#### Effect of *Camelina* (*Camelina sativa* L. Crantz) dietary inclusion on quails growth performance and carcass traits

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The present study tested the effect of a dietary inclusion with *Camelina sativa* (CAM) on the productive performance and meat quality traits of broiler quails (*Coturnix coturnix japonica*). Six-hundred 15 day-old quails (50:50 male:female) were randomly allocated into 60 battery cages and assigned to 5 different dietary treatments (12 replicated cages/diet): a control diet (K) which was a commercial feed for growing-fattening quails, a diet where soybean oil of the K diet was replaced (100%) by CAM oil (O), and three diets where the 15% CAM cake of 3 different varieties (commercial Calena-C, reduced glucosinolates content-A, low linoleic acid

content-P) was included into the K diet. Along with the trial, productive performance data were collected: live weight (LW) and feed intake (FI) were used to compute body weight gain (BWG) and feed conversion ratio (FCR). At 37 days of age, male quails ( $n=300$ ) were slaughtered at a commercial abattoir, and carcasses, breasts and legs were weighed and yields calculated. Quail breasts were analysed for pH,  $L^*a^*b^*$  colour values, cooking loss and Warner Bratzler Shear Force. Individual performance data were analysed by a two-way ANOVA that considered diet, sex and the interaction as fixed effects. A one-way ANOVA tested the diet effect on cage-based performance and meat quality traits. Live performances were strongly affected by diet and sex effects. Quails of the O group showed the best LW and BWG throughout the experimental period, whereas the worst results were exhibited by C, A and P treatments ( $p < .01$ ). The lowest FCR, thus better, was highlighted by O and K quails compared to C ones, while A and P quails were intermediate for this trait ( $p < .001$ ). Female quails provided the highest LW and BWG ( $p < .001$ ). Mortality was similar in the five experimental groups. Coherently with live performances, O and K treatments had the heaviest carcass weight ( $p < .01$ ), but carcass yield was similar in all groups. K and C treatments exhibited a higher breast yield compared to A, P and O ones ( $p < .05$ ), while the legs yield of K, C, and A quails was greater than that of P and O groups ( $p < .001$ ). Even if pH ( $p < .01$ ) and  $L^*a^*b^*$  ( $p < .01$ ) colour values were affected by the diet, all groups had acceptable outcomes. The replacement of soybean oil with CAM oil provided the best live performance, whereas the 15% dietary inclusion of CAM cake slightly impaired quail performance and highlighted variability in results depending on CAM varieties.

#### Acknowledgements

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#### O002

#### Potential use of a queen bee larvae meal (*Apis mellifera ligustica* Spin.) in animal nutrition: a nutritional and chemical-toxicological evaluation

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The aim of the present research was to test the potentiality of the larvae of honeybee queens, discarded from royal jelly production, to be used as a possible dietary supplement in animal nutrition. To this purpose, the chemical characteristics, chitin content,



amino acid, fatty acid and mineral profiles, including toxic elements, were determined on pooled samples of queen bee larvae. Queen bee larvae meal is rich in chitin, protein, essential amino acids, and some essential minerals such as phosphorous and magnesium; it is also relatively poor in fat, and with negligible levels of toxic elements such as Cd, Pb, As and Hg. However, its fatty acid profile showed a very low amount of polyunsaturated fatty acids, and the meal was poor in Ca and other trace elements when compared to the most common insect meals used in animal nutrition. Comparing the amino acid profile of QBL to the ideal amino acid profile of protein for laying hens, broilers and three commonly reared fish species, the QBL proteins appear lacking for some IAAAs for hens and broilers, while it can be noticed that it can ensure a well-balanced amino acid supply for carnivorous fish species such as gilthead sea bream and rainbow trout and common carp. Queen bee larvae have standard nutrition based on royal jelly and this could represent a great advantage to use in animal production. However, the collection of queen bee larvae does not allow to give high quantities of the final product due to both the low amount of larvae collected (an average 58.9 g/hive/month) and the relatively low yield in a meal (an average of 23.12%) recorded. Thus, the queen bee larvae meal cannot be considered as an alternative protein source in animal production but could represent a potential feed supplement to include at low doses to exploit the possible activities of gut microbiota modulator due to the high levels of chitin. This could be of potential interest for an animal but also human nutrition. For example, its use in small-scale farms, also in light of the fact that this product currently represents a production waste, could have a great potential in terms of improving a circular economy optimising the use of natural resources.

### O003

#### Effect of silkworm (*Bombyx mori*) dietary inclusion on chickens' growth performance and carcass traits

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The present experiment tested the effect of a partial replacement of soybean meal with a defatted silkworm (*Bombyx mori*) chrysalis meal (SWM-DEF) in the diets for growing chickens, on their live

performance and slaughter traits. To this aim, a total of ninety ROSS 308 broiler chickens were randomly divided into three dietary groups consisting of 5 replicated pens/diet. The first group received a control diet (commercial poultry feed; C) throughout the growing period of 42 days, the second group received a diet including 4% SWM-DEF during the starter phase (1–10 days) (SWM1) and the C diet up to slaughter, and the third group was fed with the C diet in the starter phase and the 4% SWM-DEF diet up to slaughter (SWM2). Diets were isonitrogenous and isoenergetic and birds had free access to feed and water throughout the study. During the experiment, live weight (LW) and feed intake (FI) were recorded to calculate body weight gain (BWG) and feed conversion ratio (FCR). At 42 days of age, 15 chickens/treatment were slaughtered at a commercial abattoir, carcasses were weighed, and yield was calculated. Afterwards, breasts and legs were excised, weighed and yields computed. Data were analysed by using the one-way ANOVA with diet as a fixed effect. Results highlighted that the dietary treatment affected neither the LW nor the BWG of chickens. Differently, FI was lower in SWM2 compared to the other groups ( $p < .05$ ), but only in the starter phase. In the grower and finisher phases and considering the whole cycle, FI was not affected by the diet. Despite BWG and FI were similar in treatment groups, SWM1 and SWM2 chickens displayed the lowest, thus better, FCR ( $p < .05$ ) considering the 42 days growing period. At slaughter, chicken of the three dietary groups showed comparable carcass, breast, and leg weights and yields. Overall, the present experiment indicated that SWM-DEF can be considered a promising alternative feed ingredient to replace soybean meal in chicken rations. The 4% inclusion level seems a good choice to ensure optimum chicken growth, even improving efficiency and satisfactory carcass traits. Also, different administration periods did not provide appreciable different outcomes, thus SWM-DEF can be provided in different growth phases, according to the cost-benefit ratio. Further research should focus on the nutritional characteristics of meat, a key factor for consumer's health.

### O004

#### Marine microalgal biomass from fish farming effluents: potential application in aquafeeds

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Recent research efforts for aquaculture are focused on sustainability issues such as new feedstuffs and integrated multitrophic

production systems. Aquaculture effluents contain nitrogen and phosphorus compounds that represent the natural substrate for single-cell microorganisms including microalgae. Cultivating microalgae on finfish farm effluents in a recirculating aquaculture system could realize a wastewater treatment and provide valuable biomass available for other purposes. This study was aimed to evaluate the potential of non-axenic microorganism biomass included in aquafeed formulations.

A non-axenic microalgae consortium (80% *Oocystis* sp.) was cultivated in a conventional high-rate algal pond system (HRAP 140m<sup>2</sup> and 60m<sup>3</sup>) supplied by the effluent from European seabass (*Dicentrarchus labrax*) rearing tanks providing 80g N day<sup>-1</sup> and 30g P day<sup>-1</sup> (fish biomass: 2000 fish with average body weight 80 ± 2.3g) at the facilities of Ifremer (Palavas les Flots, F). The microalgae consortium (MC) biomass was recovered and freeze-dried until used. Four isoproteic (CP 48.5 %) and isolipidic (CL 18.3 %) diets were formulated: a fishmeal, fish oil and plant protein sources basal diet was used as a control (C), two test diets including increasing levels (10% and 20%) of MC (MC10 and MC20, respectively) and a diet (NAN10) including 10% of commercial mono-cultured *Nannochloropsis* sp. biomass (GREENSEA, Meze, F) was used for comparison. The diets were offered to 636 E. seabass juveniles (18 ± 0.28 g) randomly allotted among 12 tanks/groups for 10 weeks. The effect of the microalgae dietary inclusion was evaluated on seabass zootechnical performance, morphometric indexes, in vivo nutrient digestibility and gut morphology and functionality.

The dietary MC inclusion did not hamper feed palatability, fish growth and preserved intestinal morphology ( $p > .05$ ). Diets including MC resulted in dry matter, protein and energy digestibility coefficients lower than diets C and NAN10 ( $p < .05$ ). MC also affected the maltase, sucrase-isomaltase and  $\alpha$ -GT specific enzymatic activities in the seabass distal intestine ( $p < .05$ ).

This is the first attempt utilizing a marine microalgae consortium from fish farm wastewaters in a species of commercial interest to enhance a circular economy approach in recirculating aquaculture systems. Further efforts and the safety issues connected with its utilization need specific evaluations.

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## O005

### Isoenergetic-practical and semi-purified diets for protein requirement determination in *Hermetia illucens* larvae: consequences on life-history traits

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The black soldier fly (BSF) is one of the most promising insects for intensive breeding, given its adaptability, its waste conversion efficiency, and the simple management of its life cycle. The aim of BSF breeding is not only focused on the use of waste materials as growth substrate, but also on production maximization. In order to create a sustainable insect farm, the gap between these two aspects needs to be reduced. To formulate diets that maximize the production using waste, it is fundamental to determine previously the larvae requirements in terms of macronutrients. This study aims to evaluate the effects of different, practical, semi-purified (PSP) and isoenergetic diets with increasing protein levels on larval development and mortality, and adult parameters. A total of 2000 6-day-old larvae (100 larvae/box and 4 box replicates/diet) were randomly divided into 4 PSP diets with increasing protein levels (10%, CP10; 14%, CP14; 16%, CP16; 19%, CP19), and the control diet (Gainesville; GA). To evaluate the growth of the larvae, a total of 3 samplings at 4-day intervals were carried out. At the end of the experiment, the survival rate was calculated. To assess the adult emergence, 35 pupae per replicates were positioned in boxes (1 pupae/box). Once the fly emerged, the fly's live weight (FLW) was recorded. At the death, the fly life span (FLS), the exuvia weight (EW), and the dead flyweight (DFW) were evaluated. Data were analysed by means of One-way ANOVA and the General Linear Mixed Model (IBM SPSS Statistics V20.0.0,  $p$ -value  $< .05$ ). Considering the larval stage, the CP16 treatment showed the greatest weight, while CP10 and GA groups displayed the worst growth performance ( $p < .01$ ). All the adult stage parameters were influenced by the dietary treatment, except for the FLS. As regards the FLW, experimental diets had a similar weight ( $p > .05$ ). On the contrary, the lowest DFW was recorded in the CP19 treatment ( $p < .01$ ). The GA diet showed the lowest weights in terms of both the DFW and the EW ( $p < .001$ ). Finally, the PSP diets displayed a very low emergence rate when compared to GA. In conclusion, the optimal protein level in the larvae stage can be considered 16%, while the result obtained on adult emergence in PSP diets, the protein content may be excluded as a cause. For this reason, further researches on the determination of macronutrients requirements have to be conducted in order to evaluate the diet composition effects on the BSF life-history traits.

## O006

### Effects of the inclusion of *Hermetia illucens* meal in diets for rainbow trout (*Oncorhynchus mykiss*) reared in a low-tech aquaponic system

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The study evaluated the effects of the partial substitution of fish meal (FM) with partially defatted *Hermetia illucens* (HI) meal on growth, gut morphology and fillet quality of rainbow trout reared in a low-tech aquaponic system. A total of 173 rainbow trout (initial body weight:  $156 \text{ g} \pm 39.8 \text{ g}$ ) were distributed into nine experimental aquaponic units (3 tanks per treatment, initial tank biomass  $5.74 \pm 0.44 \text{ kg m}^{-3}$ ) and fed during 76 days with three diets containing 0%, 6.3% and 12.5% HI meal and 20%, 15% and 10% FM, respectively. Therefore, HI meal replaced 0% (HI0, control diet), 25% (HI25), or 50% (HI50) of FM, respectively. During the trial, water quality was not affected by dietary treatment: temperature averaged  $19.4^\circ\text{C}$  ( $13.8 - 23.7^\circ\text{C}$ ), dissolved oxygen  $8.0 \text{ mg L}^{-1}$  ( $6.08 - 10.2 \text{ mg L}^{-1}$ ), pH 7.4 ( $6.4 - 8.5$ ), and total ammonia nitrogen  $0.13 \text{ mg L}^{-1}$  ( $0 - 0.36 \text{ mg L}^{-1}$ ), while daily water losses due to plant evapotranspiration averaged  $1.31 \text{ d}^{-1}$ . At the end of the trial, trout mortality was low (2.9%) and not affected by dietary treatment. The specific growth rate was lower in fish fed HI50 diet compared to those fed HI0 and HI25 diets after 26 days ( $1.07\% \text{ d}^{-1}$  vs.  $1.22\% \text{ d}^{-1}$ ;  $p < .001$ ) and at the end of the trial ( $0.81\% \text{ d}^{-1}$  vs.  $0.88\% \text{ d}^{-1}$ ;  $p < .05$ ). However, the dietary inclusion of HI did not affect feed conversion ratio (on average 1.53), final weight (303 g), fish condition factor (1.40), viscerosomatic index (10.9%) and hepatosomatic index (1.22%). Histological analyses of the anterior gut showed no significant differences in villi height ( $503 \mu\text{m}$ ) whereas the density of goblet cells was higher in HI50 than in HI0 trout (+11%;  $p < .05$ ). Regarding fish quality, fillet redness ( $a^*$ ) and yellowness ( $b^*$ ) were lower in HI50 than in HI0 treatments (−58% and −19%, respectively;  $p < .001$ ). Fillet proximate composition, total saturated, monounsaturated and polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid were not affected by diets, whereas the content of C12:0 and C14:0 increased with HI dietary inclusion and the index of atherogenicity was higher (+6%;  $p < .05$ ) in HI50 treatment than in HI0 and HI25 ones. In conclusion, the rearing of rainbow trout was successful in the tested aquaponic system. Fish growth, health and fillet quality were not affected when HI meal replaced 25% FM, whereas at 50% replacement rate some effects on gut histology and fillet colour and nutritional characteristics were detected, which deserve further investigations to be elucidated.

## SESSION 2 MANAGEMENT STRATEGIES TO IMPROVE ANIMAL HEALTH, WELFARE AND RESILIENCE – I

0007

### Dry period management practices in 130 dairy farms

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The dry period is a crucial time in the lactation cycle and the management practices adopted have an important role in mastitis prevention and control in the following milking period. A survey about practices applied to dry period was sent to Italian dairy farmers through different social channels (email, Facebook, web sites) and specialized magazine, a 138 questionnaire was filled and answers were analysed. The most of farms were intensive (61.8%), with more than 100 lactating cows (55.07%) and located in Lombardy (59%). Dry cows were housed on straw pack (53.4% of farms) or cubicles (32.8%). Most farms used cooling systems in the dry cow barns: 45.9% fans, 24.8% fans with a nebulizer. Half of the farmers declared duration of the dry period between 55 to 65 days, while 41.3% of farms adopted a shorter period (45–55 days). Other interesting results were obtained: 51.3% of farmers adopted gradual cessation of milking while the others declared to applied abrupt cessation; in the 39.7% of farms daily milk production at dry off was among 15 to 20 kg but in 29.01% of farms the production was higher (20–25 kg/d). Some authors found that the choice of gradual or abrupt cessation did not affect milk yield or somatic cell count (SCC) in the subsequent lactation, while the high production at dry off was associated with high SCC. The antibiotic dry cow therapy (DCT) was frequent (53.6% of farms), 37.7% of farmers declared to apply a selective DCT, 8.7% of farmers did not use any treatment. A total of 49 farmers declared average milk SCC:  $5.26 \log_{10} \text{ n cell/ml}$  for farms that adopted DCT,  $5.21 \text{ n cell/ml}$  for farms that adopted selective DCT and  $5.29 \text{ n cell/ml}$  for farms that used none treatment. Multiple correspondence analyses showed that the use of DCT was most common in farms with more than 100 lactating cows, high milk yield ( $>31 \text{ kg/d}$ ) and a gradual cessation of milking; on the contrary selective DCT was adopted in farms that housing dry cow on the straw pack with fans in the barn and applied abrupt cessation of milking. The results suggest that the application of a selective DCT could be encouraged among Italian farmers, particularly among high dimension farms. A reduction of the use of antibiotic therapy follows the EU commission (2015) indications that recommended avoiding routine DCT in order to reduce the antimicrobial resistance.

#### Acknowledgements

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O008

## Mutual relationship between technological, structural and productive features in dairy sheep farms: a multivariate approach

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The influence of different indicators on a dairy sheep farms (DSF) under extensive and semi-extensive systems can be a challenge compared to more intensive and controlled systems. The aim of the present study was to evaluate how different sets of technologies could influence the semi-extensive Italian DSFs. A questionnaire was built and performed on the farmers. Contextually, a sample from the bulk tank milk (BM) was collected from each DSF to evaluate the chemical-physical and microbiological parameters of the BM.

One-hundred and seventy-seven DSFs were included for a total of 56131 sheep. Forty technologies (T) were identified, grouped into a set of six indicators (T1-management; T2-feeding; T3-indoor welfare; T4-animal-based welfare; T5-outdoor welfare; T6-milking equipment and hygiene). Structural indicators (S) were composed of the following variables: S1-total farmland (ha); S2-number of ewes; S3-% of rented land; S4-number of ewes/total farmland. Productive indicators (P) were: P1-number of lambs born per year; P2-number of lambs sold per year; P3-milk microbiologic quality; P4-yield of fat and protein corrected milk. The value of each indicator (T, S and P) was based on the proportion of innovations implemented in each farm overall innovations identified, with values from 0 to 100%. Principal component analysis (PCA) was applied for analyzing the relationship amongst a different set of indicators. Seven principal components (PC) were extracted that explained 68% of the total variability. PCA showed the following results: PC1 was positively associated with T1 and T6 and negatively with S2; PC2 separated S2, S4, P2 from S1, S3; PC3 separated T2, T5, T6 and P3 from T3 and T4; PC4 separated P1 and P2 from T2 and T4; PC5 was positively associated with T3, T5, S3 and negatively with S1; PC6 separated T3 and S1 from P4; finally, PC7 separated T2 and P4 from T4 and P3. The main interactions were between sheep welfare, milk quality and quantity, milking hygiene, feeding and farmland management. Our results showed that small farms with small flocks, with a family-run structure, typical of Italian DSFs, were characterized by good management of the animals and higher productivity. By considering the association pattern of all the variables, the relative weight of each farm feature can be evaluated, suggesting the best strategies to improve herd productivity.

O009

## Association between liver ultrasound measurements and hematochemical indicators for early prediction of metabolic alterations in Holstein cows

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Hepatic steatosis is the most recurrent metabolic disorder in high-producing dairy cows during early lactation. The gold standard test to diagnose hepatic steatosis is the histological evaluation of liver triacylglycerol (TAG) content which is an impractical and invasive procedure. Given that even mild hepatic steatosis conditions lead to huge economic losses at the herd level, the possibility of early detects subclinical signs of liver metabolic disease becomes strategic. Ultrasound (US) imaging is a promising technique for identifying liver dysfunction, but standardized specifications of liver US-predicted parameters (i.e. texture analysis) and US measurements are needed even in physiological conditions in order to generate baseline data for subsequent clinical evaluations. In this study, we described the features of four US measurements, namely the liver predicted TAG (pTAG) content, liver depth (LD), and portal vein area (PVA) and depth (PVD), and explored the association between these traits and a set of hematochemical (HC) indicators of energy metabolism, liver oxidative stress/damage, innate immune response, and minerals in 342 clinically healthy Holstein Friesian dairy cows reared in two herds. The response variables (i.e. the US measurements) were analyzed with a linear model which considered the following effects: days in milk, parity, the HC traits (included one at a time, in classes discretized according to physiological thresholds), the interaction between HC traits and parity and the effect of herd/date. Liver pTAG was negatively associated with hematocrit ( $p < 0.01$ ) and positively with globulin ( $p < .01$ ), whereas PVA was negatively associated with thiol group levels ( $p < .05$ ), and LD positively with ceruloplasmin concentration ( $p < .01$ ). We found significant interactions between some HC parameters and parity: creatinine, thiol groups and globulin for PVA, and aspartate aminotransferase, paraoxonase and ceruloplasmin for PVD ( $p < .05$ ). The study described the fluctuations of inflammatory and oxidative stress indicators reflecting the physiological and metabolic adaptations occurring during early lactation in high-yielding dairy cows. Furthermore, we revealed the potential of a combination of minimally-invasive tools (i.e. US and HC parameters) as a useful strategy to promptly detect metabolic disorders during a routine screening in dairy herds.

## O010

### Blood biomarkers before dry-off are related to better adaptation to transition period in dairy cows

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The dry-off represents a critical phase of the lactation cycle in dairy cows, deeply influencing performance and health in the next lactation. Many changes happen in this phase, such as daily routine, social interaction, and diet. The transition from lactation to dry period requires metabolic adaptations and triggers a local and, often, systemic inflammatory response. The degree of this response is variable, and its consequences might affect the cow's health during the dry period and metabolic adaptation in the transition period. The albumin-to-globulin ratio (AG) summarizes plasma protein profile and some liver functions; it is a simple and easy index to determine, used also in human medicine. This study aimed to evaluate the relationship between AG before dry-off with the metabolic and inflammatory profile of dairy cows. Blood samples were collected from 75 Holstein dairy cows, from 7 days before dry-off to 28 days after calving to assess metabolic and inflammatory profiles. Body condition score and health status were regularly monitored. Cows were retrospectively grouped into tertiles according to AG calculated 7 days before dry-off, and data were analyzed with SAS software (PROC MIXED). AG ranged between 0.52 and 0.82 in a lower group (LO), 0.83 and 0.94 in the intermediate, (IN) and 0.95 and 1.26 in the upper (UP), reaching the highest value before calving, as a result of the shift between albumin and globulin trend. Higher values of AG are usually considered an indicator of overall better condition, as confirmed by concentrations of inflammatory markers. Haptoglobin and ceruloplasmin resulted higher in LO compared to IN and UP groups, and, on the opposite, albumin, cholesterol, retinol-binding protein, paraoxonase, and zinc resulted lower in LO compared to IN and UP. Differences in blood markers among groups observed before dry-off were kept during the dry and early lactation period. Overall, cows with high AG before dry-off had better adaptation to the next lactation, as confirmed by the reduced systemic inflammatory response than cows with low AG. In conclusion, AG before dry-off might represent a rapid and simple proxy to evaluate the inflammatory condition from dry-off to early lactation, and likely the adaptation to the transition between lactation and dry period and between dry and early lactation.

#### Acknowledgements

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## O011

### Comparison between cow milk and a high-energy whey-based milk replacer for the weaning of Holstein dairy heifers

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So far, many feeding strategies have been studied for the nursing and weaning of dairy heifers. However, there is still a lack of agreement in the literature on which type of milk (cow milk or milk replacer) should be the best choice for nursing dairy calves. This study aimed at assessing the use of a high-energy whey-based milk replacer as an alternative to whole cow milk for the weaning of Holstein heifer calves. Two groups of 10 Holstein female calves each were monitored in a dairy farm of North-eastern Italy from November 2018 to May 2019. Calves of both groups were fed their mothers' milk for the first week of age and then were randomly assigned to be fed saleable cow milk or a high-energy whey-based milk replacer (26% protein and 22% fat) according to the same feeding protocol until complete weaning at the ninth week of age. Calves of both groups were housed in single pens where solid feed (starter concentrate) and water were provided ad libitum from the first day of life. The weaning program considered the provision of 6 L of milk in two daily meals, which were reduced to a single meal from the sixth week of age on. Individual bodyweight, height at the hips, and solid feed intake were recorded weekly until the fifth-week post-weaning. Additionally, disease outbreaks during the experimental period were recorded and, in a follow-up phase of the study, age at first pregnancy was collected. Over time, the growth curves of the two groups of calves were similar, as well as the solid feed intake. The only difference was recorded on the first week after weaning when calves fed milk replacer had a higher intake of solid feed compared with calves fed cow milk. No differences were observed in the average body weight gain nor in the average height at the hips gain, whereas calves fed milk replacer had a higher average solid feed intake gain during the nursing period. Finally, no differences were found in the incidence of diseases (diarrhea) during the experimental period, and both groups of heifers got pregnant around 15 months of age. The outcomes of this study suggested that, from a nutritional point of view, a high-energy whey-based milk replacer can be a valuable alternative to cow milk for the nursing and weaning of replacement calves. Therefore, the choice of using the type of milk replacer analyzed in this study for heifer calves nutrition can be made based on the difference between its cost and the farm milk price.

## SESSION 3– ROLE AND CHARACTERIZATION OF LOCAL BREEDS

O012

### Sustainable use of local sheep breeds in Italian Eastern Alps

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This study is part of the SHEEP UP project (Rural Development Project, Veneto Region), which aims to generate added value through the sustainable use of local sheep breeds in the Veneto Region (Eastern Alps). The four local breeds are: Alpagota, Brogna, Foza and Lamon. Lamon and Foza breeds are at high risk of extinction, presenting respectively less than 400 and 150 heads. Instead, Alpagota counts 3000 heads and Brogna counts 2800 heads. Building on previous studies, a smartphone application has been developed with the aim to improve cooperation between farmers and to support mating plans to reduce the risk of inbreeding.

The sample consists of 45 farms that were surveyed: 14 for Alpagota, 13 for Brogna, 4 for Foza and 14 for Lamon. For each farm, we analysed the following data: herds' size; land use; the presence of other animal species; farm's activities and relative income; opinions on future perspectives. In addition, the analysis involved the use of focus groups on peculiarities and threats of managing flocks on mountain areas.

Results showed that the average number of heads is 95 for Alpagota, 109 for Brogna, 73 for Lamon and 13.5 for Foza breed. The farms rearing a flock of less than 20 heads is 35.7% of farms for Alpagota and 42.9% for Lamon, but 7.7% of Brogna, presenting instead 23.1% of flocks with more than 100 heads, as it is also for 28.6% of Alpagota farms, but for Lamon only 7.1%. Most farms own pastures and meadows, indicating particular ease for organic productions. The average farms surface is 24.4 ha, which is mostly represented by pastures and meadows (more than 65%). The most numerous flocks are monospecific and belong to Alpagota and Brogna certified breed, also presenting a more organized supply chain than in Lamon and Foza breeds' realities. Nevertheless, almost all participants breed multiple species of animal, thus indicating the need to diversify income flows and improve the supply chain. For the purpose, the promotion of meat and milk products, yolkly wool and the evaluation of multifunctionality is already taking place.

The final objective of the project is the development of product quality schemes based on the direct experience of marginalised livestock keepers. The involvement of farmers (in particular

smallholders) in multifactor approaches is fundamental for the conservation of Alpine sheep breeds, and efficacious marketing strategies linked to product quality labels can support the sustainable development of these farming systems.

O013

### Change of accuracy in EBVs due to genomic information in the small Rendena cattle populations

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Genomic selection has become an appealing tool in cattle breeding. In this study, we performed for the first time a genomic evaluation in Rendena cattle, a small dual-purpose Italian breed. Phenotypic information from performance-tested young bulls was used as a benchmark to assess the improvement of selection accuracy due to genomic information. Test records considered were average daily gain (ADG) measured up to 11 months of age, in vivo carcass conformation (EUROP), and in vivo carcass yield (CY), both estimated by 3 skilled classifiers at the end of the test. Conventional best linear unbiased prediction (BLUP) and single-step genomic BLUP (ssGBLUP) were then compared to verify improvement in breeding-value accuracies. Furthermore, the improvement inaccuracies due to genomic data by using an alternative single-step method was analyzed comparing weighted ss genomic BLUP (WssGBLUP) and Bayesian ss genomic BLUP (BssGBLUP) using different prior distributions (Bayes Lasso and BayesA). Phenotype and pedigree information were provided by the National Breeder Association of Rendena Cattle in a final dataset containing 1600 animals with records. Two genotyping platforms were used to genotype animals, i.e. Illumina BovineSNP33K and Illumina BovineSNP150K. The highest density panel was used for 550 males, while 1023 females and further 170 males were genotyped with the lower density SNPs panel. Genomic imputation was made with the AlphaImpute2 program and a final group of 720 genotyped males and 654 females with a density of 113,280 SNPs were accounted. Accuracy in each scenario was estimated for genotyped animals by cross-validation. As expected, the addition of genomic information increases the accuracy of genomic prediction. The initial accuracy of BLUP (0.205, 0.330, and 0.361 for ADG, EUROP, and CY, respectively) was increased for the same traits to 0.250, 0.400, and 0.399, respectively by ssGBLUP. Moreover, the improvement in accuracy using models that assumed unequal variance among markers, such as WssGBLUP and BssGBLUP, appeared to be beneficial for all performance phenotypes. A noticeable gain was observed in CY and EUROP, which indicates the presence of relevant quantitative trait loci for these traits. This suggests that both WssGBLUP



and BssGBLUP are appropriate methods for GEBV prediction in traits controlled by a large number of quantitative loci.

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## O014

### Histochemical muscle fibre features and gene expression in Maremmana and Aubrac steers

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It is widely accepted that skeletal muscle tissue is composed of at least three types of muscle fibres. Their frequency and size are important factors affecting both muscle growth potential and meat quality. This study aimed to characterise the muscle fibres features from two bovine breeds, reared on grazing or feedlot system: Maremmana (MA), an autochthonous breed from Tuscany (Italy), in comparison to Aubrac (AU), from the Massif Central (France). A total of 40 steers were considered for this purpose and allotted in 10 animals' groups depending on the breed and rearing system adopted. From each steer, Triceps brachii (TB) and Semimembranosus (SM) muscles were collected immediately after the killing procedure. All the steers were slaughtered between 20 and 22 months of age. The histochemical analysis allowed to distinguish type I, IIA, and IIB fibres, moreover the cross-sectional area (CSA) and the percentage of CSA occupied by each fibre type were determined. The expressions of *atp1a1*, *mt-atp6*, and *capn1* genes on total RNA extracted from SM muscle were evaluated, in order to find potential associations with the above-mentioned parameters. In SM and TB muscles, the MA steers exhibited a greater frequency of oxidative fibres (type I and IIA), while AU steers had a higher frequency of glycolytic fibres (type IIB). In both muscles, all muscle fibre types from MA and AU steers reared on pasture had the larger CSA values in comparison with feedlot steers, but without a significant difference. As regard of gene expression, the higher *atp1a1* expression in MA might be positively associated with the greater total CSA occupied by oxidative fibres expressed as a percentage (~45% MA vs. ~30% AU). Conversely, AU had a higher *capn1* expression that whether combined with the impact of glycolytic fibre on the tenderisation process, doesn't give more insights on meat quality aspects. The grazing activity was probably insufficient to

determine significant differences both for histochemical muscle characteristics and for *atp1a1* and *capn1* gene expressions. Unexpectedly, only Feedlot MA steers presented the highest *mt-atp6* expression compared to other steers. However, these findings further the knowledge of skeletal muscle properties belonging to cattle breed less genetically improved and less studied, and the effect of voluntary physical activity on pasture, since few studies are available in this regard.

## O015

### Deciphering climate-mediated adaptation in European sheep

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After livestock domestication and dispersal, environment-mediated selective pressure has shaped phenotypic variation and left specific signatures in the genome of locally adapted breeds. The identification of genes containing adaptive variation is of strategic importance for the livestock sector, to guide selection in a period of rapid climate change. Among domesticated species, sheep (*Ovis aries*) have established a wide geographic range due to their rusticity, manageable size, adaptability to nutrient-poor diets, and tolerance to highly diverse environments and climatic conditions. Within the European project 'Innovative Management of Animal Genetic Resources' (acronym IMAGE), we used high-density SNP genotyping data from 80 autochthonous sheep breeds, spanning from North Africa to Scandinavia to sample a wide range of climatic scenarios. We investigated the structuring of genetic diversity, and combined genotype-environment association and selection signature analyses to pinpoint genomic regions candidate for adaptation to different climates. Common signals between the two methods pointed at genes involved in adaptation to extreme environments (ARHGAP26, CMYA5) and energy management (STARD13, SOCS2). Further, we identified genes that play a role in modulating thermoregulation through fat deposition, skin thickness,

and hair follicle differentiation as the homeobox genes HOXC11, HOXC12, and HOXC13. Our findings shed light on the genomic mechanisms of climate adaptation and provide molecular information useful to allow selection for improved resilience and welfare under climate change.

## O016

### Prion protein (PRNP) gene polymorphisms in Valle del Belice sheep breed

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Scrapie is a transmissible spongiform encephalopathy, and its susceptibility is associated with polymorphisms in the prion protein gene (PRNP). Classical scrapie susceptibility in sheep has been linked to three polymorphisms at codons 136, 154, and 171 in the PRNP gene, whereas atypical scrapie susceptibility is related to polymorphisms at codon 141. These polymorphisms play a crucial role in terms of incubation period and/or susceptibility to scrapie. Genotype ARR/ARR is resistant to scrapie infection while genotypes carrying ARQ or VRQ alleles are associated with a high susceptibility to scrapie. Nowadays, ARR/ARR genotype is not present with a high frequency in all sheep breeds as reported in many studies. In this work, sequence analysis of the PRNP gene was performed with the main goal to obtain information about the genetic variability of the Valle del Belice breed at this locus, and the frequency trend on resistant and non-resistant genotypes. A total of 1197 individuals of the Valle del Belice breed belonging to five different farms were investigated for PRNP gene polymorphisms. Analyses of the obtained sequences showed the presence of the four main alleles ARR, ARQ, AHQ and VRQ with frequencies of 0.523, 0.450, 0.020, and 0.007, respectively. Within our breed, the most frequent genotype was ARR/ARQ with the frequency of almost 42% followed by ARR/ARR with 28.5%, ARQ/ARQ with 22%. X<sup>2</sup> test for Hardy–Weiberg equilibrium was performed ( $p < .05$ ). All the other genotypes ARR/AHQ, ARQ/VRQ, ARQ/AHQ, and ARR/VRQ were present with an overall frequency of 5.2%. At codon 141, only 0.5% of individuals presented the F amino acid with respect to the L. Moreover, five additional amino acid changes M112T, G127V, M137T, H143R and R231W were found, as well as, one synonymous polymorphism at codon 237 which does not cause amino acid change. These changes were present as M112T with the frequency of 8.7%, G127V with 0.7%, M137T with 2.3%, H143R with 1.3%, and R231W with 7.9%. It is interesting that individuals heterozygous at codon 231 were heterozygous to codon 237. Genetic selection is currently the most effective way for the eradication of the susceptible VRQ allele in favor of the resistant ARR allele. Application of molecular protocol

for PRNP gene could assist the farmers to improve ARR/ARR genotype frequency in Sicilian farms and help them to take precautionary measures against scrapie.

## O017

### Physico-chemical characteristics of milk from autochthonous cattle breeds of the Emilia Romagna region

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The safeguard of autochthonous dairy cattle breeds goes through the characterization and valorization of their milk. In this context, the objective of this research was to study the physicochemical characteristics of bulk milk of autochthonous cattle breeds of the Emilia Romagna (ER) region: Reggiana (Re), Modenese (Mo), Garfagnina (Ga), Ottonese (Ot) and Pontremolese (Po). One herd was selected for each breed and milk sampling was carried out throughout one year. Bulk milk samples collected for each breed were 15, 12, 9, 7 and 5 for Re, Mo, Ga, Ot, and Po, respectively. In the same period, 3 bulk milk samples from an Italian Friesian (IF) cattle herd located in ER were collected as well. The contents of crude protein and casein were assessed with the Kjeldahl method and those of fat and lactose by MilkoScan. The content of P was measured by the colorimetric method and those of Ca and Mg with atomic absorption spectrophotometer. The somatic cells count (SCC) was determined by the Opto-fluorometric method. The significance of the differences between the breeds was tested by one-way analysis of variance, considering as fixed factor the breed (7 levels, one for each breed). The highest contents of crude protein and casein were found in Ot milk (3.58 and 2.79 g/100g respectively), while the lowest in IF milk (3.30 and 2.55 g/100g respectively). The highest content of lactose was recorded in Po milk (5.01 g/100g) and the lowest in Ga milk (4.71 g/100g). Po milk was also characterised by the lowest SCC value (37,103 cells/ml) that confirm the negative correlation between the values of lactose and somatic cell count in milk. On the contrary, the highest values of SCC were observed in the milk of Ot and Mo (343 and 337 103 cells/ml, respectively). The highest content Ca, P were observed in Re milk (123.14 and 96.56 mg/100g, respectively), while the lowest in Ga (117.99 and 93.03 mg/100g) and IF milk (116.18 and 92.80 mg/100g). Po milk showed the highest Mg content (10.60 mg/100g) followed by Ot milk (10.03 mg/100g). No differences were observed between the milk of other breeds, which values ranged from 9.91 (Mo) and 9.67 mg/100g (IF). Compared to the milk of the cosmopolitan and

highly selected IF, those from autochthonous cattle breeds seem to have an elevated cheese yielding ability, a characteristic that should be exploited for the purpose of their valorisation.

#### SESSION 4 – NUTRITIONAL PROFILE OF FOOD – I

O018

### Lipid metabolism in different Italian poultry breed and crossbreed

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Italian chicken breeds are an important source of poultry biodiversity. Due to their low productive performance, pure breeds are not competitive from a productive viewpoint. However, they have unique and ancestor metabolic pathways, that positively affect the quality of the products (meat and egg). The objective of the present research was to investigate the differences in the chicken lipid metabolism of a commercial meat-type breed, two Italian pure breeds and their crossbreeds. For this purpose, 225 chickens (15 animals/strain/replicate) belonging to Ross 308 (R), Robusta Maculata (RM), Bionda Piemontese (BP), RM × Sasso (RM × S) and BP × Sasso (BP × S) were used. Chickens were raised in different indoor pens and subjected to standard conditions of temperature (20–32 °C) and humidity (65–72%); all the chickens were fed *ad libitum* with the same standard diet. Fifteen chickens/group were slaughtered at 45 d (all groups), 81 d (only R) and 140 d (only pure breeds and crossbreeds), and the liver was sampled for fatty acids (FAs) determination (by GC-FID). The data were analysed with a linear model to evaluate the effect of breed and age (within breed) by comparing Ross (45 d vs. 81d) and pure breed and crossbreed (45 d vs. 140 d) to compare chickens with the different maturity levels. Results demonstrated that pure breeds and crossbreeds have a different lipid profile than R one. In particular, the R strain showed a higher level of ALA at 45 d (0.83 % FA;  $p < .04$ ) with respect to the pure breeds and crossbreeds, where BP exhibited the lowest value (0.37 %). The same trend was obtained for the n-3 and n-6 long-chain polyunsaturated fatty acids (LCP) content with the highest value in R strain (7.82 and 13.20%, respectively) whereas, BP was characterized by the lowest concentration of n-3 LCP (6.20%) and RM by the lowest level of n-6 LCP (12.20%  $p < .036$ ). In all the chickens studied, the n-3 LCP amount of liver was higher at 45 d with respect to older ages (81 and 140 d, respectively in R and the pure breeds and crossbreeds). However, it is important to underline that the different strains studied were not significantly different for the efficiency in converting ALA into n-3 LCP and LA to n-6 LCP. Our data suggested that the pure breeds and crossbreeds were probably characterized by a rapid mobilization of these FAs from the liver to other body tissues.

O019

### Detailed mineral profile of milk from cows, buffaloes, goats, sheep, dromedary camels, and donkeys and minerals recovery in cheese

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Milk is an important source of essential minerals in the human diet. Bovine milk is the most commonly consumed milk worldwide, but in some areas of the world milk from other species represents an important contribution to the overall consumption. This study aimed to compare the detailed mineral profile of milk from cows, buffaloes, goats, sheep, dromedary camels, and donkeys and the minerals recovery in their cheese. Ten 2-liter samples of bulk milk were collected from different Italian farms for donkey, cattle, buffalo, goat, and sheep species, while 10 milk samples were collected from dromedary camels reared in two different areas in Algeria. We processed the samples individually using the same model cheese-making procedure and the wheels obtained were ripened for  $95 \pm 5$  d in the same conditions (15 °C and 85% RH). Samples of milk and ripened cheese were analyzed for the detailed mineral profile using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). The percentage of milk minerals recovery in cheese was also calculated. Milk minerals and their recovery in cheese were analyzed using a generalized linear model, where the species was considered as a fixed effect. The results showed that donkey milk had the lowest concentration of macro- and micro-minerals except for Sr. Considering the macro-mineral profile, the dromedary milk had the highest concentration of Na and was similar to the cow's and goat's milk in terms of Ca, K, P, and S, but it was more similar to the donkey milk in terms of Mg. Compared to the bubaline and ovine milk, cow and goat showed a lower content of many macro-minerals but had a higher concentration of K. In terms of micro-minerals, the results showed greater variability (from  $0.02 \pm 0.017$  mg/kg for Ti to  $5.80 \pm 2.017$  mg/kg of Zn) and every species was characterized by a different profile. The donkey milk was the only one that did not coagulate in the same cheese-making conditions of the other species. The recoveries showed that about 80% of Ca, 60% of P and S, and 45% of Mg from milk were transferred to the cheese, whereas almost 90% of K was lost in



the whey. The recovery of the micro-minerals was more variable, but in many cases over 50% of milk content. The buffalo's and sheep's milk often showed a higher recovery efficiency than the other species, except for some micro-minerals. Further investigations should be done on the relationships between minerals and other milk and cheese nutrients.

## O020

### Animal and environmental effects on protease and lipase activity of bovine milk

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Bovine milk contains a range of proteases and lipases that can impact the quality and stability of dairy products. Proteolytic and lipolytic activities are both responsible for typical characteristics of dairy products but they can also lead to the production of unfavourable or rancid off-flavour. The aims of the study were: (i) to characterize the presence of native protease and lipase enzymes in individual bovine milk samples and to assess (ii) the effects of two specific pre-treatments (skimming and freezing) and (iii) the effects of days in milk (DIM), order of parity and farming system on protease and lipase enzymes variability. In total 1256 Brown Swiss cows reared in 80 farms located in Trento province were sampled and then analyzed for milk components (fat, protein, casein, lactose, urea, SCS and pH) and enzyme activity (protease and lipase). Herds were selected and classified considering farm management, feeding system and herd location. The study consisted of two subsequent trials with respect to the pre-treatment tested (T; T1,  $n = 598$ ; T2,  $n = 658$ ). In the T1, enzymes were analyzed on fresh full-fat and skimmed milk samples and, in the T2, on full-fat and skimmed milk samples previously frozen at  $-80^{\circ}\text{C}$ . Milk components were analyzed on fresh full-fat samples for both trials. Milk composition was analyzed using a linear mixed model including DIM, parity, season, farming system and trial (T1 vs. T2) as fixed factors and herd as a random effect, while for enzymes trial effect was excluded. Milk components were not affected by the trial effect indicating that samples from both trials were equal in terms of composition. Our results showed that sample pre-treatments caused important differences in protease and lipase activities. In particular, pre-freezing affected mainly protease variability, whereas skimming caused significant differences for both protease and lipase activities. Enzyme activity was overall quite affected by animal and herd factors, especially by DIM ( $p < .01$  for lipase activity).

Days in milk increased enzyme activity, especially lipase in the final phase of the lactation period, whereas parity did not exhibit any influence on those traits. Among herd factors, the effect of the dairy farming system was negligible while the individual herd was high for both trials and enzymes. This study provides useful elements to better understand the variability of native enzymes in milk with respect to lab pre-treatments, herd practices and cow characteristics.

## O021

### Associations among somatic cell count, differential somatic cell count and milk protein profile in Holstein cattle

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Udder health in dairy herds has become in recent years a very important issue to ensure animal welfare and the production of high-quality milk. The somatic cell count (SCC) is the most widespread indicator for the assessment of udder health status. However, recently, the differential somatic cell count (DSCC), defined as the sum of polymorphonuclear neutrophils and lymphocytes, has been proposed as a new indicator for a better evaluation of intra-mammary infection dynamics. Although recent studies have provided insights on the associations between SCC and DSCC and milk composition and milk technological properties, no information is currently available for milk protein fractions. Hence, this study aimed at evaluating the relationship between SCC and DSCC and the detailed milk protein profile in a population of 497 Holstein cows.

A validated reversed-phase high-performance liquid chromatography method was used to quantify five caseins (CN), namely  $\alpha\text{s}1$ -,  $\alpha\text{s}2$ -,  $\kappa$ -, and  $\beta$ -CN, and three whey protein fractions, namely  $\beta$ -LG,  $\alpha$ -LA and LF, which were expressed both quantitatively (g/L) and qualitatively as a percentage of total milk nitrogen content (%N). Data were analysed with a linear mixed model including the fixed effects of cows' days in milk, parity, SCC and DSCC and the random effect of herd/date.

Considering the protein fractions expressed as g/L, SCC showed a negative association on almost all casein fractions, except for  $\kappa$ -CN, and a positive association with the whey protein  $\alpha$ -LA ( $p < .01$ ). Regarding DSCC, it showed associations with  $\alpha\text{s}1$ -CN ( $p < .05$ ),  $\beta$ -CN ( $p < .001$ ) and  $\alpha$ -LA, ( $p < .01$ ) with an opposite pattern with respect to the SCC. Moving from the quantitative to the qualitative data, we observed the same pattern as previously reported, confirming the opposite behaviour of SCC and DSCC towards milk protein fractions. Briefly, SCC negatively affected casein proportion ( $p < .001$ ),  $\beta$ -CN proportion ( $p < .001$ ) and

positively  $\beta$ -LG proportion ( $p < .001$ ). DSCC showed a positive association with  $\beta$ -CN and a negative one with  $\beta$ -LG ( $p < .001$ ). In conclusion, these results confirmed the unfavourable effect of SCC on milk caseins, probably related to the proteolytic activity of plasmin and add new insights on the association between DSCC and milk proteins.

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### SESSION 5 – ALTERNATIVE FEEDS AND WASTE RECYCLING – II

#### O022

#### Peanut (*Arachis hypogaea*) crop within a circular economy perspective: potential use of biomass as forage

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The use of annual peanut (*Arachis hypogaea*) as a dual-purpose crop providing both seeds for human consumption and fodder for ruminant feeding may be of interest to connect food and feed chains in a perspective of circular economy and agricultural waste valorization. In this study, we examined the yield and chemical composition of epigeal biomass of peanut as influenced by both cultivar (Cv) and cutting height. Three late-maturing peanut Cv, namely IPG 1288, AG 44, and Lotus, were sown in three 1-ha fields near Caserta (Italy) in May 2020. All Cv had resistance to tomato spotted wilt virus and to leaf spot. At harvesting maturity of pods (September 2020), three 2 × 1 m strips/field were randomly selected, and the plants ( $n$  10) were manually cut to a stubble height of 5 cm. The plants were weighted to determine biomass yield and then fractionated into 3 layers corresponding to the upper (first 20 cm in height), middle (20–40 cm), and basal (over 40 cm) parts of the plants. Each layer was further separated into stems and leaves to assess plant traits and yield components. The samples so generated were oven-dried to determine chemical composition. Data on plant height, whole plant crude protein (CP) content, and dry matter (DM), and CP yield ha<sup>-1</sup> were analyzed by one-way ANOVA with Cv as a factor. Data on the leaf to stem ratio and chemical composition were analyzed by a two-way ANOVA (proc GLM) with Cv, layer, and their interaction as factors. The Cv IPG 1288 was taller than AG 44 and Lotus ( $p < .01$ ) and showed the highest DM and CP yield ha<sup>-1</sup> ( $p < .001$ ) followed by AG 44 ( $p < .001$ ) and Lotus ( $p < .001$ ). The CP and NDF contents of the whole plant did not differ among Cv and averaged on a DM

basis  $18.3 \pm 0.38$  SEM and  $40.3 \pm 0.79$ , respectively. The interaction Cv\*layer was always significant. All Cv was leafier in the upper layer with the higher value observed for IPG 1288 followed by AG 44 ( $p < .001$ ) and Lotus ( $p < .001$ ). Consequently, also the CP content was higher in the upper layer of IPG 1288 followed by AG 44 ( $p < .001$ ) while Lotus presented the greatest protein content in the middle layer. The basal layer was the poorest in proteins with the lower value observed for IPG 1288. Overall, the DM and CP yield of peanut biomass are quite variable in relation to the botanical variety, but chemical composition can be significantly modified through a careful choice of the cutting height. The chemical characteristics propose biomass peanut as medium quality legume forage.

#### O023

#### Agronomical traits and chemical characterization of whole plant and botanical fractions of six varieties of hemp cultivated in the Veneto region

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Hemp (*Cannabis sativa* L.) is an annual plant, globally distributed and cultivated in the past as a source of fiber. Recently, the interest in hemp cultivation has significantly increased, considering its positive environmental impact and several application fields. Over 70 hemp varieties, with different morphological, anatomical and productive characteristics are included in the EU Common Catalogue of Varieties of Agricultural Plant Species. In this experiment, 6 varieties (Codimono, Futura 65, CS, Carmaleonte, Felina 32 and USO 31) were obtained from outdoor cultivation at CREA-CI of Rovigo (northeast Italy), were analysed for agronomic and chemical traits. The results of the partition of whole plants in the different botanical fractions showed a proportion (on DM) of stems, leaves and seeds varying respectively from 38.8 to 69.6% of DM, from 18.0 to 36.6% and from 9.5 to 29.0%. In hemp stems the content of NDF was very high (from 76.3 to 86.9% on DM) and the fiber resulted highly lignified. On the contrary, the leaves of hemp could be considered a good forage, with CP levels changing from 11.8 to 17.5% on DM and NDF contents from 24.0 to 29.7% on DM. In hemp whole seeds, the crude protein (CP) and lipid contents varied respectively from 19.2 and 23.6% on DM and from 18.2 to 28.7% on DM, showing a large variability among varieties. In animal feeding, full-fat seeds but also the products obtained by cold mechanical pressing of seeds (oil and cake) can

be used. In this experiment, the percentage of oil extracted from seeds of different varieties varied from 11.6 to 27.7% on a DM basis. The oils were subjected to analysis of fatty acids profile by chromatographic way. The total saturated fatty acids (SFA), mainly represented by palmitic acid, varied from 9.3 to 16.4% of total FA. Over 90% of the monounsaturated FA (MUFA), which varied from 12.9 to 15.5% of total FA, was represented by oleic acid. The percentage of polyunsaturated FA (PUFA) was very high in the hemp oil and reached values close to 75% of the total FA (from 66.2 to 72.3% of total FA). Among PUFA, linoleic acid (C18:2 n6) and alpha-linolenic acid (C18:3 n3) are the most represented FA (on average 57.0 and 14.6% of the total FA). The n-6/n-3 ratio ranged from 2.61 and 4.04. In conclusion, the wide variability of the chemical composition of the products obtained from the tested hemp varieties allows their suitable use in the feeding of monogastric animals and ruminants.

## O024

### Partial replacement of soybean meal with self-produced whole-plant soybean silage in lactating dairy cows diet

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The high reliance of the EU livestock sector on imported soybean meal, especially from South America, poses environmental problems, like greenhouse gas emissions for transportation and land-use change with the loss of carbon stock and biodiversity. Self-produced whole-plant soybean silage can be an alternative protein source with positive agronomic side effects and lower exposure to market cost fluctuations. The aim of the present study was to evaluate the partial substitution of soybean meal with whole-plant soybean silage in the diet of dairy cows.

The experiment was conducted in the experimental farm Angelo Menozzi, University of Milan (Italy). The crop was harvested at the full seed stage (R6), with 26.2% DM. Thirty-six lactating Holstein cows were divided into two groups, according to a change-over design, and were fed alternatively, for 3 weeks, a control diet (CON) based on corn silage and soybean meal as a protein source, and a diet with the inclusion of 12.5% soybean silage on total DM (SBS) in substitution of 35% of the soybean meal of CON. Urine purine derivatives were used to estimate DMI, while undigested fibre (uNDF) at 288 h *in vitro* incubation was used as an internal marker to determine total tract digestibility. Net energy for lactation and NDFD of the whole-plant soybean was 5.09 MJ/kg DM and 38.6%, respectively. The dietary treatment did not affect DMI, milk production and dairy efficiency, averaging

among the two treatments 23.7 kg/d, 33.0 kg/d, and 1.40, respectively. By contrast, milk quality was influenced by diet. Cows fed SBS resulted in lower milk crude protein (3.43 vs. 3.55%,  $p < .001$ ), higher milk urea (30.5 vs. 28.7 mg/dL,  $p = .002$ ), and higher fat yield (1.50 vs. 1.41 kg/d,  $p = .024$ ) in comparison with CON. Nutrients digestibility was lower for SBS than CON; particularly, DMD was 65.2 vs. 68.6% ( $p < .001$ ), OMD was 66.4 vs. 69.8% ( $p < .001$ ), and NDFD was 31.5 vs. 38.8% ( $p < .001$ ). Urine N excretion (% N intake) was higher for the cows fed SBS than CON (32.3 vs. 28.9%,  $p = .005$ ) and the efficiency of N utilization (N milk/N intake \*100) was higher for CON than SBS (32.7 vs. 31.3%,  $p = .003$ ).

Soybean silage did not penalize feed intake and milk production. However, to fully exploit this forage, digestibility and N utilization efficiency should be improved, evaluating different agronomical strategies in the harvesting of the crop and including more rapidly fermentable carbohydrates in the diet.

## O025

### Biorefinery approach to valorise vegetable waste from the wholesale market

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The biorefinery can be a valid approach to enhance food waste, especially fruit and vegetable of which 40–50% is lost and wasted. This biomass is suitable for the development of other value-added products becoming an important raw material of valuable phytochemicals such as Dietary Fibre (DF) and other natural bioactive substances such as polyphenolic compounds. DF consists of soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) and in monogastric animals, it has different chemical properties and functions within the gastrointestinal tract. DF can positively influence the composition of the intestinal microbiota by stimulating the proliferation of health-promoting bacterial species. Furthermore, the polyphenolic compounds have positive nutritional properties due to their antioxidant, anti-inflammatory, anti-viral, anti-microbial activities including their ability to modulate lipid metabolism.

This study is focused on the evaluation of the content of DF and total polyphenol of fruit and vegetable waste from the Wholesale Market of Milan, the largest in Italy, where fruits and vegetables are sold to retailers. The unsold products (i.e. those not redistributable by charities and those damaged, spoilt and bruised) become waste and are evaluated about 1700 tons/year. Representative samples composed of a mixture of fruit and vegetable waste collected across the year were considered in this study. Total, soluble, and insoluble dietary fibres were quantitated



by the enzymatic-gravimetric method (AOAC 991.43–1994, 2000), while the total polyphenols were determined with the Folin–Ciocalteu method and data expressed as gallic acid equivalents (GAE).

Across the year the DF was evaluated as  $26.4 \pm 6.6\%$  on DM basis of which the IDF was  $19.47 \pm 7.4\%$  on DM basis and SDF as  $6.93 \pm 2.3\%$  on DM basis, while total polyphenols were evaluated as  $2.23 \pm 0.7\%$  GAE on DM basis. Results indicated that fruit and vegetable waste represents a good source of DF and polyphenols.

These data show that fruit and vegetable waste can represent an excellent feed ingredient and can be considered an important resource for specific nutritional needs aimed at maintaining animal health and improve gut functions. This can stimulate new investigations focusing on feed production from this biomass.

## SESSION 6 – MANAGEMENT STRATEGIES TO IMPROVE ANIMAL HEALTH, WELFARE AND RESILIENCE – II

0026

### Quarter-level analysis of mastitis infection and relationships with milk traits in Holstein cattle

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Mastitis is the most common disease in dairy cattle leading to large economic losses at various levels of the dairy chain. The aim of this study was to investigate the relationships between sub-clinical mastitis infection and milk composition and fatty acids (FA), udder health indicators, and milk technological traits at the quarter level. The dataset comprised 450 Holstein cows belonging to three different dairy herds which showed the presence of mastitis from three pathogens: *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Prototheca* spp. A cross-sectional study based on three different sampling time (T) for each animal was set up. In total, after an initial bacteriological screening (T0), 613 quarter records for 2 different sampling times (T1 and T2, 1 month after T1) were available to follow animals' responses over time. The response variables (i.e. the milk traits) were analyzed with a hierarchical linear model which considered the following effects: days in milk, parity and herd (tested on the animal

variance) and bacteriological examination (positive and negative) and time of sampling (T1 and T2) which were tested on the residual variance. Mastitis infection decreased milk production ( $p < .01$ ), increased milk conductivity ( $p < .05$ ), decreased casein index and lactose ( $p < .05$ ), increased milk pH ( $p < .01$ ), and increased somatic cell score (SCS;  $p < .001$ ). Mastitis infection did not affect differential somatic cell count (DSCC) expressed as a percentage of polymorphonuclear neutrophils-lymphocytes (PMN-LYM, %) but it had a significant effect on DSCC expressed as PMN-LYM and macrophages (MAC) count ( $p < .05$ ). In particular, similarly to SCS, a positive bacteriological examination was associated with an increase in both PMN-LYM and MAC count to a different extent. Mastitis infection worsened milk coagulation properties, as it extended rennet coagulation time ( $p < .05$ ) and decreased maximum curd firmness and potential curd firmness ( $p < .001$ ). Regarding cheese-making traits, mastitis infection decreased the curd nutrients recovery ( $p < .01$ ). In summary, we confirmed the unfavorable associations between mastitis infection and milk composition, udder health indicators, and milk technological traits. Besides this, our results suggest the PMN-LYM and MAC count could be useful indicator traits of mammary gland inflammation.

#### Acknowledgements

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0027

### Effects of microalgae and insect meal supplementation to diets high in soybean meal, deprived of fish meal, on gut health of seabass (*D. labrax*) and sea bream (*S. aurata*)

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To meet the sustainability challenge the search for functional aquafeed ingredients able to promote fish growth and welfare represents a great opportunity for the aquaculture industry, mostly when diets high in veg-proteins are used. In this respect, microalgae and insects, deserve great attention for their nutritional properties besides their nutrient supply. The aim of the present study was to evaluate the effect of insect meal from *Hermetia illucens* larvae (Hi) and microalgae dry biomass (a blend of *T. lutea*:*T. suecica* in a 2:1 w/w ratio; MAmix) as supplements in fish meal-free diets high in soybean meal during

Gilthead sea bream (GSB) and European seabass (ES) culture. Four iso-proteic (45%) and iso-lipidic (20%) diets were formulated. A diet denoted CF was designed to obtain 90:10 and 67:33 weight ratios between fish and vegetable protein and lipid, respectively. On the opposite, a diet coined CV was formulated with the reversed proportion between fish and vegetable protein and lipid ratios. Two other diets (H10 and MA10) were prepared by replacing 10% protein from veg. sources of CV diet, by Hi and MAMix protein respectively. The diets were randomly assigned in triplicate to 12 groups (18 fish/group) of juveniles per fish species. Fish were kept in fiberglass tanks (250-L) in an indoor marine recirculating aquaculture system at T, 23.5 °C and 30 psu salinity. Fish were daily fed to visual satiety over 12 and 18 weeks for GSB and ES, respectively. At the end of the feeding trials, besides growth performance and efficiency, fish were sampled for gut and liver histology, gene expression analysis and FTIR-spectroscopy. Compared to CV and CF, fish-fed MA10 diet resulted in the worst growth performance and feed conversion ratio while the H10 diet showed the best performance in GSB and did not differ from CV and CF in ES. Gut histology in both fish species fed diet CV, high in soybean meal, showed slightly inflamed distal intestine, a condition that was partially reversed in fish fed H10 and MA10 diets. Several inflammatory marker genes were down-regulated in GSB fed H10 diet, while in ES this effect was more evident in the fish fed MA10 diet. The FTIR analysis showed a different modulation of nutrient absorption and liver composition in response to the Hi and MAMix dietary supplementation. The results obtained so far showed Hi and MAMix to be beneficial on gut health; however, the use MAMix should be better refined to avoid growth performance.

## O028

### Heatwaves effects on Italian Brown Swiss milk production

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The temperature-humidity index (THI) has been effectively used to estimate the effects of the environment on dairy cows' efficiency and welfare. Recent studies stated that environmental climatic conditions are able to affect dairy cows' production also

in relation to the duration of this condition, introducing the focus on heatwaves (HW). The present study aims to estimate the effect of HW on the Italian Brown Swiss (IBS) population. The definition of HW was adapted to the study's aims considering it as a period lasting from 2 to 5 consecutive days with a THI (minimum, mean and maximum) over the known threshold for the IBS population. The aim of this study was to estimate the effects of HW of different duration (from 2 to 5 days) in IBS on fat corrected milk yield (FCM), protein and fat yield, protein and fat percentage. Ten years of data from the test day record system at the national level were merged with data from 76 weather stations. The dataset was subdivided according to parity in 4 categories: first, second, third, and  $\geq$  fourth parity. A mixed-effects model for repeated measures was applied to evaluate the effects of HW on each production trait. Month, parity class and days in milk class were considered as fixed effects, while herd and year were considered as random effects. When THI exceeded the maximum THI threshold, cows yielded from 32 (2 days) to 40 g (5 days) of daily protein less ( $p < .0001$ ) for each THI point over the threshold. When THI exceeded the minimum THI threshold, cows produced from 30 (2 days) to 32 g (5 days) daily protein less ( $p < .0001$ ) for each THI point over the limit. The FCM was lower in cows subjected to HW over the maximum THI, worsening in relation to the HW duration (from 554 g for a duration of 2 days to 850 g at 5 days lasting HW, for each point over the threshold;  $p < .0001$ ). The same trend, but with different absolute values was recorded considering THI over the minimum THI threshold: from 774g for each THI over the threshold for an HW of 2 days to 841 g for each THI over the threshold for HW of 5 days ( $p < .0001$ ). Although Brown Swiss is more tolerant to heat stress than other breeds, the present contribution highlights how HW may affect the efficiency of this breed too. Moreover, although from a physiological perspective minimum and maximum THI values have a different meaning, both showed to be equally effective in estimating how HW affects milk production traits in IBS.

## O029

### Dietary supplementation with nucleotides in calves: effects on oxidative balance and immune response

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Mammals may recover nucleotides through the diet, by the salvage pathway, or by de novo synthesis. The latter one is a metabolically costly process, thus, the salvage pathway to recycle dietary nucleotides is more favorable, especially during the

growth phase. Dietary nucleotides have been reported to positively affect a plethora of biological functions (e.g. immune response, cell development and repair, and maintaining of the redox state homeostasis). This study aimed to evaluate the effects of dietary nucleotides supplementation on the immune response of calves and their ability to counteract oxidative stress. Twenty male Holstein Friesian calves were randomly divided into two groups: one was individually and orally supplemented from birth to 25 days with 5g/head/day of an additive containing 40% of yeast-free nucleotides 5' mono-phosphate (NG); the other group represented the control (CG), supplemented with 20ml/head/day of freshwater. All the animals were fed with the same natural colostrum, milk replacer, calf starter and hay, and with the same standardized protocols. Blood samples were collected at 0, 3, 7, 15 and 25 days to assay some markers of oxidative and immunological profiles. In plasma from NG, superoxide dismutase and glutathione peroxidase activities showed an increasing ( $p < .01$ ) trend, whereas their activity remained almost stable in CG. The total antioxidant capacity of plasma (ABTS and FRAP assays) was higher in the experimental group compared to the control one ( $p < .01$ ). Overall, peripheral blood mononuclear cell (PBMC) viability did not show differences according to dietary treatment, even after stimulation with  $H_2O_2$ . Untreated PBMC showed the lowest levels of intracellular reactive oxygen species (ROS) in NG ( $p < .05$ ). Under  $H_2O_2$  stimulus, intracellular ROS levels significantly rose ( $p < .05$ ) in PBMC from CG, while in NG there was a slight increase. The levels of ROS and reactive nitrogen species (RNS) in PBMC supernatants were found at the lowest levels in NG ( $p < .05$ ). The serum levels of matrix metalloproteinases-9 (MMP-9) were influenced by feeding supplementation. In particular, lower levels ( $p < .05$ ) of MMP-9 were observed in NG compared to CG. The feeding treatments did not affect the serum concentrations of MMP-2. Concluding, dietary supplementation with nucleotides exerted a beneficial role on calves' oxidative imbalance and immune function modulation.

### O030

#### The effect of cocoa husks supplementation on fatty acids profile and blood and milk antioxidant status of dairy ewes

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The effect of cocoa husks (CH) containing theobromine on milk fatty acids and milk and blood oxidative status in dairy ewes was investigated. The experiment was carried out with 24 dairy sheep divided into three homogeneous groups fed 0, 50 or 100 g/d per

head of CH (group CON, CH50 and CH100, respectively). The trial lasted 8 weeks with 3 weeks of adaptation period and 5 weeks of the experimental period. Blood and milk samples were analyzed for the determination of total antioxidant capacity and oxidative stress biomarkers. Blood samples were analyzed for the activities of superoxide dismutase (SOD), glutathione transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GSH-Px), while milk samples were analyzed for SOD, GR and lactoperoxidase (LPO). Milk fatty acid profiles were determined by GC. Orthogonal polynomial contrasts (linear and quadratic) were used to investigate the effect of the diet. The C16:0 and MCFA (medium-chain fatty acids) decreased, whereas C18:0 and LCFA (long-chain fatty acids) increased quadratically with the dose of CH. The supplementation of CH decreased linearly the OBCFA (branched-chain fatty acid). Regarding antioxidant results, the CH diet did not affect the Ferric Reducing Ability of Plasma (FRAP) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays, GST, GR and malondialdehyde (MDA) in blood plasma. Mean protein carbonyls (PC) decreased linearly (2.89 vs. 2.67 vs. 2.23 nmol/ml) and SOD activity tended to increase linearly (19 vs. 19.4 vs. 22.98 units/ml) in blood with increasing CH dose. Meanwhile, antioxidant analysis of milk samples showed that CH supplementation increased quadratically the LPO (0.45 vs. 0.81 vs. 0.36 units/ml) and tended to decrease quadratically the MDA level (0.49 vs. 0.41 vs. 0.49  $\mu$ M).

In conclusion, the addition of CH as a supplement to the diet of ewes could modify the milk FA profile and could improve the antioxidant capacity of blood and milk, but only at a low dose. For this reason, more studies are necessary to explain better the dose-response effects showed by using this by-product in lactating ewes diet.

### O031

#### Effect of two different ways for milk replacer administration on the growth performance and welfare of piglets

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In high prolific sows, large litters are characterized by a huge variation in piglets' weight that associates with the competition for the teats and represents a risk factor for impairment of litter health. The use of milk replacers (MR) is a promising strategy to manage these litters. The study aims to evaluate the effect of two ways of MR (DanMilk™) administration on the performance,



health, and behaviour of piglets during the suckling and post-weaning period. 30 litters from DANBRED crossbreed sows (for a total of 398 piglets) were included in the trial. Litters were divided into three groups: i. Control group (CO), only sow milk; ii. CO plus MR administered automatically in a continuous flow (AA); iii. CO plus MR is administered manually twice a day (AM). The milk supplement was available from d0 to d14, thereafter only creep feed was provided until the weaning (d21 of the study). At weaning, pigs were regrouped in boxes of about 30 pigs each. Piglets were individually weighed at the beginning of the trial (d0), d14, d21 and d31 (ten days post-weaning) of life. Behaviour and lesions were scored at d4, d11 (end of the milk administration) and d18 (after milk suspension). Statistical analysis was conducted in the R environment using car, lsmeans and lmer packages and on SAS software using the PROC MIXED method. Treatments did not affect the growth performance during suckling and post-weaning. No significant differences were observed in post-weaning mortality among groups. During suckling, the AA group significantly increased the survivability of pigs compared to CO and AM groups both at d0–d14 and at d0–d21 (AA vs. CO,  $p < .0001$ ; mortality rate: 3.1% vs. 8.4% and 3.1 vs. 9.21%; AA vs. AM,  $p < .05$ ; mortality rate: 3.1% vs. 6.0% and 3.1% vs. 6.7%). No differences between the treatments were observed in behavioural and lesion scores during suckling. The treatment affected other behavioural indicators. Specifically, at d4 piglets from the AA group tended to explore the pens more than the CO group ( $p = .08$ ; 19.3% vs. 3.3%). While AM group has higher scores in negative social behaviour compared to the CO group ( $p < .05$ ; 5.7% vs. 1.4%), this can be due to the competition for access to the MR. Overall, the results suggested that MR supplementation contributes to increasing pig survivability pre-weaning, with an additional advantage observed with the automatic system probably due to the continuous availability of the MR. This effect seems not directly related to the welfare status of the litter. Additional studies can disclose the role of MR to sustain the gut health of piglets.

## SESSION 7 – GENOMIC AND PHENOMIC TOOLS TO IMPROVE LIVESTOCK ANIMALS – I

O032

### A genome wide association study for beef production traits in five Italian cattle breeds

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Beef cattle production has a high economic impact worldwide, being the third most important livestock species after poultry and pig. In 2018, 72,438,200 million tons of beef were produced around the world, and the European Union was the third-biggest producer. In 2018 Italy ranked as the third producer in European Union, with 980,906 tons of beef, exporting 123,385 tons to other countries. This study investigates the genetic determinism of growth and muscle development traits in five autochthonous Italian beef cattle breeds selected by the National Association of Italian Beef Cattle Breeders (ANABIC). The traits were recorded on 3581 young bulls representing five of the main Italian beef cattle breeds: Chianina (CHI,  $n = 909$ ), Marchigiana (MAR,  $n = 879$ ), Romagnola (ROM,  $n = 904$ ), Maremmana (MRM,  $n = 334$ ), and Podolica (POD,  $n = 555$ ). Samples were collected at the ANABIC genetic station during the performance test from 1985 to 2019. We genotyped the animals in 2019 using the GeneSeek® Genomic Profiler™ Bovine LDv4 33K chip (Illumina Inc., San Diego, CA, USA) and performed a genome-wide association study (GWAS) using the GEMMA software. The GWAS highlighted significant associated SNPs in chromosome 2 of MAR breed for the muscularity trait. At the chromosome-wide level, a total of 40, 19, 15, 11, and 11 significant SNPs were identified considering all analyzed traits for MAR, CHI, ROM, MRM, and POD, respectively. After performing a gene functional analysis, we identified different genes (e.g. MSTN, COL5A2, SLC40A1, ANKAR, ASNSD1, TRHR, CTGF, CAPN1, and IGFBP6) located close and within the significant SNPs, which are involved in growth and muscle metabolic pathways. In this study, for the first time, all the main Italian beef cattle breeds were investigated by a genomic approach for traits related to beef production. The availability of new genomic tools in the selection of these breeds, preserving the history and the traits that distinguish them, allows us to identify different markers of interest. The significant SNPs found in this study, especially those which are in common between breeds, can be used hereinafter for the selected programs and the improvement of the Italian beef cattle breeds.

#### Acknowledgements

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## O033

### Complex genetic pattern supported by genome wide SNPs data in Mediterranean goat breeds

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The Mediterranean Sea has long been one of the most important natural hubs for the expansion of humans and cultures, representing a crossroads of human migrations since the Neolithic times. Neolithic civilizations probably introduced new livestock species with peculiar productive traits and genetic structure to southwest Europe. However, little is still known about the evolutionary dynamics that shaped them. In this study, a picture of 67 European, North African, and Middle Eastern goat breeds (1378 individuals) was provided through SNPs data obtained with the Illumina Goat SNP50 BeadChip from different collaborative projects (Italian Goat Consortium -IGC, ADAPMAP and Nextgene) or obtained in the present study. To define the genetic macro-structure, multidimensional scaling, Neighbor-net dendrogram, and Admixture analysis were performed. Concerning the latter analysis, coefficients of individual admixture were displayed on a geographic map using the approximate coordinates of the sampling area, through an interpolation process implemented on tess3r, R statistical package. Multidimensional scaling and admixture events highlighted gene flow among geographically close breeds. The Neighbor-net dendrogram showed a clear partition in three main clusters based on the geographic origin: Africa and the Middle East, Alpine region, and France (except for the Provençale and Corse breeds), and all the other European breeds. Some suggestive patterns of genetic relationship were evident between Provençale, Corsican, and Italian breeds (particularly Sarda, Montecristo Wild, and Garfagnina) as also supported by the Admixture analysis. Additionally, Admixture analysis

showed African ancestry in the south European breeds (especially the Spanish ones). Our results also pinpointed additional interesting patterns, such as (i) a clear clustering of Italian peninsular breeds with the Romanian and the Greek breeds, and (ii) a distinct group of Spanish breeds with Moroccan breeds, possibly reflecting the strict, reciprocal, geopolitical influence between the two countries along the centuries. The east border of Italy with Croatia seems a gateway between southern Italy and the Balkan region; surprisingly, no evidence of admixture and the geographical connection was observed between Turkish and Greeks breeds.

## O034

### Preliminary results of genome wide association study with milk functional molecules in different cow breeds

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Today, consumers have mixed views about milk: the negative one, due to adverse effects that are directly linked to excessive milk consumption, and the positive one, due to the presence of functional molecules with prebiotic and protective effects. To help the dairy industry, 'precision breeding' schemes, using marker-assisted selection, could be used to produce milk with more and more benefits for human health. In this respect, a genome-wide association study (GWAS) might allow identifying genomic markers associated with milk functional traits. Different milk molecules, such as sialic acid (N-Acetylneuraminic acid – Neu5Ac; N-Glycolylneuraminic acid – Neu5Gc), oligosaccharides and antioxidant agents (thiols,  $\alpha$ -lipoic acid and glutathione), were evaluated in milk collected at around 60 and 120 days after calving from different breeds (Holstein – HO, Simmental – SM, Simmental  $\times$  Holstein crossbred – SM  $\times$  HO, and Podolica – POD). The animals were genotyped using different SNP chip platforms, mainly GeneSeek GGP Bovine 150K. After quality control and marker harmonization among panels, the GWAS dataset consisted of 113 animals (26 HO, 30 SM, 27 SM  $\times$  HO and 30 POD) and almost 37,000 markers. Different GWAS methods were preliminary tested considering single and multi-breed scenarios, and the two lactation periods. GCTA software was used for the single-SNP GWAS and gene-based approach (fastBAT). In humans, Neu5Gc is recognized as an exogenous molecule and

causes health problems. So, the production of milk with lower levels of Neu5Gc would be beneficial for human health and increase milk commercial value for breeders. Neu5Gc and Neu5Ac content was significantly associated with breed: POD animals showed a lower level compared to HO, SMxHO and SM. The GWAS identified suggestively associated regions on BTA 4 and 10. When Neu5Ac was analysed, signals on BTA 7 and 24 were identified. In particular, *GALNT1*, a gene indirectly linked with sialic metabolism, was identified as suggestively associated in the BTA24 region. About the glutathione, an SNP that explains 20% of the phenotypic variance was identified on BTA26. Further studies are needed to confirm the preliminary results here obtained.

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## O035

### Donkey worldwide diversity based on control-region data and entire mitogenomes

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Since domestication ca. 5000 years ago donkeys (*Equus asinus*) have been widely used in agricultural activities and for the transportation of people and goods, but the growing diffusion of mechanization of the last century largely spoiled their role as burden animals. As a consequence, donkey breed number and population size have decreased dramatically, particularly in developed countries, and diversity contributed by autochthonous gene pools was progressively eroded. In this context, 1350 donkeys mitochondrial (mtDNA) control region sequences and 65 complete mitogenomes, representing animals from four continents (i.e. Africa, Asia, Europe

and Southern America) have been analysed to i) carry out a world-wide analysis of diversity, ii) evaluate geographical patterns of variation, and iii) propose a new nomenclature for mtDNA haplogroups. A Maximum Parsimony (MP) tree was reconstructed from coding region data of complete mitogenomes, while diversity metrics, mismatch distribution and phylogenetic networks were assessed using control-region data. The MP tree topology confirmed the two previously identified major clades, i.e. Clades 1 and 2, resulting from two independent domestications in Northern Africa. Clade 1, which derives from the Nubian wild donkey *E. a. africanus* was renamed as Haplogroup A and Clade 2 as Haplogroup B to harmonize haplogroup nomenclature with the standard currently adopted for other livestock species. Control-region haplotype (*h*) and nucleotide diversity (*n*) values varied considerably between geographical areas (*h* = 0.403–0.922, *n* = 0.011–0.025), but were highest in North-eastern Africa (*h* = 0.922, *n* = 0.025) confirming this region as the likely domestication center. Mismatch distribution curves indicate early population expansion in this area and later demographic increase in other regions of the world following the donkey spread out of the domestication center. Phylogenetic networks confirm the co-occurrence of both haplogroups in all sampled populations, while differences at the regional level point at the joint effects of demography, past human migrations and trade. Despite the strong decline of donkey populations in many areas of the world, the remarkable mtDNA variability we detected emphasizes the need for an extensive characterization also of nuclear genome variation to identify hotspots of diversity and aid the conservation of donkey breeds worldwide.

## SESSION 8 – NUTRITIONAL PROFILE OF FOOD – II

## O036

### Evaluation of oil from *Camelina sativa* in feeds for gilthead sea bream (*Sparus aurata*): effects on fish growth, fillet fatty acid profile, and gut microbiota composition

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Alternatives to fish oil (FO) in aquaculture feeds have been explored intensively in the last decades and the commercial feed industry has progressively reduced the level of FO in feeds by replacing FO with more sustainable vegetable oils. Among these, *Camelina sativa* contains up to 40% of the omega-3 (n-3) precursors, such as  $\alpha$ -linolenic acid (ALA) and linoleic acid (LA). Moreover, Camelina oil (CO) is rich in mono and polyunsaturated fatty acids as well as in antioxidants.



Accordingly, a 90 day-feeding trial was set using 600 gilthead sea bream of  $32.92 \pm 0.31$ g mean initial weight. Fish were distributed in triplicate tanks per diet (50 fish/tank) in order to study the effects of replacing FO with CO on growth performance, fillet fatty acid profiles, and gut microbiota. Four diets were formulated: control diet (15.3% FO); 20% CO (12.4% FO); 40% CO (9.3%FO); and 60% CO (6.0% FO).

The NGS Illumina platform for sequencing of 16S rRNA gene and Mothur pipeline were used to identify bacteria in the faeces, gut mucosa and feeds in addition to metagenomic analysis using PICRUST.

The growth parameters (feed conversion rate and specific growth rate) were not affected by diet, whereas final weight resulted significantly lower in fish fed the 60% CO diet ( $p < .05$ ). Reduced final weight was attributed to lower levels of EPA and DHA in the CO ingredient. The lipid profile of fillets was similar among dietary groups with regard to total saturated, monounsaturated, PUFA (n-3 and n-6), and the ratio of n-3/n-6. Levels of EPA and DHA in the fillet reflected the progressive replacement of FO by CO in the diet and the EPA was significantly lower in fish fed the 60% CO diet ( $p = .006$ ), whereas ALA was increased.

Alpha and beta-diversities of gut bacteria in both faeces and gut mucosa were not affected by the dietary treatment. However, a lower abundance of lactic acid bacteria, specifically *Lactobacillus*, in the gut of fish fed the 60% CO diet may indicate a negative effect of this diet on gut microbiota.

PICRUST analysis revealed similar predictive functions of bacteria in the faeces and mucosa, although a higher abundance of *Corynebacterium* in the mucosa of fish fed 60% CO diet increased the KEGG pathway of fatty acid synthesis and may act to compensate for the lack of dietary fatty acids.

In conclusion, this study demonstrated that diets could replace up to 40% of FO with CO without negative effects on growth performance, fillet fatty acid profile and gut microbiome of *S. aurata*.

molecules. In the MIQUALAT project milk recording data, fatty acid profiles, sialic acid, glutathione, thiol compounds and lipoic acid and 1H-NMR spectroscopy-based metabolomics profiles have been determined in the milk of different cattle breeds, at two lactation points (60 and 120 days by calving). The herd established at CREA, made by three groups of lactating cows (purebred Holstein (HO), purebred Simmental (SM) and crosses (SMxHO)), and Podolica (POD) breed, as an outgroup, have been enrolled in the project. Statistically significant differences in biomarkers content among the four genetic groups have been found by GLM procedure. POD has shown a higher content of thiols and lipoic acid than HO and SMxHO ( $p < .01$ ), while the concentration of urea and N-Glycolylneuraminic acid (Neu5Gc) was lower ( $p < .01$ ). N-Acetylneuraminic acid (Neu5Ac) content was higher in SM than HO ( $p < .01$ ), and in HO than POD and SMxHO ( $p < .01$ ). The analysis of fatty acids has revealed that POD milk was enriched in short fatty acid (SFA),  $\omega 6/\omega 3$ , polyunsaturated fatty acid (PUFA), conjugated linoleic acid (CLA), branched and ODD fatty acids (BCOFA) with healthy properties for human consumption. Cis-vaccenic acid content (C18:1 cis11) was higher in SMxHO vs. HO, SM ( $p < .01$ ), while SM had a higher content of linolenic acid (C18:3 cis9-12-15) than HO ( $p < .01$ ). The glutathione, CLA, linolenic acid (C18:3 cis9-12-15) and docosapentaenoic acid (C22:5) had higher concentrations at 60 days while, palmitic acid (C16:0), Neu5Ac, Neu5Gc, linoleic acid (C18:2trans9-12) and eicosapentaenoic acid (C20:5) were higher at 120 days ( $p < .01$ ). A multivariate exploratory technique of NMR data showed that twenty-seven bins differentiated the four breeds. Statistically significant differences of metabolites content (corresponding to the identified bins) between breeds were highlighted as follow: SM vs. POD and HO for isobutyrate 2-Hydroxyvalerate ( $p < .01$ ), POD vs. the other breeds for creatine-P/creatinine, lactose, glycerol-3-phosphocholine, dichloromethane and formate ( $p < .01$ ), HO vs. other breeds for lactose ( $p < .01$ ). Genetic analyses (GWAS and RNA-seq) will help to shed light on the biological mechanisms underlying the observed phenotypic divergences among breeds.

#### Acknowledgements

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## O037

### Evaluation of milk quality in different cow breeds by biological markers

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Milk and dairy products are an integral part of human nutrition and they are considered as the carriers of higher biological value

O038

### Antioxidant capacity and polyphenol content of goat milk: comparison among autochthonous breeds

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The aim of this study was to evaluate and compare the polyphenols content and antioxidant activity in milk from three autochthonous goat breeds at risk of extinction: Garganica (G), Derivata di Siria (DS), and Ionica (I) breed. The goats used in this study (50 subjects for each breed), belonging to the same mounting groups within the considered breed, were reared under an extensive system on the same farm in the province of Potenza (southern Italy). Milk samples were collected by each goat the last week of June, and on individual milk samples total phenolic and flavonoid content was determined by the Folin–Ciocalteu and aluminum chloride methods, respectively; whereas, the antioxidant activity of milk was measured using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays. The same analysis was carried out on a forage sample representative of the pabular phytomass in the grazing period. The statistical analysis was performed by means of ANOVA, using the GLM procedure of SAS. The forage sample showed a phenolic acid and flavonoid content of 11.28 mg gallic acid equivalent (GAE)/g and 20.72 mg quercetin equivalent (QE)/g, respectively. Moreover, it resulted characterized by the antioxidant activity of 17.96 and 8.40 mg trolox equivalent (TE)/g for ABTS and FRAP assays, respectively. Overall, milk from a goat on pasture showed a good phenolic acid and flavonoid content (22.26 µg GAE/g milk and 0.239 µg QE/g milk; respectively), which is closely associated with its antioxidant activity (20.99 and 6.72 µg TE/g milk for ABTS and FRAP assays, respectively). Comparing different autochthonous breeds it emerged that I milk showed lower total phenol and flavonoids content (20.87 µg GAE/g milk and 0.212 µg QE/g milk, respectively;  $p < .05$ ) than G milk (23.30 µg GAE/g milk and 0.246 µg QE/g milk, respectively;  $p < .05$ ) and DS milk (22.62 µg GAE/g milk and 0.259 µg QE/g milk, respectively;  $p < .05$ ). Furthermore, DS milk showed the highest ABTS and FRAP values (22.04 and 7.36 µg TE/g milk for ABTS and FRAP assays, respectively;  $p < .05$ ), followed by G milk (21.18 and 6.23 µg TE/g milk for ABTS and FRAP assays, respectively;  $p < .05$ ), and I milk (19.76 and 6.56 µg TE/g milk for ABTS and FRAP assays, respectively;  $p < .05$ ). The differences highlighted in this study could be ascribed to the genetic peculiarities of the genomes of the three considered breeds, which are the result of a selective process linked to adaptation, therefore a complex interactive phenomenon.

O039

### Fatty acid profile and endogenous bioactive compounds of beef as affect by the duration of linseed supplementation

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The ability of oilseeds on improving meat fatty acid profiles is well known however no data are available regarding variation induced in other meat components such as bioactive compounds. It is also useful to evaluate the necessary time to achieve a significant amount of n-3 polyunsaturated fatty acids (PUFA) into steer meat. This study was carried out to assess the effects on the fatty acid profile, cholesterol and bioactive compounds of meat from bovine affected by the duration of linseed supplementation. Treatments were arranged in a 2 × 3 factorial design, 54 Friesian steers were randomly assigned during the finishing period into 6 experimental treatments. The 6 treatments consisted of two diets: the control diet (CO) with no supplemental fat and the linseed diet (LS) with 10% whole linseed. Both dietary groups were treated for 3 different feeding duration: 40, 75 and 120 days. Steers from each group were slaughtered at the end of the finishing period and, after 8 days of aging, samples were taken from the longissimus thoracis muscle for meat nutritional profile evaluation. The fatty acid composition was mainly modified by linseed inclusion, increasing monounsaturated fatty acids ( $p < .05$ ), CLA ( $p < .05$ ) and n-3 PUFA ( $p < .001$ ), and reducing the proportion of saturated fatty acids and n-6 PUFA ( $p < .01$ ). Particularly, meat from steers fed with linseed showed a decrease in the n-6/n-3 ratio from 40 to 75 days of feeding (from 4.61 to 3.60;  $p < .001$ ) due to the increased percentage of n-3 PUFA, linolenic acid, EPA and also of vaccenic acid, CLA 9c,11t, and total CLA which, however, declined at 120 days. The highest content of bioactive compounds such as creatine (3.15 vs. 2.72 mg/g;  $p < .05$ ), carnosine (3.68 vs. 2.38 mg/g;  $p < .001$ ) and anserine ( $p < .001$ ) was observed in meat from the linseed group. Creatine concentrations were greatly influenced by the duration of feeding ( $p < .05$ ) with an increase in the LS group from 40 to 75 days of feeding. Feeding linseed did not modify the cholesterol content on average although, after 75 days of the administration, the lowest cholesterol content was found in the LS group ( $p < .05$ ). These results demonstrate that, due to the economic sustainability, a short-term diet manipulation could be a good practice to improve bovine meat nutritional properties, bioactive compounds and subsequently the commercial value of these animals.



O040

## Colostrum composition of Sarda Dairy Ewes

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Colostrum intake is of crucial importance for the protection of newborn lambs against infectious agents during their first days of life. Its composition, especially the immunoglobulin (Ig) content of colostrum plays an important role in the success of the transfer of passive immunity. However, little is known about the colostrum composition in Sarda dairy ewes. Colostrum composition was surveyed via sample and data collection from 8 dairy sheep farms in the North-Sardinia. Four pregnant ewes were selected in each farm for a total of thirty-two samples. Colostrum was collected within 48 hours from parturition and analyzed for total nitrogen, immunoglobulin G (IgG), fat and fatty acids profile. Data were submitted to a descriptive statistic, in order to characterize the colostrum samples. One-way ANOVA was applied to all parameters to evidence the differences between farm samples using MINITAB® software. The total nitrogen (NT) content in the colostrum was  $14.48 \pm 3.98\%$  and the IgG concentration was  $43.6 \pm 20.2$  g/l and were significantly different among farms ( $p < .01$ ). The values of IgG were lower than previously reported in the same bred by previous surveys. However, some research evidenced that lambs should intake an average content of 28–30 g of IgG in the first 24 h from birth to reach values of serum IgG that ensure them correct passive immunity. The fat content was  $8.19 \pm 3.45\%$ , that contains different types of fatty acid (g/100 g of total fat) in which the most representative were C14:0 ( $11.11 \pm 2.83$ ), C16:0 ( $29.13 \pm 5.65$ ), C18:1c9 ( $29.38 \pm 7.88$ ) and C18:0 ( $7.06 \pm 2.02$ ). The concentration of C18:1 t11 ( $0.64 \pm 0.26$ ) and conjugated linoleic acid CLAc9t11 ( $0.68 \pm 0.24$ ) were lower than that usually reported in milk. Most of the fat in colostrum is made up of short medium-chain fatty acids (MCFA,  $48.93 \pm 9.44$  g/100), followed by long-chain fatty acids (LCFA,  $45.99 \pm 10.62$ ) and short-chain fatty acids (SCFA,  $5.09 \pm 1.58$ ). The groups of fatty acids showed a content of monounsaturated fatty acids (MUFA,  $36.31 \pm 7.63$ ), saturated fatty acids (SFA,  $58.12 \pm 8.54$ ) and polyunsaturated fatty acids (PUFA,  $5.56 \pm 1.16$ ), PUFA6 ( $2.91 \pm 0.61$ ) and PUFA3 ( $0.75 \pm 0.23$ ). Almost all fatty acids contents were significantly different among farms ( $p < .01$ ). In conclusion, this survey provides new data about the fatty acid profile of colostrum in Sarda dairy ewes. Further investigations are necessary to evaluate the factors of variation associated with ewe colostrum quality, especially those associated with IgG concentration variability.

### Acknowledgements

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## SESSION 9 – NON-CONVENTIONAL LIVESTOCK SYSTEMS

O041

## Food for forest: an integrative silvi-pastoral system with semi-free-ranging pigs

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Silvi-pastoralism can be a sustainable management practice for the restoration of degraded forests. Semi-free-ranging pigs are commonly farmed in silvi-pastoral systems due to their ability to feed on understory multiple resources (fruits, plant leaves and underground organs, etc.). The project 'Food For Forest', funded by the RDP of Piedmont Region (NW Italy), aimed to evaluate the effectiveness of pig grazing for the restoration and economic valorisation of degraded temperate forests. Specifically, pig grazing was targeted to control undesirable species (e.g. bramble), to facilitate a profitable silvicultural use through understory clearing, and to reduce fattening costs. The study was carried out in two semi-abandoned hill chestnut stands managed with a compound coppice system. Here a rotational grazing system with 20 Nero di Parma barrows was applied from April to December (240 days) in 2019 and 2020. Plant feeding selection, grazing on post-cut tree resprouting, and pig growing performances were evaluated. Feeding selection was assessed on 33 plant species by direct observations. *Corylus avellana*, *Hedera helix*, *Robinia pseudoacacia* and *Rubus* spp. were the preferred species. Among others, the species consumed according to their availability were *Castanea sativa*, *Cornus sanguinea* and *Ulmus minor*, while avoided ones were *Fraxinus ornus*, *Quercus cerris*, *Q. pubescens* and *Q. robur*. After a silvicultural renovation cut, removing 36% of the total available stock, the impact of grazing on 1045 sprouts was evaluated. Swine grazed firstly buds (mainly of *C. avellana* and *C. sativa*), while later in the season both buds and leaves were consumed. The average stump height of most species remained unvaried from May to August. Exceptions were *F. ornus* and *R. pseudoacacia*, which had few sprouts showing signs of grazing (4% and 20%, respectively) and heights increasing through time. On average, pigs grew from 59.69 to 157.13 kg LW (6 to 14 months age), reaching the maximum LW gain in June (0.77 kg/d). The reduced feed supplementation (–30% compared to conventional systems) and the high meat selling price (+40 to 100% than the average market price) are expected to balance the slow weight gains and ensure economic sustainability to farmers. Furthermore, preliminary results do not highlight

negative impacts on plant community or soil erosion effects. The positive outcomes of this multifunctional silvi-pastoral approach suggest possible applications in similar environments.

## O042

### Investigating dairy grazing patterns and soil microbial trends in the nitrogen cycle across traditional mountain landscapes

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This study investigated grazing impacts on soil biodiversity and soil health indicators on three summer farms in the Paneveggio Natural Park and two permanent farms in Cansiglio. The study area included semi-natural grasslands characterized by a historical human presence. We compared grassland features between management practices (including transhumance in Paneveggio and both meadow and constant presence in Cansiglio) and differences in geographic location. The mean area of the summer farms was 266 ha with a standard deviation of 62 ha while the permanent farms had a mean area of 112 ha and a standard deviation of 9 ha. The study utilized 48 soil sampling locations in each farm for three sampling periods over the course of one year. Cattle grazing patterns were tracked using GPS collars on grazing cattle over the course of summer transhumance from July to September for Paneveggio Natural Park's summer farms. Points were studied and correlated to slope, distance from concentrated flow paths, and time spent at locations across each summer farm. Soil samples were taken from each summer farm at three sampling times: before, during, and after grazing. Microbial populations were analyzed for diversity and abundance in realtime-PCR while soil characteristics included N, C, pH, and texture analysis. Genes used included *nosZ* for the final step of denitrification and *amoA* for ammonification. The analysis highlighted great variability in terms of abundance for all genes among all study areas, but the *nosZ* presence showed no significant difference in pastures between sampling times. These preliminary results outline a constant presence of a complete denitrification process in alpine semi-natural grasslands. Using these results to develop reliable indices for ecosystem services can help researchers, farmers, and policymakers understand the importance of grasslands and their use in creating more sustainable ecosystems.

## O043

### Human choices, slope, and vegetation productivity determine pattern of traditional Alpine summer grazing

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Grazing patterns in extensive pastures influence both animal productivity and conservation of grasslands ecosystem services. This study used Global Positioning Systems (GPS) tracking to describe the daily movement patterns and habitat selection of Simmental and Alpine grey lactating cows in the summer pastures of 'Malga Ombretta', located at 1900 m a.s.l. in the Dolomites, eastern Italian Alps (46.424549; 11.880871). During the study period (5 July–5 August 2018), the pastures (approximately 35 ha) were grazed by 14 Simmental and 7 Alpine grey cows (stocking rate = 0.6 LU/ha). Each morning (8.00–9.00 am) the farmer-led the cows to graze in a different section of the pasture until 12.30–1.00 p.m. when cows were left free until they returned spontaneously to the barn (5.30–6.30 p.m.), where they spent the night. Nine Simmental and 4 Alpine grey cows were equipped with GPS collars collecting one position/minute. Recorded positions were edited to exclude the in-barn night periods (final database = 174,208 records) and used to describe grazing patterns at two temporal scales. At the daily scale, total distances walked varied from 2.0 to 8.9 km (mean: 4.7) across dates and increased with longer daily outdoor periods, reflecting the farmer's choices of when/where to move the herd, but showed also a remarkable variation across individual cows, possibly because of individual features. No differences were found between breeds. At the step scale (i.e. for each interval between subsequent locations), slopes and altitudes used were higher, and walking speed was lower, in morning hours when cows were guided by the farmer than in afternoon hours when they were free. Slopes steeper than 30° were very seldom used, possibly indicating a threshold that cows are unwilling to trespass. Alpine grey cows used slightly higher slopes and altitudes and moved slightly faster than Simmental cows in the afternoon, suggesting a better adaption to difficult terrain conditions. Finally, in morning hours cows of both breeds used grassland patches with lower vegetation abundance, as indicated by the satellite-derived Normalized Difference Vegetation Index, than in afternoon hours. In this study, using high-frequency GPS tracking outlined the great variability of grazing patterns, and helped to understand how they can be influenced by human (farmer's decisions) and animal (breed, but also individual) choices.

O044

## Application of Virtual Fencing for the management of Limousine cows at pasture during spring–summer season: preliminary results

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In virtual fencing (VF) system, physical fences are replaced by virtual ones and, when the animals approach the boundaries, they receive a paired stimulus: an audio cue followed by a low electrical shock if animals cross over the fences. The study aims are: i) to evaluate the animal's ability to learn, and then respond positively, to an aversive stimulus; ii) to evaluate the VF efficiency to manage the herd within grazing areas virtually delimited. The study area was a pasture of 20 ha located in Mugello (Tuscany). The experiment took place in spring–summer 2020. Twenty Limousine cows were endowed with VF-GPS collars (Nofence AS, Batnfjordsør, Norway). The trial started with inactive collars to let the animals get acquainted with them. After eight days, an experimental step trial was conducted by progressively widening three pasture areas via VF: i) Trial (T1) of 8 ha for three days; ii) Trial (T2) of 12 ha for four days; iii) Trial (T3) of 13 ha, where boundaries were moved longitudinally to the pasture area, for three days. Number of sounds (S) and shocks (Z) delivered by the collars, length of sounds (ms) emitted without the animals receiving the shocks (Wd), number of escapes from the VF area (E), were the parameters recorded. All data were analyzed using the general linear model of SAS Software. Results show a significant difference either in S (T3 = 9.47 vs. T1 = 29.95 and T2 = 26.40,  $p < .0001$ ) and Z (T1 = 15.88 vs. T2 = 10.00 vs. T3 = 4.13,  $p < .0001$ ). Significant difference was also observed in E among the three sessions (T1 = 3.06 vs. T2 = 1.60 vs. T3 = 0.90,  $p < .0001$ ). Moreover, Wd was significantly reduced in the T3 (T3 = 26319 ms vs. T1 = 65483 ms and T2 = 83413 ms  $p < .0002$ ). Lastly, a reduction ( $p < .0250$ ) in the S/Z ratio was observed between T1 (1.73) and T3 (3.03), with T2 (2.80) being like both. Thus, the lesser S and Z occurred during T3 seem correlated with the animals' positive response to the system. The progressive decrease in E indicates the animals' attitude to stay within the virtual boundaries. Last, cows were more responsive to the audio cue in T3, indeed, thanks to their previous experiences, they learn

to turn back quickly before taking a shock. The differences observed in the studied parameters between the following sessions suggested that animals were able to learn how to correctly interact with VF, and therefore the effectiveness of such technology in managing beef cows at grazing.

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## SESSION 10 – MANAGEMENT STRATEGIES TO IMPROVE ANIMAL HEALTH, WELFARE AND RESILIENCE – III

O045

## Relationship among production traits, milk conductivity, somatic cell score and THI in the Italian Mediterranean Buffalo

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Thanks to iconic traditional dairy products like the Mozzarella di Bufala Campana, the Buffalo production system is increasing its relevance not only in Italy but worldwide. However, the impact of some environmental effects on milk yield and quality deserves additional researches.

The objective of this study was to investigate the relationship among production traits, conductivity, somatic cell score and THI in the Italian Mediterranean Buffalo. Data consisted of 5411 test-day records collected on 808 buffalo cows at a commercial buffalo farm located in Southern Italy. Production traits such as milk yield (MY) in kg/d, protein percentage (PP), fat percentage (FP), somatic cell count (SCC) and electrical conductivity (EC) of milk were used. In order to cope with non-normality SCC were log-transformed into somatic cell score (SCS). Ambient temperature and relative humidity were recorded and used for THI calculation. A set of mixed models were used to investigate the relationship between MY, PP, FP, SCS, EC and THI. The models included the following fixed effects: THI class, the interaction between stage of lactation and parity, year-season of calving and the linear regression on SCS, EC or MY, depending on the analyzed variable. All models included a random animal effect. Moreover, EC's data collected during 3 days before the test day were used to investigate both the relationship between EC<sub>day3</sub> and SCS at test-day and a change in EC on SCS at test-day.



Pluriparous buffalo cows had higher daily milk yields than primiparous until approximately 200 days in milk (DIM). Fat content increased across lactation and, on average, was higher for primiparous than pluriparous. The trend for protein content was specular to milk yield with a nadir around 80 DIM and highest values after 200 DIM. The EC increased across lactation, especially for pluriparous buffalo cows. In primiparous buffalo cows, EC remained stable below 8.7 across nearly all lactation. SCS increased steadily for all parities until 150 DIM and afterwards the rate of increase changed according to parity. All effects included in the models were significant apart from year-season of calving for FP. Interestingly the effect of THI was more evident at low values (THI <55), affecting MY, PP and EC. The regression of EC on SCS at test-day using different EC parameters was always significant except when the EC value was included in the model as slope obtained from a linear regression of EC on the 3-days period.

## O046

### Suckling of dairy calves by their dams: trade-off between ethical and economic sustainability

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The sustainable development of livestock farming systems raises several issues. In dairy production, one of the relevant common welfare concerns is the early separation of calves from their dams. Therefore, research interest in cow-calf contact rearing systems during the milk-feeding period is increasing. This practice was studied mainly from the animal welfare and performance point of view but the economic consequences are still poorly documented. At INRAE experimental farm 'Herbipole', four different suckling strategies were compared to calves separated from dams immediately after birth and fed with an automatic milk feeder until weaning. The suckling strategies consisted in: 1/ dam-calf contact allowed for 20 min before morning milking until weaning at 13 weeks (2017); 2–3/ dam-calf contact allowed 6 or 9 h/day between morning and evening milkings until weaning at 11 weeks (2018 and 2019); 4/ dam-calf contact allowed 6 h/day between morning and evening milkings during 3 weeks, and automatic milk feeder until weaning at 11 weeks (2019). Regardless of the duration of the contact period, the daily milk ingestion of suckling calves is about 11% of their weight until weaning, and it takes five weeks for suckled cows to reach the milk production of control cows after separation from the calves.

During the suckling period, milk yield at the parlor was reduced from 21 to 46 %. Suckling systems also affected milk fat content (from –5.8 to +3.4 g/kg), milk protein content (from +1.0 to +2.3 g/kg) and calves' weight at weaning (from –3.5 to +15.1 kg). Starting from these technical results, we also considered workload and cowshed design to simulate the impact of this innovative practice on the economic viability of intensive, extensive and organic farming systems, in France and Italy.

## O047

### Modeling of milk fatty acids profiles in Holstein dairy cows during early and mid-lactation

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Detection of cows' health problems has become important especially during early lactation when animals experience a stressful period that often impairs their welfare status and productive response. Milk composition analysis has been recognized as a tool to detect cows that are exposed to critical feeding or management situations. In particular, the proportions of different groups of milk fatty acids (i.e. de novo, preformed and mixed) are considered promising biomarkers of cows' welfare and proper feeding. Understanding the trend of these groups of milk fatty acids (FA) in healthy cows could be useful to identify animals in critical health conditions, even before clinical signs are visible. To define reference trends and confidence intervals for the three groups of FA in Holstein dairy cows according to days in milk (DIM), 300 individual milk samples were collected from 10 different herds belonging to Grana Padano (GP; 6 herds) and Parmigiano Reggiano (PR; 4 herds) dairy chains. In each farm, 10 multiparous cows (6 with DIM between 5 and 45 and 4 with DIM >45) in good health status were monthly sampled from August to October 2020. Milk samples were analysed for milk composition and FA profiles with the MIR instrument. Analytical data were statistically pre-processed and modelled including the effects of DIM and type of milk. To model the trend of FA, linear mixed models with nested random effects (i.e. cows and farms) were used to account for repeated measures. The average milk yield was 37.9 and 37.2 kg for GP and PR, respectively ( $p = .54$ ). Milk composition showed a higher fat (3.76 vs. 3.42%) and a lower protein (3.02 vs. 3.17%) content for GP, compared to PR. The percentage of de novo FA (m/m) had an increasing overall trend according to DIM, while the trend was opposite for preformed FA. No significant differences in de novo, mixed and preformed FA trends were detected between GP and PR. However, the percentage of de novo in PR milk tended to be always lower ( $p = .12$ ) than GP, as a possible sign of a greater risk of subacute ruminal

acidosis promoted by PR diets through a potential increase of cows' feed selection activity. Reference intervals for the three groups of FA were estimated according to DIM and type of milk, and they could help farmers assessing cows' state of well-being during early and mid-lactation.

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## O048

### Growth performance and welfare status of gilthead sea bream (*Sparus aurata*) fed low fish meal diets

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Aquaculture nutrition progressed over the past two decades through innovation in feed formulations and the replacement of fish meals with more sustainable protein sources. This study evaluated the growth and welfare of Gilthead sea bream-fed low fish meal diets inclusive of innovative ingredients as partial substitutes for conventional protein-rich plant ingredients (PP). Eight isoproteic and isolipidic diets (45–20% as fed) were formulated by replacing different proportions of PP in a control plant protein-based diet (CV) with insect meal from *Hermetia illucens* larvae (IM), poultry by-product meal (PBM) alone and in combination with IM, red swamp crayfish *P. clarkii* meal (RCM), and dried microalgae blend (MA). A positive control, fish meal-based diet (CF) was also formulated. Diets were tested in triplicate, over 12 weeks. Dietary effects on growth, physiological and immunological functions were evaluated by analysing growth indices, blood chemistry profile, innate immune parameters and liver histology.

Weight gain and feed conversion ratio improved in fish fed diets with 40% protein inclusion from IM (IM40) and PBM (PBM40)

alone or in combination (PBM30IM10) compared to fish fed CV and CF diets ( $p < .05$ ). Growth and FCR value worsened in fish-fed MA diet where 10% PP was replaced with a blend of *T. lutea* and *T. suecica*, compared to control diets ( $p < .05$ ). Intermediate growth performance was observed in fish-fed RCM diet containing 10% protein from crayfish in substitution of the same PP amount. Nutritional and metabolic conditions reflected growth performance, resulting in more favourable in fish-fed PBM40, IM40, PBM30IM10 diets than in those fed MA and RCM diets. In particular, fish-fed MA diet showed higher plasma cortisol and lower protein, lipid and minerals levels compared to the other groups. Moderate hepatic steatosis was observed in fish-fed diets PBM40, IM40, PBM30IM10, consistent with high blood lipid and hepatosomatic index. The respiratory burst was triggered in fish fed IM40 diet compared to those fed CV, IM10, IM40 and P30IM10 diets. No differences in serum peroxidase activity ( $p > .05$ ) were found among groups, due to high individual variability. Overall, the results highlight the suitability of IM and PBM as good alternatives to PP in low fish meal diets for sea bream and the beneficial effects of their combination on growth and fish welfare as well as on improving the sustainability of diet formulation.

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## O049

### Description of the effects of reducing protein content diet on piglets post-weaning intestinal health: a meta-analysis

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Weaning transition is often associated with the occurrence of post-weaning diarrhoea syndrome (PWDS). Among the available nutritional strategies to counteract the PWDS, the reduction of dietary crude protein (CP) level in the weaning diet has been intensively exploited, mainly thanks to the availability of industrial feed-grade amino acids for livestock feed formulas. While the effect of dietary CP reduction on economic cost and environmental impact has been deeply investigated, less is known about the effect on gut health. Therefore, the aim of the present meta-analysis is to depict the effect of a reduction in dietary CP on intestinal parameters related to gut health, besides growth and diarrhoea in weaning piglets.

To this end, a structured search was performed on PubMed. A total of 71 articles were found, of which 26 (published between 2006 and 2020) were selected. Parameters (growth, faecal score,



intestinal morphology, markers of inflammation, bacterial metabolites, intestinal pH, microbiota diversity) were extracted, expressed as a percentage of the control diet and analysed using a general linear model that included, the trial, the reduction in CP (expressed in percentage points) and the dLys/CP as factors, using Minitab® software. The decrease in CP was associated with a decrease in fecal score ( $p = .002$ ), ammonia ( $p < .0001$ ), pH ( $p = .039$ ), total SCFAs ( $p = .027$ ) and some biogenic amines including cadaverine ( $p = .034$ ) and putrescine ( $p = .030$ ) in the intestinal contents. Growth of pigs, intestinal crypt depth, villus height and microbiota diversity remained unchanged while the intestinal expression of TLR4 was significantly reduced ( $p < .0001$ ) by a reduction of dietary CP.

In conclusion, this study supported that a reduction in dietary CP drops an excess of bacterial protein fermentation in the gut resulting in a decrease in risk of diarrhea and beneficial effects on the gut health of weaning piglets.

## O050

### Preliminary investigations on functional longevity in Italian Mediterranean Buffaloes (*Bubalus bubalis*)

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Productive life refers to the animal's ability to not be culled due to low production, low fertility or health reasons. In the past, longevity was not considered as a breeding objective because such information was available only when the animal was culled. However, longevity in livestock has been experiencing growing economic importance over the past two decades, including the Italian Mediterranean Buffalo (IMB). A survival analysis applying proportional hazards models was used to investigate the non-genetic factors affecting the longevity of the IMB. The data included records of reproductive, productive and type traits provided by the Italian National Association of Buffalo Breeders. Data from 180,155 buffalo with the first calving from 1992 to 2019 were used. Functional survival was defined as the number of days from the first calving until culling and the censored animals until the date of the last functional control. The Weibull model included time-dependent effects of herd-year-season, the year and season of calving, parity and milk production and yield as within-herd deviations, as well as time-independent effects of age at first calving, year of birth, herd size, late production in the first 60 days and morphological traits under teat and feet and legs. The average duration of productive life was 1378 d. Regarding the effects, the herd-year season had a strong effect on the risk of culling while the year of birth and year of calving had the greatest effect on longevity,

followed by production-related effects. The age at first calving clearly showed an intermediate optimum, with corresponding lower risk values between 35 and 38 months. Looking at morphological traits, females with lower scores in the under teat and feet and legs condition showed the greater culling risk. The variation in herd size also had an effect on the risk ratio, being the intermediate classes with less risk. The parity shows a completely linear and inverse trend, in which the risk decreases toward higher lactation classes. Regarding the season of calving, the highest risk was shown in the months with the highest environmental temperatures. In summary, the survival model applied in this study described that longevity of IMB was influenced by many of the non-genetic factors studied while accounting for important time-dependent and independent explanatory variables.

#### Acknowledgements

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## SESSION 11 – GENOMIC AND PHENOMIC TOOLS TO IMPROVE LIVESTOCK ANIMALS – II

## O051

### Mining livestock genomes for a One Health perspective in the management of animal genetic resources

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Viruses constantly and silently circulate, emerge and re-emerge in human and animal populations causing mild to severe diseases. Some examples are derived by coronaviruses and include the severe acute respiratory syndrome coronavirus (SARS-CoV-1) and the recently emerged SARS-CoV-2, the Bovine coronavirus (BCoV) the Porcine Epidemic Diarrhea Virus (PEDV) and the Middle-East respiratory syndrome coronavirus (MERS-CoV). Several animal species can act as reservoirs of these viruses that have already demonstrated the ability to easily cross species barriers. This suggests that in livestock and companion animals, within-species genetic variability determining susceptibility or resistance to viral infections should be evaluated as part of a global One Health perspective to reduce the risk that these infecting agents may pose for animal and human populations. In addressing this challenging objective, we investigated the genetic variability of hundreds of candidate genes of the host genomes that can serve as receptors for priming the infection of viruses or are involved in the subsequent progress of the viral diseases, including the well-known ACE2, ANPEP, DPP4 and TMPRSS2 involved in the progression of coronavirus infections. For this

purpose, we mined a few thousands of sequenced genomes of four main livestock species (*Bos taurus*, *Sus scrofa*, *Gallus gallus* and *Oryctolagus cuniculus*), retrieved from public sequence repositories or obtained from newly generated datasets. We then in silico modelled the relevance of the identified DNA variants in conferring potential differences in susceptibility to viral diseases, considering a comparative analysis with the homologous human gene and protein structure. At the inferred protein level, some of the detected within-species mutations in the host genes are expected to modify the virus-host interaction dynamics that could alter the response of the host to the infections. Overall, the obtained results can be useful to establish risk evaluation systems in a One Health approach that includes within-species genetic diversity as potential risk factors to be considered in selection and conservation programs of animal genetic resources.

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## O052

### Machine learning and variable selection methods for Fourier transform infrared prediction in Holstein cattle

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The usefulness of Fourier-transform infrared (FTIR) spectroscopy in dairy cattle has prompted efforts to develop new and improved statistical methods, to accurately predict different phenotypic information. Hence, this study was carried out to compare the predictive ability of two machine learning methods, random forest (RF), and gradient boosting machine (GBM), and penalized regression against PLS regression for predicting three different gold-standard phenotypes in Holstein-Friesian cattle under two cross-validations (CV) scenarios. The dataset comprised phenotypic information from 471 Holstein-Friesian cows, and three target phenotypes i) body condition score (BCS), ii) blood -hydroxybutyrate (BHB, mmol/L), and iii) kappa-casein expressed as a percentage of nitrogen (k-CN, %N). The data set was split considering two CV scenarios namely 'samples-out' randomly split in 10-folds and 'herd/date-out' within which the population

was randomly assigned as training and testing set based on herd/date from which the samples were collected. The predictive ability of the different statistical methods was assessed by Pearson's correlation ( $r$ ) between the observed and predicted phenotypes, root mean square error (RMSE). The slope of the linear regression of the observed and predicted values in each model/cross-validation scenario for the phenotypic traits evaluated was used to assess the model unbiasedness. Using 'samples-out' CV the GBM increased the predictive ability, defined as Pearson correlation, by 7.46%, RF by 5.42%, and elastic net (EN) by 3.29% and statistically differed ( $p < .05$ ) against the PLS model. On the other hand, considering a 'herd/date-out' CV GBM and RF model tended to outperform PLS regression in predictive ability around 7%, and EN around 2.7%. Although machine learning techniques outperformed PLS in 'herd/date-out' CV, no significant differences were observed in terms of predictive ability due to the large standard deviation observed in predictions. Leaving Herd/Date-out CV, records from a different farm, resulting in an 8% reduction in model performance compared with 'samples-out' random CV. Overall, GBM achieved the highest accuracy, with the lowest RMSE and less unbiased (slope near 1) of FTIR predictions for different phenotypic traits across the CV scenarios and provided a promising method to calibrate FTIR spectral data to predict novel phenotypes.

## O053

### Noncoagulating sheep milk characterization through Fourier-Transform IR spectroscopy

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Milk coagulation ability is important for the sheep dairy industry because all sheep milk is destined for cheese processing. The aim of this work is to investigate relationships between milk composition, physical properties and Fourier-Transform Infra-Red (FTIR) spectra and the occurrence of non-coagulating (NC) sheep milk. Milk was collected from 1018 Sarda ewes farmed in 47 flocks located in Sardinia. Measured variables were milk yield, composition and coagulation properties (MCP): rennet coagulation time (RCT), curd firming time (k20) and curd firmness (a30). The FTIR spectra were also acquired. About 9% of samples did not coagulate within 30 min. A linear mixed model (LMM) analysis was performed to test whether milk composition differs between coagulating and NC samples; fixed effects of parity (3 levels), DIM (5 dim classes), lambing month (4 levels) and the random effect of flock-test-date (67 levels) were included in the model. Principal component (PC) analysis was carried out on FTIR

spectra. PC scores were used as the response variables with the same MLM. Coagulating and NC showed differences for all traits ( $p < .01$ ), except for milk yield and fat%. NC samples showed higher values for protein, casein, somatic cells score (SCS), chloride and pH, and lower for lactose and urea, respectively. Seven PC explained 90% of the variance. Interestingly, PC2 and PC3 were associated to the coagulation class ( $p < .05$ ). Two regions (2290–2657, 3663–3732  $\text{cm}^{-1}$ ) were highly correlated with PC2. PC3 loadings showed values higher than 0.5 in two spectral regions (1096–1469 and 2668–2826  $\text{cm}^{-1}$ ). High loadings of PC2 and PC3 correspond to regions highly correlated with fat and casein, and with lactose and chloride contents, respectively, including a large region (from 1780 to 2825  $\text{cm}^{-1}$ ) often not used in prediction models as scarcely informative. Significant differences in the milk composition and FTIR spectra between normal and NC milk samples were observed in the present study, likely related to the variables often associated with the mammary gland health status, that seems a very important factor influencing milk coagulability. The study of the individual milk FTIR spectra is crucial in order to predict the coagulability of future samples. This may contribute to the development of useful applications for the dairy sheep industry.

## O054

### Genomic prediction of VNN resistance, body weight, cortisol concentration and antibody titer in European sea bass (*Dicentrarchus labrax* L.)

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In European sea bass (*Dicentrarchus labrax* L.), traits like viral nervous necrosis (VNN) mortality (MORT), post-stress cortisol concentrations (HC), antibody titer (AT) against nervous necrosis virus (NNV) and body weight (BW) show significant heritability estimates which make selective breeding based on genomic data a possible option for improvement while overcoming difficulties related to individual phenotyping.

An experimental population ( $N = 650$ ) generated by a commercial sea bass broodstock was subjected to a confinement stress procedure followed by a VNN challenge test.

The fish were phenotyped for MORT, HC, AT, and BW and genotyped with a genome-wide SNP panel (16,075 markers).

Performances of three Bayesian linear regression methods (Bayes B, Bayes C and Bayesian Ridge Regression) and of a machine learning algorithm (Random Forests, RF) were compared in the prediction of the estimated breeding value (EBV) or the phenotype for the investigated traits. The accuracy in the prediction of such methods was evaluated by computing the correlation between the predicted and observed EBV (phenotype) in 16 independently generated 5-fold cross-validations. Several metrics were used to also evaluate the degree of misclassification when the genomic predictions generated by the methods were used to classify the phenotype for MORT.

Bayesian methods exhibited greater prediction accuracies than RF. The prediction accuracy of the EBV for MORT was approximately 0.90 whereas the prediction accuracies of the EBV and the phenotype were 0.88 and 0.22 for HC, 0.76 and 0.26 for AT, 0.71 and 0.38 for BW, respectively.

When the genomic prediction of the EBV for MORT was used to classify the phenotype for the same trait, it showed better classification performances when compared to the genomic prediction of the MORT phenotype. The predictions obtained for HC, AT and BW did not provide any added value in the classification of MORT.

Difficulties arising from individual phenotyping of traits like MORT, HC and AT make the implementation of traditional selective methods in European sea bass breeding programs troublesome. The prediction of genetic merit based on genomic models may help in overcoming such difficulties. However, the results of this study need to be validated in future investigations based on a larger experimental population.

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## O055

### Genomic prediction of dry-cured ham weight loss in Italian heavy pigs

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Large ham weight losses (HWL) during dry-curing lead to a loss of marketable product and penalize the quality of the dry-cured ham. Genetic selection for reducing HWL is challenging because measures of HWL require individual traceability of hams throughout the dry-curing process. Also, they can be acquired only after 12 months of seasoning, resulting in long generation intervals. Infrared spectroscopy has recently enabled cost-effective, high-throughput phenotyping for HWL by providing early predictions of HWL that are highly genetically correlated with the actual HWL. The objective of this study was to assess the accuracy of



genomic prediction models for infrared-predicted HWL (IR-HWL) in a heavy pig population.

Infrared prediction of HWL at the end of dry-curing was obtained in 1056 crossbred pigs enrolled in the sib-testing program of the Goland C21 sire line (Gorzagari, Fonzaso, Italy). The infrared prediction model included the following explanatory variables: i) on-site visible-infrared spectra collected on the transversal section of the subcutaneous fat of raw ham, ii) carcass traits (carcass weight, backfat depth, lean meat content, weights of the raw hams), iii) ham quality traits (ham subcutaneous fat depth, and linear scores for ham round shape, subcutaneous fat thickness, and ham marbling).

Pigs were genotyped with a low-density GGP-Porcine BeadChip and imputed to 60K. After editing, 29,570 SNPs were retained for the genomic prediction models. Accuracy of genomic predictions for IR-HWL was evaluated by using a 5-fold cross-validation scheme and considering five models: Bayesian Ridge Regression, Bayes A, Bayes B, Bayes C, and Bayesian Lasso, and two types of response variables: estimated breeding value (EBV), and phenotype adjusted for fixed effects.

The pedigree-based heritability of IR-HWL was 0.31, whereas its genomic heritability was 0.21. The accuracy of genomic prediction of IR-HWL was moderate to high and prediction of genomic breeding values from EBV was more accurate than from adjusted phenotypes. All the models yielded very similar results, with prediction accuracies ranging from 0.69 to 0.70 when EBV was used as a response variable, and from 0.51 to 0.52 when using the adjusted phenotypes. Results indicate that genomic prediction models have the potential to be used in future selection programs aiming at reducing HWL.

of Wooden Breast meat. For this purpose, a total of 48 P. major muscles have been selected from the same flock of Ross 308 chickens (males, 48 d of age, 2.8 kg at slaughter), packaged under vacuum, and divided into 4 experimental groups ( $n = 12$ /each): NORM (unaffected muscles), WB (muscles affected by Wooden Breast myopathy), NORM-T and WB-T (unaffected and affected muscles, respectively, subjected to LFU treatment). LFU treatment was performed at 24 kHz, 300 W for 40 min at  $4 \pm 1$  °C. Meat colour, pH, water holding capacity, protein denaturation enthalpy (measured through Differential Scanning Calorimetry) as well as 40% compression force were assessed immediately after the treatment (T0) and after 5 (T1) and 10 (T2) days of storage (2–4 °C). Overall, pH, colour and water holding capacity of WB muscles were not affected by LFU treatment, while NORM-T muscles showed significantly higher lightness, yellowness (at T0,  $p < .01$ ) and drip loss values (at T1,  $p < .05$ ) if compared to their untreated counterparts. As concern texture, a tendency ( $p = .07$ ) has been observed after 10 days of storage, with WB-T showing lower compression forces if compared to WB (9.2 vs. 11.9 kg), while LFU did not affect the textural traits of NORM-T muscles. Accordingly, at T2, total protein denaturation enthalpy was found to be significantly reduced in WB-T samples if compared to WB (2.50 vs. 2.78 J/g,  $p < .05$ ). In this regard, the contribution of stromal proteins (namely collagen) to the total denaturation enthalpy was found to be significantly lowered in the WB-T group (–20.6%,  $p < .001$ ). Taken together, these results suggest that after 10 days of storage LFU treatment might have tenderized Wooden Breast meat by weakening its connective tissue. However, further treatment conditions should be tested to achieve a greater and faster tenderization process, without affecting neither the appearance nor the water holding capacity of meat.

## SESSION 12 – FOOD PRODUCTION AND PROCESSING

### O056

#### Innovative approach for the tenderization of Wooden Breast meat in broilers

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Wooden Breast is a well-known myopathy occurring in fast-growing broilers and characterized by an extreme hardening of the *Pectoralis major* muscle, which is usually downgraded due to its unwilling visual aspect and impaired technological properties. In this regard, the application of innovative techniques might represent a valuable tool to improve textural traits of Wooden Breast meat and mitigate the economic losses related to the downgrading of affected muscles. Thus, this study aimed at exploring the effect of Low-Frequency Ultrasounds (LFU) on the tenderization

### O057

#### Effects of rosemary powder and salt additions in goose meat burgers

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The effects of freeze-dried rosemary powder and salt addition in goose burgers on the microbiological and chemical-physical quality were evaluated. Four different formulations of burgers were prepared (meat only – control; 0.25% freeze-dried rosemary powder – R; 1% salt – S; 0.25% freeze-dried rosemary powder + 1% salt – RS). The shelf life of burgers was tested on samples stored at 4 °C for 6 days. Microbiological analyses included evaluation of total mesophilic bacterial load, total psychophilic bacterial load, *Pseudomonas* spp., *Brochothrix thermosphacta*,

Enterobacteriaceae, lactobacilli and yeasts and moulds. Burgers were chemical-physical analysed for the evaluation of pH, colour parameters (external-internal), drip loss, antioxidant capacity, lipid and protein oxidation. From a microbiological point of view, the development of psychrophilic microorganisms, *Pseudomonas* spp. and *Brochothrix thermosphacta* was lower in burgers containing rosemary powder than the control ( $p < .001$ ), confirming their bacteriostatic action. The addition of salt, however, caused a partial variation in colour and an increase in the values of lipid (TBARS) and protein (carbonyls) oxidation. Rosemary had an essential effect on colour, partially modifying L\*, a\* and b\* parameters ( $p < .01$ ), and had a strong effect on the antioxidant capacity of the products (ABTS, DPPH and FRAP values,  $p < .05$ ). Rosemary powder modified the burgers aspects with a wide decrease ( $p < .01$ ) of a\* (19.83 for C vs. 16.87 and 16.03 for R and RS, internal) b\* (11.42 for C vs. 10.93 and 9.96 for R and RS, internal) and parameters due to its natural green pigments. The results obtained showed that the use of these ingredients, salt and rosemary, can result in better shelf life in this type of product. Indeed, the use of the two ingredients in combination showed to have a combined bacteriostatic and antioxidant effects. Moreover, RS burgers showed to be positively affected by rosemary powder addition ameliorating the negative effects of salt. Antioxidant capacity of RS burgers (ABTS, DPPH and FRAP) were similar to R burgers and higher than C and S burgers ( $p < .05$ ), while lipid ( $S \geq C \geq RS > R$ ,  $p < .001$ ) and protein ( $S > C > R = RS$ ,  $p < .05$ ) oxidation were partially affected. Goose meat products are still poorly studied, both in terms of nutritional and quality characteristics, such as shelf life. The results obtained represent a base on which more in-depth research could be performed.

## O058

### Effects of cooking with Superheated Steam on organic Maremmana breed beef

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Generally, meat is cooked before consumption. The purpose of cooking is to make meat more digestible, palatable, and microbiologically safe. However, cooking is a critical point in industrial and domestic meat processing. In fact, cooking methods, as well as time-temperature parameters, can lead to undesirable changes. Wrong heat treatments produce a reduction in nutritional value, vitamins and minerals losses, lipid and protein oxidation, and the production of some toxic compounds. Meat from grazing animals contains a high percentage of unsaturated fatty acids, as well as a high level of vitamins that are typical of this kind of breeding system. Thus, the SHS cooking system could be useful

to process meat with high nutritional value. The objective of this study was to evaluate the effects of two different drying treatments on organic beef of the Maremmana breed: superheated steam (SHS) and traditional steam oven (C). Total lipids content, fatty acid (FA) composition and lipid oxidation analyses were investigated. Concerning the last point, the following analyses were made: thiobarbituric acid reactive substances test (TBARS), total cholesterol oxidation products (COPs), 7-ketocolesterol, volatile aldehydes, and alcohols. The cooking method affected none of the parameters of the proximate composition of meat. This is a quite surprising result, as other authors demonstrated SHS effective in decreasing lipid content. The explanation of our findings could be that organic meat shows a high water-holding and this limits the ability of SHS. Concerning the lipid peroxidation, SHS limited significantly ( $p < .05$ ) the oxidation of polyunsaturated FA (TBARS was 0.69 vs. 1.05 mg/kg of meat, in SHS and C respectively) and monounsaturated FA (hexanal was 433.06 vs. 1112.71 ppm, in SHS and C respectively). The SHS cooking method affected also the content of volatiles aldehydes (722.27 vs. 1582.28 ppm, in SHS and C respectively;  $p < .05$ ) and alcohols (477.52 vs. 880.51 ppm, in SHS and C respectively;  $p < .05$ ). Finally, the cooking method did not affect both COPs and 7-ketocolesterol. The preliminary results of the research confirmed the effectiveness of SHS as a safe method to cook meat and to preserve its nutritional value. However, it would seem that the effectiveness of the treatment depends on meat composition. To express the whole potentiality of this method, further studies to find the best set-up of cooking parameters are needed.

## O059

### Innovative feeding strategies for the heavy pig: effects on technological and quality traits of dry-cured hams

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To explore the influence of 4 feeding strategies on the quality of the seasoned dry-cured ham, 116 Goland C21 pigs, barrows and gilts of 90 kg body weight (BW), were divided into 4 groups, assigned to one of four treatments, and housed in 8 pens equipped with automated feeding stations. The pigs of the conventional group (CONV) were fed restrictively medium-protein feeds till 170 kg BW, and hence slaughtered at 265 d of age. With the LP treatment, the pigs were fed restrictively low protein feeds till 170 kg BW, and hence slaughtered at 278 d of age. The other two



groups were fed ad libitum high protein feeds (HP), but one group was slaughtered at 170 kg BW (HP170; 237 d of age), and the other at 265 d of age (HP9m; 194 kg BW). According to the Prosciutto Veneto production guidelines the hams were dry-cured and seasoned for 607 d. Hams were weighed before and after seasoning and deboning. Seasoned hams were scored by an expert for round shape (1 to 3, 1 = low; 3 = high), fat cover depth (1 to 3, 1 = low; 3 = high), fat hardness (1–3, 1 = low; 3 = high), fat color (1 to 3, 1 = white, 3 = yellow); marbling (1 to 5, 1 = low; 5 = high), meat colour (1 to 5; 1 = pale; 5 = dark), hardness at touching (1 to 5, 1 = soft, 5 = hard). Piercing, with a horse-bone needle, was practised to score the hams for meat hardness (1 to 5, 1 = soft, 5 = hard) and detect off-odours in different anatomical points. Data were analysed with a model that considered sex (1 d.f.), treatment (3 d.f.) and sex X treatment as fixed factors. Contrasts were run to compare CONV with the other groups. LP had lower round shape ( $p = .046$ ), greater veining ( $p = .007$ ), and tends to have greater marbling ( $p = .06$ ), and meat hardness at piercing ( $p = .07$ ) compared to CONV. The HP170 hams had greater veining ( $p = .037$ ), and lower hardness at piercing ( $p = .036$ ) than CONV. Major differences were detected between CONV and HP9m. The HP9m hams had less seasoning weight losses (269 vs. 298 g/kg,  $p = .004$ ), and were heavier (8.32 vs. 7.08 kg;  $p < .030$ ) than the CONV hams. In addition, they had greater fat cover depth (3.97 vs. 2.72,  $p = .004$ ), greater veining (2.25 vs. 1.64,  $p = .014$ ), and marbling (3.09 vs. 2.44,  $p = .045$ ), but paler meat colour (1.55 vs. 2.33,  $p = .030$ ) and lower meat hardness at piercing ( $p = .036$ ) than CONV. Off odours at piercing were always absent. These results will impact the conventional rearing practices because of the expected changes in the production disciplinary.

## O060

### Goat milk-based predictive formulas for different measures of cheese yield

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The aim of the study was to develop predictive formulas for cheese-yield (%CY) traits in goats based on milk composition. The %CY traits were run in duplicate from 600 goats, by using individual laboratory cheese-making procedures, for a total of 1200 observations. Goats were reared in 36 herds and belonged to six breeds [Saanen ( $n = 99$ ), Murciano-Granadina ( $n = 89$ ), Camosciata delle Alpi ( $n = 96$ ), Maltese ( $n = 101$ ), Sarda ( $n = 122$ ) and Sarda Primitiva ( $n = 76$ )]. Fresh %CY (%CYCURD),

total solids (%CYSOLIDS) and water retained (%CYWATER) in fresh cheese were used as response variables. Firstly, single/multiple linear regression models were tested via different combinations of milk components (fat, protein, casein) and indirect udder health indicators (UHI; lactose, SCS, pH, LBC). The two %CY observations within an animal were averaged and a cross-validation (CV) scheme was adopted, in which 80% of observations were randomly assigned to training (TRN), and 20% to validation (VAL) set. The procedure was repeated ten times to account for sampling variability. Fitting statistics of the models were assessed by the coefficient of determination of validation (R2VAL) and the root mean square error of validation (RMSEVAL). Further, the model presenting the best prediction accuracy in CV was used in a second analysis to assess the repeatability (REP) of the laboratory cheese-making procedure, where the first %CY observation within animals was used to predict the second %CY observation (within animal validation, WAV). Finally, a stratified CV (SCV) was applied to assess the formula prediction accuracy across goat breeds, in which five breeds were included in TRN, and the remaining one in the VAL set, using the average of the two observations within the animal. In the CV, the formula with the best prediction accuracies for all %CY traits included fat, casein and UHI (R2VAL = 0.65, 0.96, and 0.23 for %CYCURD, %CYSOLIDS and %CYWATER, respectively). The WAV procedure showed R2VAL higher than those obtained in CV, evidencing the high REP of the cheese-making procedure. In the SCV, large differences of RMSEVAL were observed among breeds (ranging between 0.30 and 1.57) suggesting that breed is a critical factor to be considered when using formulas to predict %CY traits. These formulas may be useful to monitor milk composition and quantify the influence of milk traits in the composite selection indices of specific breeds, or for the direct genetic improvement of cheese production.

## O061

### Effect of species on oxidative stability of ozone-treated milk cream

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This study aimed to evaluate the effect of species (cow and buffalo) on oxidative stability of milk cream after different exposure times to ozone treatment, using peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) methods. The colorimetric characteristics of ozone-treated milk cream were also detected by spectrophotometric and CIE (Commission Internationale de l'Eclairage) L\*a\*b\* methods. For each species, cream (~45% w/w fat) was obtained from the higher-fat layer

after separation for centrifugation of fresh skim milk. For ozonation treatments, milk cream samples were divided into portions (3g) spread homogeneously as a thin layer (0.2 cm) on glass dishes ( $\varnothing$  5 cm) and placed in a box handmade with a total volume of 2730 cm<sup>3</sup> (dimensions: 21 × 13 × 10 cm). Ozone treatment was performed at room temperature using a generator with a flow of 300 mg O<sub>3</sub>/h, for six exposure times (10, 20, 30, 40, 50, and 60 min) under the continuous stream. One milk cream sample not treated was used as a control. The statistical analysis was performed by means of ANOVA, using the GLM procedure of SAS. Overall, the ozonation process significantly affected lipid oxidation of milk cream ( $p < .05$ ) which increased as the ozone exposure time increased. In particular, PV and TBARS values ranged from 0.208 ( $t = 0$  min) to 3.858 ( $t = 60$  min) meq O<sub>2</sub>/kg fat and from 1.233 ( $t = 0$  min) to 7.524 ( $t = 60$  min) mg MDA/kg, respectively. Considering the species, before ozone treatment, buffalo milk cream showed the lowest PV and TBARS values (0.188 meq O<sub>2</sub>/kg fat and 1.051 mg MDA/kg, respectively;  $p < .05$ ). Furthermore, at the end of O<sub>3</sub>-exposure time, PV was significantly higher in cow milk cream compared to buffalo milk cream (4.086 and 3.623 meq O<sub>2</sub>/kg fat;  $p < .05$ ), whereas no difference was detected for TBARS value (7.587 and 7.462 mg MDA/kg for cow and buffalo samples, respectively). Overall, the colour characteristics ( $L^*$ ,  $a^*$ , and  $b^*$  values) of milk cream samples decreased as the ozone exposure time increased ( $p < .05$ ). Considering the species, at the end of O<sub>3</sub>-exposure time, buffalo milk cream showed the highest colorimetric values ( $p < .05$ ). The finding of this study highlighted that, although ozone treatment is an extremely useful method to reduce the microbiological load of foods, the duration of ozone treatment and the species are key factors to determine the capacity of the ozonation process to balance microbial and chemical quality of foods.

## SESSION 13 – MONITORING AND MITIGATING IMPACTS ON ENVIRONMENT – I

### O062

#### Environmental impact of Italian beef suckler to finish system

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At COP21 countries agreed to keep the increase of the global temperature below 2 °C and to cut at least 30% of the greenhouse gas (GHG) emissions by 2030. In order to reach this goal, the beef sector, similarly to any other human activity will be required to implement effective and high-impact measures to drive down total GHG, sustain biodiversity and promote carbon sequestration. The aim of this study was to evaluate the environmental impact

in 17 Italian beef sucklers to finish farms located in Piemonte and to identify the factors that can contribute to the reduction of the emission intensity by improving on-farm production and through better input and resource utilisation. This evaluation was carried out using a multi-indicator approach, which combines environmental impact categories computed with a cradle-to-farm gate Life Cycle Assessment (LCA) with productive, reproductive and food efficiency indicators. LCA was performed utilizing CAP2ER<sup>®</sup> (Niveau 2) developed at the French Institut de l'Elevage. The functional unit was 1 kg of live weight gain (LWG) and the environmental categories were: global warming potential (GWP), acidification potential (ACP), eutrophication potential (EUP) and energy consumption (EC). Feed conversion ratio (FCR) was calculated as the ratio between animal feeds used in the farms (kg DM) and total live weight produced (kg/year). Multiple regression analysis was applied to determine the farms' characteristics that best explain the LCA results. Statistical analyses were performed using R, software version 3.4 (R Development Core Team, 2008) and significant differences were declared at  $p < .05$ . Farms' mean size was 54 ± 32 ha and 215 ± 184 places; the average number of suckler cows was 65 ± 41 and the total live weight production was 59 ± 81 T/year. Estimated GWP was 20.14 ± 6.77 kgCO<sub>2</sub>eq/kg LWG; ACP was 0.07 ± 0.03 kg SO<sub>2</sub> eq/kg LWG; EUP was 0.10 ± 0.07 kg PO<sub>4</sub><sup>3-</sup> eq/kg LWG and EC was 39.20 ± 17.76 MJ/kg LWG. Results of multiple regression analysis showed that the variables that most influence the GWP are the live weight at slaughter and ADG of heifers and FCR ( $R^2 = 0.71$ ,  $p < .001$ ).

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### O063

#### Polluting compounds in Po Valley dairy system: amount, variation and some determinants in Veneto region farms

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This study, part of the wider project Mitigactions (Rural development program 2014–2020, Regione Veneto), aimed to quantify the environmental footprint (Life Cycle Assessment) of milk production in Veneto region specialized dairy farms and to identify its main determinants to address specific mitigation strategies. Data originated from 28 dairy farms (103 ± 95 lactating cows, 9092 ± 1235 kg fat protein corrected milk - FPCM/cow in 305-d lactation). System boundaries included herd and manure management, on-farm feedstuffs production, purchased

feedstuffs and materials. Global warming (GWP), acidification (AP), and eutrophication (EP) potentials were assessed as impact categories. The functional unit was 1 kg FPCM, with biophysical milk vs. meat allocation (IDF method). Mean impact values per 1 kg FPCM were  $1.10 \pm 0.15$  kg CO<sub>2</sub>-eq,  $17.1 \pm 2.5$  g SO<sub>2</sub>-eq and  $6.0 \pm 1.0$  g PO<sub>4</sub>-eq. The greatest contributors to GWP, AP, and EP were enteric fermentation (43% of total emission), manure storage (50%) and feeds production (65%) phases, respectively. General linear models (GLM) were used to investigate separately the effects of farm management (MAN\_F), diet (DIET\_F), and animal response factors (ANI\_F) on the variation of impact categories. Among MAN\_F, both GWP and AP linearly decreased ( $p < .10$ ) at increasing stocking rate (SR). All impact categories were quadratically related with feed self-sufficiency (FSS), and the lowest emissions were associated with medium FSS (around 50% of dietary DM) compared to low or high FSS (average dietary DM 20 and 70%, respectively). Among DIET\_F, GWP linearly decreased at increasing the amount of dietary cereal silages. Acidification potential and EP linearly decreased at the decreasing of dietary CP content ( $p < .05$ ), which was in turn quadratically related to GWP. Among ANI\_F, both GWP and EP were significant influenced by FPCM ( $p < .05$ ). Namely, they decreased moving from low to medium productivity herds (7800 vs. 9100 kg average FPCM, respectively), whereas high productivity herds (average FPCM 10800 kg) had mean GWP and EP comparable to medium productivity ones. GWP linearly increased at increasing culling rate and when average age at first calving of the herd exceeded 24 mo. AP and EP were lower in herds characterized by age at first calving  $\leq 24$  mo. In conclusion, some farm, diet, and animal management factors were significant related to impact categories and can be used as targets or aims when considering mitigation strategies of dairy farms.

emissions (EME) using milk Fourier-transform infrared (FTIR) spectroscopy. Therefore the objective of this study was to assess the effects of breed, farm intensiveness, and cow productivity on enteric methane emissions directly predicted at the population level using FTIR-spectra (Direct-IR) and predicted indirectly using the milk fatty acids (Indirect-IR). Data included 1,759,706 test-day (TD) records from 6430 herds with 291,129 lactations of dairy cows (Holstein (HO), Brown Swiss (BS), Simmental (SI) Alpine Grey (AG)) during 8 years. The average daily milk energy output (dMEO, MJ/d) was used to classify the herds according to their intensiveness level (HL, factor of 5 levels), and the cows according to their productivity within herds (CL, factor of 5 levels), using a linear mixed model. The EME traits studied were daily methane production (d CH<sub>4</sub>, g/d), methane yield per unit dry matter intake (CH<sub>4</sub>-DMI, g/kg), and methane intensity per kg of corrected milk (CH<sub>4</sub>-CM, g/kg), of fresh cheese (CH<sub>4</sub>-FC, g/kg), and of cheese solids (CH<sub>4</sub>-CS, g/kg). A linear model was fitted including the effects of parity, days in milk (DIM), year, month, HL, CL and breed, and the interactions between HL\*Breed y CL\*Breed. All EME traits showed a sinusoidal circannual pattern with a nadir value in spring and a zenith value in autumn. All EME traits showed an increase during the early lactation and d CH<sub>4</sub> a strong decrease after the peak of lactation. The methane yield CH<sub>4</sub>-DMI decreased strongly with increasing HL in the case of HO and SI cows, but not for BS and AG cows and decreased much less with increasing CL. Also, the 3 methane intensity traits decreased more with increasing HL than CL. Daily methane production increased with increasing HL and CL much more using indirect-IR than Direct-IR. In conclusion, the traits studied related to methane emissions have different trends and patterns depending on the breed and the interaction with the herd intensiveness and cow milk production.

## O064

### Effect of breed, farm intensiveness and cow productivity on predicted enteric methane emissions predicted at the population level

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The relationship between rumen fermentation and milk fatty acid profile allows the indirect prediction of enteric methane

## O065

### Effect of summer cooling of dairy cows on carbon and water footprint intensity of milk

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The study was aimed at assessing the effects of summer cooling operations (SCOs) on carbon (CF) and blue water footprint (bWF) intensity of cows' milk. Six dairy farms equipped with fans and sprinklers for SCOs were enrolled in the study. The analysis was carried out using a cradle-to-farm gate Life Cycle Assessment approach and one kg of fat and protein corrected milk (FPCM) as a functional unit. The information acquired was the number of heads, milk yield, housing, cooling and manure management,



feeds, crops activities, energy consumption, transports and water utilization for crop production and animal management. Methane from enteric fermentation and manure management, nitrous oxide from manure and soil management, CO<sub>2</sub> from energy consumption and soil organic carbon sequestration (C-sink) were considered and expressed as kg CO<sub>2</sub>eq. The bWF embedded the water used for crops irrigation, sprinklers, cleaning parlour equipment and drinking of animals and was expressed as litres. The effect of SCOs on CF and blue WF intensity was evaluated considering the summer-winter (SW) production rate that measures the efficiency of SCOs on milk yield. Specifically, the CF and bWF were modelled using the value of SW referring to no cooling conditions which was then compared to the CF and bWF of the farms under study.

The CF intensity was 1.247 kg CO<sub>2</sub>eq/kg FPCM and it ranged from 1.014 to 1.619 kg CO<sub>2</sub>eq/kg FPCM. When the C-sink was included, the range of CF lowered from 0.972 to 1.576 kg CO<sub>2</sub>eq/kg FPCM with an average impact of C-sink of about -1.3%. The higher sources of emissions were enteric fermentation and feed production (about 81%). The CF modelled for the no cooling scenario resulted in higher (0.8%) than the cooled one. The energy for SCOs contributes only to the 0.4% of the total CF. The bWF intensity was 161 litres/kg FPCM and it ranged from 35 to 276 litres/kg FPCM. The irrigation (both on-farm and extra-farm feed) was the main contributor (98%), whereas the water for SCOs accounted for 0.2% of total bWF. In general, SCOs of the selected farms reduced the bWF by 1.3% compared to the modelled scenario of no cooling operations.

Summer cooling management of dairy cattle is an important adaptation strategy to warming climate scenarios and, although fans and sprinklers increase energy and water consumption, the improvement of animal welfare has a positive effect on milk production and therefore on carbon and water footprint intensity.

## O066

### Farm-level mitigation strategies to improve sustainability of milk production: effects on GHG emissions

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Greenhouse gas (GHG) emissions per unit of milk have declined over the past 50 years, due to improvements in production efficiency and animal performances. However, the decline in emission intensity may be insufficient to offset the rising absolute emissions from increasing global demand for animal products. In this context, it's important to know which dairy systems

combine high productivity and low environmental impact. Recent studies pointed out that farm sustainable intensification strategies, e.g. improvement of feed efficiency and crop production, are related to more sustainable milk production. The aim of the study was to evaluate the effect on GHG emissions, related to milk production, of mitigation strategies (MS) based on the optimization of forage production, conservation and use in the cow diet. Between 2016 and 2020, in 20 dairy cattle farms, representative of the most widespread forage systems (FS) of northern Italy, different MS were applied. Life Cycle Assessment, accounting for changes in GHG associated with changes in farm management, was performed to evaluate the environmental impact of milk production, both in terms of emission intensity (CF, carbon footprint, expressed as kg CO<sub>2</sub>eq/kg FPCM, fat and protein corrected milk) and absolute emissions (kg CO<sub>2</sub>eq/hectare).

The results pointed out that High-Quality FS is the most favourable system in terms of GHG emission intensity (1.17 kg CO<sub>2</sub>eq/kg FPCM), while, considering absolute emissions, less intensive systems, such as Mixed Forages and Parmesan Dry Forage FSs, seem to be more sustainable (21,104 and 16,699 kg CO<sub>2</sub>eq/ha, respectively). The Mixed FS showed an improvement of CF within the two years (from 1.38 to 1.17 kg CO<sub>2</sub>eq/kg FPCM), even though absolute emissions didn't change. The reduction of GHG emission intensity in Mixed FS may be probably related to the increased area cultivated with forage legumes, together with the partial replacement of soybean in the ration. Multiple correspondence analysis revealed that the reduction of CF seems to be associated with the inclusion in the diet of forages with high nutritive value, the increasing of forage conservation efficiency and the replacement of soybean meal with forage legumes in the diet.

In conclusion, MS-based on optimization of forage production, conservation and use in the diet seem to contribute to improving the environmental sustainability of milk production.

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## O067

### A tool to efficiently replace dairy heifers using genomic information

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Genomic testing is an efficient tool for breeders, providing useful information for their management and selection decisions. An approach that is increasing worldwide is genotyping the entire

herd as a way to increase genetic gain. For breeders, it is important to discover the genetic merit of their animals before animals begin to produce milk. Choosing only the best animals to keep as replacements heifers and using beef semen to breed low genetic merit cows, can avoid herd overcrowding conditions with positive effects in terms of animal welfare and sustainability. ANAFIJ starts to develop a herd management tool that aims at optimizing dairy replacement heifer's management. A crucial parameter that affects the number of heifers needed is the age at first calving. The aim of this paper is to provide an overview of how the average age at first calving affects the number of annually required replacement animals in a herd, assuming no herd size growth. The algorithm used is based on the Heifer Replacement model (Cabrera 2009). The model considers a number of adult cows, calf–heifer culling rate, the average age at first calving and adult cow culling rate as input data. The model is herd-based. We also want to evaluate the economic and environmental impact of a reduction in the number of heifers needed. Given a herd with 297 adult cows, 24 months average age at first calving, 21% adult cow culling rate, 7% calf–heifer culling rate, the required replacement animals are 145 (animals/year) (1). Reducing age at first calving at 22 months (others parameters are unchanged) the number turns out to be 131 (animals/year) (2). Economically calculations have been done as reduced feed costs for heifers' maintenance (assuming feed cost 2 €/head/d) in the amount of €36,478 and greater gross income for milk sold in the amount of 735 €/head/productive life. In these calculations, we do not consider heifer replacement cost. Then, we assume that lowering the required heifers to keep as replacements lead to an effluent reduction. Considering 350 kg as average BW and free-stall barns using straw as bedding material, comparing the number of animals between the situation (1) and (2), displays  $\Delta 77.91 \text{ m}^3/\text{y}$  as the amount of effluent and  $\Delta 73.5 \text{ m}^3/\text{y}$  as the amount of manure. Regarding nitrogen production (net of  $\text{NH}_3$  emission losses) results are 6343.75 kg/y (1) and 5731.25 kg/y (2) with a  $\Delta$  of 612.5 kg/y (Calculation based under the Lombardia region nitrates Directive of March 2020 (2020–2023)).

## SESSION 14 – BREEDING STRATEGIES TO IMPROVE ANIMAL HEALTH, WELFARE AND RESILIENCE

0068

### Lifetime profitability assessed through multivariate health index measured on primiparous Italian Simmental cows

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The herd life is of major importance in dairy cattle. Direct or indirect indicators of health, fertility, feed efficiency, etc. are often used for selecting animals for reducing input costs. Many factors affect both the efficiency and the short/long-run profit of the dairy herds. The broad idea behind this contribution may be defined by the highlight 'the healthier is the cows the higher the longevity and the profit in the long run'. Thus, breeding for healthier cows can accommodate both animal welfare and a sustainable system of milk production. This study aimed at developing a health index on primiparous cows and to study its relationship with cows' longevity. Data were from the Italian Simmental breeder association (ANAPRI). From an initial set of 957716 individual lactation records (11476 herds) a set of 131317 (5959 herds) of primiparous cows, born between 1992 and 2018, were extracted (90–600 DIM). Each cow was scored for: milk (MY), fat (FY), protein yield (PY) and fat:protein ratio (F:P) (effective or within the first 90 DIM), somatic cell score (SCS), days open (DO), size (SZ), muscularity (MU), udder (UD) and feet and legs (FL) scores. Moreover, for each cow retrospectively were computed the number of lactations (NL) performed, the longevity as the difference between the date of the last test day and date of first calving (LON, d), lifetime milking days (LMD, d), lifetime MY (LMY, kg) and lifetime daily average milk production (LAP, kg/d). Multivariate factor (F) analysis of scored traits was performed. The longevity (LON & LMD) and lifetime production (LMY & LAP) were analysed with the following mixed linear model: random effect of the herd (5959 levels); fixed effects of year of calving (27 levels) and classes of F scores (3 levels: high, medium, low) built according to their distributions. The NL ranged from 1–13 (mean  $2.81 \pm 1.73$ ). Average LON, LMD, LMY and LAP were  $1116 \pm 666 \text{ d}$ ,  $848 \pm 509 \text{ d}$ ,  $19203 \pm 13069 \text{ kg}$  and  $22.15 \pm 4.98 \text{ kg/d}$ , respectively. Four common F were retained (64%  $\sigma^2$ ). F1–F4 were associated with production, size, health and NEB, respectively. The F3 (22%  $\sigma^2$ ) is regarded as a health index since its positive association with SCS (0.65) and logDO (0.58) and negative correlation with AP (–0.48) and UD (–0.56). The health index (F3) exerts a statistically significant effect on LON, LMD, LMY and LAP ( $p < .001$ ). For instance, LON raises from  $1039.7 \pm 11.17 \text{ d}$  to  $1225.56 \pm 11.18 \text{ d}$  ( $+0.78 \pm 0.81 \text{ NL}$  and  $+1.44 \text{ kg/d}$  of LAP) passing from the favourable to unfavourable health scores classes.



O069

## Genetic aspects of immunoglobulins G concentration measured with gold standard and predicted from infrared spectra in bovine colostrum

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The concentration of immunoglobulins G (IgG) is the criterion usually adopted to classify the quality of the colostrum administered to calves. Although the reference analysis of IgG (g/L) is time-consuming and expensive, no studies have evaluated the ability of infrared spectroscopy to predict colostrum IgG concentration so far. In this study, colostrum was collected on 693 Holsteins within 6 h after calving following a specific protocol. Samples were analysed through the reference analysis (radial immunodiffusion), which was carried out according to the manufacturer's instructions (Triple J Farm, Bellingham, US) after dilution of colostrum (1:5 v/v) in pure water. Near-infrared spectra (400–2500 nm) were collected on all samples using the DS2500 (Foss, Hillerød, Denmark). After spectra quality editing, the final dataset accounted for 685 samples. The calibration set included 195 samples representative of the 9 herds and cows from parity 1 to 6, and the best prediction model for IgG (RMSE = 14.2 g/L;  $R^2$  = 0.84) was reached through 20-fold cross-validation and multiplicative scatter correction and second derivative as mathematical treatment. Finally, IgG was predicted in the validation set which accounted for the remaining 490 samples (RMSE = 19.4 g/L;  $R^2$  = 0.73). Variance and covariance components were estimated for both reference and predicted IgG (validation set) using a linear model which included the fixed effects of parity (5 levels: 1, 2, 3, 4, and 5 + 6), the season of calving (4 levels), year of calving (2 levels), and herd (9 levels), and the random effects of additive genetic animal (6714 individuals in the pedigree) and the residual. Means (coefficient of genetic variation) of reference and predicted IgG were 92.4 g/L (14.8%) and 90.5 g/L (15.1%), respectively. Heritabilities of IgG were  $0.18 \pm 0.15$  (reference) and  $0.21 \pm 0.15$  (predicted). The phenotypic correlation between the two traits was positive and strong ( $0.86 \pm 0.01$ ), as well as the genetic correlation ( $0.99 \pm 0.11$ ). Overall, results indicated that IgG can be predicted from colostrum spectra with moderate accuracy and the genetic correlation between the reference and predicted IgG is close to 1. Therefore, IgG predictions can be potentially exploited as phenotypes to improve the IgG content of bovine colostrum, leading to potential practical positive consequences on calf health.

O070

## Genetic modelling of heat stress in Italian Holstein cows

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European Mediterranean countries are characterized by exposure to considerable heat between three and six months annually. The combination of ambient temperature and relative humidity, causes the effective temperature of the environment to be above the thermo-neutral zone of the animals and therefore heat stress occurs. Heat stress is one of the limiting factors in dairy production in hot climates. The interest of our study is to investigate if heat stress results in different effects on Italian Holstein performance due to genetic differences in heat tolerance. Heat stress was modelled by using data from weather stations. Daily maximum temperature and relative humidity were collected from 1994 till now. Weather measurements (777.980 records) were collected from 137 stations distributed across the national territory. Geographical coordinates and altitudes from all weather stations and the community of the farm were available. Distances between weather stations and farms were computed. Milk test day records were linked with the nearest weather stations within 80 km. Weather stations more than 500 m above or below the farm were omitted. Weather estimates for the farm were computed as a weighted average for the geometric center of the chosen weather stations accounting for the distance from the farm. The average distance between the geometric center of the chosen nearby weather stations and the farms was 13.5 km. The aim of this study was to determine if a threshold of the temperature-humidity index (THI) is evident for fat and protein corrected milk (FPCM) in Italian Holstein dairy cows. Because longer periods of heat stress might have a severe effect than shorter periods, 2-, 4-, 5-, 7-, 10- and 14-days were considered using average weather data measurements. For this study, we sampled from the whole Italian population 100 herds with a total of 172748 records belonging to 11150 cows in a period of 17-yr (2003–2020). Fixed regression analyses were based on models that included a herd-year season of recording (HYS), class of dim by parity and THI as a fixed effect. As random effects were considered the cow because there is repeated information per cow, the animal and the residual error. Preliminary results indicate that there is a drop in production in terms of milk energy above 19° of THI index expressed on a Celsius-like a scale in all periods considered. Further study is in progress to estimate the genetic component of heat tolerance.

O071

## Implementation of SCC breeding values in Italian Jersey breed

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In the framework of the LATTECO project, ANAFIJ (Associazione Nazionale Allevatori della Razza Frisona e Jersey Italiana) has been working on improving animal welfare, biodiversity and sustainability. Focusing on animal welfare, ANAFIJ has implemented a genetic evaluation for somatic cell count (SCC) in the Italian Jersey population. The aim of this study is to describe this implementation which became official in December 2020. It is expected that breeding values for SCC will help farmers to produce a progeny more resistant to mastitis, with a consequent reduction of veterinary costs and a valorisation of milk in quality payment systems. The dataset included 284,904 records of SCC collected on 19,305 cows belonging to 774 herds during the official test day milk recording program. Data ranged from 2005 to 2016. Means of SCC and somatic cell score (SCS), calculated as  $3 + \log_2(\text{SCC}/100)$ , were  $277.47 \pm 677.43$  cells/ $\mu\text{L}$  and  $3.14 \pm 1.76$ . Bulls' breeding values were estimated using a repeatability animal model which included the fixed effects of parity (first, second and third lactation), the season of calving (4 classes, 3 months each), days in milk (10 classes of 30-d each, ranged from 4 to 305 d), and herd-test-day (26,866 records) as a contemporary group. The random effects were an additive genetic animal, permanent environment and the residual. Breeding values for 187 bulls with a minimum of 10 daughters in at least 10 herds were estimated and they were referred to ANAFIJ genetic base, which is updated every 5 years and at the moment of the study, included animals born between 2010 and 2015. Somatic cell score was standardised to mean 100 and standard deviation 5. Breeding values reliability averaged  $0.80 \pm 0.13$  and the mean of daughters' phenotype for SCC was equal to  $275.41 \pm 92.81$  cells/ $\mu\text{L}$ . Bulls were divided into 3 groups according to their breeding values for SCS: as expected, bulls with higher ebvs had lower mean SCC; while a decrease in ebvs was associated with increased SCC. In January 2021, the new breeding value was submitted to Interbull for the test-run routine to validate it; this will allow ANAFIJ to have an international result for the SCC breeding value of the Italian Jersey population.

O072

## Development of a selection index for resistance to subclinical ketosis in Holstein Friesian dairy cows

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At the onset of lactation, high-yielding dairy cows could often experience a period of negative energy balance. This is reflected in a loss of body condition, due to body fat mobilization, and an increase of circulating ketone bodies, particularly  $\beta$ -hydroxybutyrate (BHB). This condition, known as hyperketonaemia, can result in (sub) clinical ketosis with negative implications on cow productivity and functionality, including health and fertility. The objective of the present study was to develop a genetic evaluation of resistance to subclinical ketosis for Holstein dairy cattle using data routinely available from the national milk recording system and linear classification. Milk BHB and fat-to-protein ratio (FPR) was available on more than 2.2 million test-days records belonging to Holstein cows in the first 90 days-in-milk from first up to the third lactation. These records were subsequently matched to the closest linear classification date when body condition score (BCS) was measured by an expert evaluator. The pedigree of cows has traced back up to 6 generations. (Co)variance components were estimated using trivariate linear mixed models; in particular, for BHB and FPR the fixed effects of herd-test-day, the two-way interaction between the week of lactation and parity, and the three-way interaction between classes of age at calving, parity and year of calving were considered. The linear model for BCS included the fixed effects of herd-year-round of classification, year of calving and the two-way interaction between age at calving and stage of lactation. The additive genetic effect and, only for BHB and FPR, the permanent environment were the two random terms. Due to computational constraints, (co)variance components were estimated on ten different subsets including 400 herds each, and subsequently averaged. Milk BHB and FPR and BCS averaged 0.056, 1.152 and 2.99, respectively. Heritability estimates were 0.093, 0.090 and 0.157 for BHB, FPR and BCS, while repeatability estimates were 0.179 (BHB) and 0.209 (FPR). The genetic (phenotypic in parenthesis) correlations were 0.159 (0.279; BHB vs. FPR),  $-0.161$  ( $-0.038$ ; BHB vs. BCS) and  $-0.140$  ( $-0.049$ ; FPR vs. BCS). The present study suggests that an exploitable additive genetic variation exists for milk BHB, and it could be used to set up breeding strategies aiming at improving resistance to subclinical ketosis through genetic selection.

## O073

### Can SNPs associated with the variation of stress biomarkers be used for the selection of stress-resilient dairy cows?

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Despite the progressive improvement in management practices, animals are still exposed to physiological and environmental stressors, which are exacerbated by ongoing climate change and poor husbandry. Selecting and rearing stress-resilient animals can increase animal production efficiency and enhance animal welfare. At the physiological level, stress causes a change in homeostasis. Metabolism, liver functionality and immune system activity are all modulated during stress-response, which may be assessed by measuring the plasma levels of several biomarkers identified by scientific literature.

A previous study identified a significant association of SNP markers with the plasma level of three proteins synthesised by the liver (ceruloplasmin – CP, paraoxonase – PON and gamma-glutamyl transferase – GGT) in Italian Holstein and Italian Red Pied breeds sampled around mid-lactation. In all cases, variation in the levels of these biomarkers was mainly driven by genetic variants mapping within or nearby genes coding for the proteins themselves. The aims of the present study were to confirm these associations in a larger and different set of animals and to understand if the SNPs associated with the level of biomarkers are also predictive of the response of dairy cows to stress. A total of 1000 Italian Holstein dairy cattle were sampled at one farm in northern Italy and genotyped with the GGP bovine 100K SNP panel (Neogen). Single-SNP, gene and haplotype-based GWAS were conducted on all cows utilizing plasma-biomarker levels as intermediate phenotypes for stress response. Results confirmed the genetic association between SNPs and plasma levels of paraoxonase (on BTA4) and gamma-glutamyl transferase (on BTA17). A novel association was discovered between SNPs and alkaline phosphatase (on BTA2), while the association with CP was not confirmed.

100 cows having opposite homozygote genotypes at SNPs previously associated with CP, GGT and PON have been identified and are being sampled during the stressing peripartum period (–7, +5 and +30 days from delivery). Analyses on post-partum animals will assess their metabolic response to parturition and early lactation stresses. The results of this investigation will shed light on the utility of intermediate phenotypes (stress biomarkers) as a proxy

of complex traits (stress response) and the convenience of their inclusion in genomic assisted breeding programs as novel traits.

## SESSION 15 – FEED EFFICIENCY AND GROWTH PERFORMANCE – I

## O074

### Residual feed intake and its association with growth performance and blood metabolic profile in preweaning Simmental calves

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The RFI is defined as the difference between measured and predicted feed intake [calculated using linear regression of measured intake on metabolic body weight and average daily gain (ADG)]. Hence, identifying potential regulators of feed efficiency from a physiological standpoint in calves is warranted. The aim of this study was to investigate the immunometabolic profile of calves categorized as most-efficient (M) or least-efficient (L) using RFI divergence during the preweaning phase.

Twenty Simmental calves were monitored through 60 d of age. Calves received 3 L of colostrum from their respective dams. Until 53 d of age, calves were fed milk replacer twice daily, whereas from 54 to 60 d (weaning) calves received only one meal. Calves had ad libitum access to concentrate and intakes were recorded daily. The measurements of BW and blood samples were performed at 0, 1, 7, 14, 21, 28, 35, 45, 54, and 60 d.

The RFI coefficient was –0.11 and 0.10 kg of DMI/d for M and L calves, respectively. Overall, no differences were found for BW ( $88.37 \pm 12.73$  for L and  $88.96 \pm 9.41$  kg for M calves at 60 d) and ADG (overall,  $0.68 \pm 0.35$  for L and  $0.69 \pm 0.28$  kg/d for M calves). M calves had a greater gain-to-feed ratio compared with L calves. The concentrate DMI was greater for L calves compared with M calves and at weaning resulted in 1.63 kg/d for L calves vs. 1.16 kg/d for M calves. Overall, plasma ceruloplasmin, myeloperoxidase and reactive oxygen species were greater in L than M calves. In addition, L calves had greater plasma haptoglobin at 21 and 60 d than M calves. M calves showed greater globulin and Zn, greater GGT at 1 d, greater FRAP at 7 d, and lower BHB at 28 d compared with L. Retinol and urea were greater in L than M calves due to the higher concentrate intake.

The divergence in RFI during early life is associated with the immunometabolic response. It can be envisioned that a low grade oxidative stress and a lower liver production of biomarkers related to the systemic inflammation (ceruloplasmin and haptoglobin) in most efficient calves is associated with better efficiency of



nutrients utilization. This idea is supported by the similar performance obtained in M calves that consumed less concentrate than L calves, where the latter likely had a greater energy expenditure to maintain the higher activation of the immune system mirrored by oxidative stress and inflammation biomarkers.

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## O075

### Residual feed intake of Italian Simmental bulls in performance test

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Improving feed efficiency involves a reduction in production costs and containment of greenhouse gas and pollutant emissions. In the beef cattle sector, residual feed intake (RFI) has become the most widely used measure of feed efficiency. RFI is calculated as the difference between actual dry matter intake (DMI) and predicted DMI, based on animal performance. It is therefore independent of body size (BW) and daily gain (ADG). This study aimed to evaluate the variability of RFI in Italian Simmental bulls and to study the effect on the main traits of feeding behaviour. For the trial, 272 young Simmental bulls subjected to performance tests at the ANAPRI Genetic Center were considered. The bulls started the trial on average at 275.8 days and 358.5 kg and finished at 332.4 days and 447.9 kg. Each pen was equipped with two electronic troughs (RIC system; Hokofarm Group, Marknesse, The Netherlands) capable of recording DMI and feeding behaviour for individual animals. The bulls were classified on an RFI basis and divided into two groups with divergent RFI according to SD (Low RFI < -0.787,  $n = 39$ ; High RFI > +0.787,  $n = 47$ ). The average DMI was 10.35 kg DM/d or 2.58% BW. RFI had values ranging from -2.55 to +1.86 kg/d. Estimated excretions of methane, N urinary and N faecal were 223.7, 99.4 and 60.3 g/d, respectively. The bulls ate for 113.1 min/d with an average intake of 231.3 g DM per visit. The missing event, that is the access to the trough without feeding, was 8.65 per day for a time of about 6 min/d. With the same ADG (average 1.58 kg/d), Low RFI bulls had a lower DMI of 2.30 kg/d than the high RFI group ( $p < .01$ ). This difference allows the most efficient group to had a significant ( $p < .01$ ) reduction in methane (20%), N faecal (23%) and N urinary (15%) excretions. In terms of feeding behaviour. The High RFI group had a higher number of FE ( $p < .01$ ) than the low RFI group but a very similar FT (average 122.3 min/d). This resulted in a different time

per FE (3.22 vs. 2.52 min/visit;  $p = .02$ ). The high RFI group had a significantly higher feeding rate (+19 g DM/min;  $p < .01$ ) than Low RFI group. Missing events tend to be lower ( $p = .10$ ) in low RFI but do not differ in time per day. The study allowed a first broad evaluation of the feeding efficiency in the Italian Simmental breed, providing useful information also in view of the upcoming inclusion of RFI in the selection index.

## O076

### Growth performance and gut health of rainbow trout fed commercial diets containing black soldier fly larva meal

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The administration of fishmeal (FM)-based diets containing *Hermetia illucens* (HI) meal to rainbow trout has been reported to influence both growth performance and gut health of fish, but the potential of using HI meal in commercial diets has rarely been assessed. This study aims to investigate the effects of increasing levels of HI meal in commercial diets for rainbow trout, focusing on growth performance and selected gut health parameters. A total of 600 fish were allotted to 4 experimental dietary treatments (3 tanks/diet with 50 fish/tank): HI0 (the control diet without HI meal) and HI25, HI50 and HI100 (with 8, 16 and 32% of HI meal inclusion, respectively, as a replacement of 25, 50 and 100% of FM). At the end of the trial (133 days), the growth parameters were recorded, and 12 fish/diet (4 animals/tank) were killed by over anaesthesia, with samples of the anterior and posterior gut, as well as posterior intestine content, being collected. Gut morphology was evaluated through morphometric measurements of villus height, while intestinal microbiota was assessed by 16S rRNA amplicon-based sequencing. Data were analyzed by R software ( $p < .05$ ). Growth performance was unaffected in the HI-fed fish ( $p > .05$ ). Gut morphology was not influenced by dietary HI meal inclusion ( $p > .05$ ), with the posterior gut showing higher villi than the anterior one in all the dietary treatments ( $p < .05$ ). Dietary HI meal inclusion increased the Chao1 index of the gut



microbiota, but, at the same time, reduced the Shannon index ( $p < .05$ ). The HI25 and HI50 fish also displayed a higher relative abundance of Actinobacteria (comprising chitin degrading bacteria) when compared to the other dietary treatments, as well as decreased Bacteroidetes (False Discovery Rate [FDR]  $< 0.05$ ). Furthermore, *Actinomyces*, *Bacillus*, *Enterococcus*, *Staphylococcus* (recognized as chitinolytic genera), and *Oceanobacillus* resulted to be enriched in the posterior gut microbiota of the HI-fed fish (FDR  $< 0.05$ ). Differently, dietary HI meal inclusion determined a reduction of *Campylobacter* and *Listeria* (common agents of food-borne diseases), as well as *Clostridium*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, unclassified members (U.m.) of Peptostreptococceae, Weissella, and Vagococcus and Lactococcus (whose distinct species are potential pathogens). In conclusion, dietary HI meal inclusion exerted a positive influence on the gut microbiota of rainbow trout, without compromising either growth performance or gut morphology.

## O077

### Rumen microbiota and growth performance in Aubrac and Maremmana steers reared in pasture and feedlot

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Maremma (MA) and Aubrac (AU) are rustic cattle well suited for extensive beef production. Rumen microorganisms are the key players during the conversion of plant biomass in ruminants. The characterization of the rumen microbiota is pivotal for the development of feeding strategies to increase the sustainability of livestock production. The activity of rumen microbiota can be modulated by the diet, but several studies reported that the rumen microbiome composition can be influenced by host genetics. This evidence suggests the possibility of using breeding strategies to select rumen microbiomes with desirable traits, but data on rustic cattle breeds are scarce. In this study, the rumen microbiota of MA and AU steers reared in different systems (feedlot and grazing) was characterized and correlated to both the lipid composition of rumen liquor and growth performance.

Forty 4.5-month-old AU and MA steers were allotted into 2 experimental groups: grazing (10 animals for each breed) and feedlot (10 animals for each breed) and the average daily weight gain (ADG) was calculated. When the animals were slaughtered the

rumen contents were collected. The fatty acid (FA) profiles were characterized by GC-FID. The bacterial communities were characterized by high-throughput sequencing of 16S rRNA gene amplicons. All experiments in this study were performed in accordance with guidelines from the European directive 2010/63/UE and DL 4/03/2014 n 26.

Higher ADG was recorded for the AU steers, regardless of the rearing system. Stearic acid (C18:0) was the most abundant FA, with the lowest concentrations being observed in the rumens of grazing MA steers. The rumen bacterial communities were influenced by the rearing system but the main factor that shaped the communities was the breed. The genus *Succiniclasticum*, the genus *Fibrobacter* and the Rikenellaceae RC9 gut group were positively correlated to the growth performance in MA steers. In the rumen liquor collected from the AU steers the only bacterial group with a positive correlation to growth performance was *Succinivibrionaceae* UCG-002.

Our data suggest that, in both cattle breeds, and regardless of the bacterial groups involved, animal performance is influenced by pathways involved in short-chain FA production, and by the presence of H<sub>2</sub> sinks that provide an alternative to methanogenesis.

## O078

### Effect of salty or sweet food leftover-based diets on growth performance and digestibility in piglets

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In recent years, animal nutrition has received deep attention, especially for sustainability. Former Foodstuffs (FFPs) are foodstuffs which were manufactured for human consumption in full compliance with the EU food law, but which are no longer intended for human consumption for practical or logistical reasons and which do not present any health risks when used as feed. For this reason, biscuits, bread, chocolate bars, pasta, savory snacks and sweets, high in energy content in the form of sugar, starch, oil or fat can be considered an appealing alternative feed ingredients. Although FFPs composition, may vary to a large extent, they have been indicated as energy sources mainly. In this study, FFPs were divided into two main categories (sweet

and salty) and they were used in post-weaning piglet's diets in order to replace standard cereals and investigate the effects of these alternative feed ingredients on growth performance and digestibility. Three experimental diets were formulated: 1) Control diets (CRT), 0% FFPs; 2) 30% conventional cereals substituted for 30% confectionary FFPs (FFP sweet); 3) 30% conventional cereals substituted for 30% bakery FFPs (FFP salty). The diets were iso-energetic and iso-nitrogenous and met NRC (2012) requirements. The trial lasted 42 days and 36 weaned female pigs (28 d of life,  $6.70 \pm 1.07$  kg) were housed in individual pens. After one week of adaptation, pigs were fed 1 of the 3 experimental diets. Fresh water and feed were available ad libitum throughout the whole experimental period. Individual pig body weight (BW) and feed intake (FI) were measured weekly. To determine apparent total tract digestibility (ATTD), also stools were collected. In addition, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were being calculated. The results of this experiment showed no differences in BW between groups ( $p > .05$ ). Also, the results revealed that ATTD values did not differ between CTR and FFPs based diets. However, ATTD was higher ( $p < .05$ ) in a salty FFP based diet compared to a sweet FFP based one ( $p < .05$ ). Overall ADG, ADFI, and FCR were not affected by any dietary treatments. In conclusion, the use of FFPs up to a level of 30% in post-weaning diets has no detrimental effects on pig growth performance, although more studies are needed to confirm these results.

#### Acknowledgements

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### SESSION 16 – FOOD SENSORY QUALITY, LABELLING AND VALORIZATION

O079

#### Feed insects for aquaculture. Use of *Hermetia illucens* L. meal for *Sparus aurata* L.: Emerging trends of advanced sensor-based instruments (E-eye, E-nose, E-tongue) for fish quality

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The use of *Hermetia illucens* L. (HI) in aquaculture seems to be a promising candidate as an innovative and sustainable protein source. With the aim of evaluating the effects of a diet containing HI meal in partial substitution of fishmeal (FM) on organoleptic quality of *Sparus aurata* L. fillets, 324 fish were randomly divided

into 4 groups with 3 replicates each. Fish were fed 4 diets containing increasing levels of HIM in FM substitution: HIM0, HIM25, HIM35 and HIM50 with 0%, 25%, 35% and 50%, respectively. After 180 days, a subsample of 18 specimens per diet (n. 6 fish per tank) were randomly sacrificed, filleted and analyzed using a sensor-based instruments platform consisting of E-eye, E-nose with 18 MOS sensors and a potentiometric E-tongue with 7 chemical sensors. The artificial sense platform was used to evaluate the difference between the 4 groups of fresh fillets in terms of color, volatile fraction and taste. A principal component analysis (PCA) of all sensor data was performed and the discrimination was assessed based upon the Discrimination Index (DI) that gives the discrimination quality through an indication of the surface between groups. Then, a discriminant function analysis (DFA) from selected sensors was performed and to validate the model, the recognition rate was determined by cross-validation score (VS). E-Eye analysis revealed that PCA represents 81.2% of the variance between sample measurements, but the DI was negative (277). Four sensors with the highest discrimination power were selected to build the DFA model and increase the discrimination capacity, but the performance obtained was not improved, showing a VS of 38, too low to distinguish the 4 groups. Similar results were obtained for E-Nose. PCA discrimination for volatile compounds explained 93.2% of the variance but showing a negative DI (113). DFA analysis with the 3 best-performing sensors improved the VS (41) showing a higher discrimination power, but not sufficient to distinguish the 4 groups. All the  $D^2$ -Mahalanobis distances, for color and volatile components, were not statistically significant. E-Tongue results were similar to those obtained with E-Eye and E-Nose, with a  $DI = -243$  and  $VS = 39$ . The  $D^2$ -Mahalanobis distance between HIM0 and HIM35 groups was the highest ( $p < .01$ ). Overall, the organoleptic properties were similar among the groups analyzed, with slight variations in taste for fillets of the HIM35 group. These results suggest that the use of HIM does not alter significantly the organoleptic properties of *Sparus aurata* L. fillets.

#### Acknowledgements

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O080

#### To eat or not to eat: consumer behavior towards sustainable fish

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Nowadays recent studies point up how it is important to understand better in which way people evaluate the sustainability of

different food aspects, in particular in fish consumption. In recent years, the decrease in the availability of fishmeal and fish oil prompted the detection of sustainable alternatives for aquaculture feeds. Recently, studies on the replacement of fishmeal with alternative protein sources in the diet of fish have emerged and the promising results have encouraged further research. In line with these researches, different alternative feed ingredients, such as insect's meal, poultry by-products meal, dead microalgae biomass and the meal obtained by processing the invasive alien Louisiana red swamp crayfish, are considered a potential ingredient of feed and, due to their 'growing ability' and great availability, a product of an environmental-friendly farming process. For some of these sources, their production can be renewable and cost-effective since they can be produced also on waste. The aim of this study is to explore and investigate the attitude of a sample of Italian citizens ( $N = 1200$ ) towards the opportunity to purchase fish, feed with fish diets largely based on suitable candidates for providing sustainable proteins, as part of fish diets. A theoretical model with the aim to analyze the relationships between latent constructs in consumer behavior, investigating the applicability of the Theory of Planned Behavior (TPB), is proposed. A Structural Equation Model (SEM) is implemented to test and measure the consumer reactivity towards this kind of information on fish sustainability and the strength to affect the buying intention. The results of this study support the particular positive attitude towards sustainable alternative sources for aquaculture feeds as feed for fish and consequent positive influence on buying intention of fish farmed using alternative meals in their diets. An efficient communication on fishes farmed using sustainable alternative sources meals has positive effects on attitudes, buying intention and on their acceptability. Highlighting these direct benefits for consumers will likely increase their willingness to pay for sustainable fish and reduce environment-unfriendly consumption patterns.

## O081

### Effects of diets with different protein sources on carbon and nitrogen isotopic signatures in *Hermetia illucens*: potential zootechnical implications and traceability of insect meals

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The use of insects as feed has attracted the attention of the agri-food sector of many countries for years, also thanks to the presumed advantages that insect rearing could entail compared to the breeding of traditional animals. For example, insects could potentially be reared on waste substrates, in order to reduce the disposal costs of such substrates and allow sustainable rearing. Nevertheless, such uses of insects may be subject, in different countries, to restrictions that prevent the insects from being successfully adapted for feed purposes. In order to contribute to making insect feeds safer and more spreadable, it is imperative to develop effective methods to track and detect the use of different types of substrates for their rearing.

Our study investigated the possibility of observing, by means of isotope-ratio mass spectrometry (IRMS), divergences in the isotopic carbon ( $\delta^{13}C$ ) and nitrogen ( $\delta^{15}N$ ) ratios of larval and pre-pupal samples of *Hermetia illucens* L. (Black Soldier Fly, BSF). Three different experimental trials lasting 21 days each were performed, each including five different groups of 6-day-old BSF larvae fed on a reference diet (RD) and two experimental diets including a specific plant protein source (soft wheat gluten diet, GD) and an animal protein source (bovine blood meal diet, BMD). At the end of the trials, the larvae were grounded and submitted to IRMS. By means of ANOVA, for the carbon isotopic signature we observed a clear incremental trend:  $-18.22\text{‰}$ ,  $-17.58\text{‰}$  and  $-15.28\text{‰}$  for the RD, GD, and BMD, respectively ( $p < .0001$ ), indicating a shift of BMD-reared insects toward higher isotopic signatures, as expected from an animal protein source. One-way ANOVA Contrasts for whole BMD pre-pupae against the average of the other two diets together (coefficients:  $+2$  for BMD and  $-1$  for both GD and RD) showed significance for  $\delta^{13}C$  ( $p < .001$ ). Surprisingly, the trends for nitrogen lacked consistency and therefore did not appear to be decisive in allowing trace insects reared on the animal-derived diet, presumably due to the different metabolism caused by different diets, which may have influenced isotopic fractionation of nitrogen.

According to our observations, the study of isotopic ratios could be a promising method for the traceability of insect meals. However, it is desirable that, given the lack of studies on the topic, the subject is examined in more depth, especially as far as the nitrogen isotopic abundance in insect-derived proteins.

## O082

### Dry vs. Vacuum aging in horse meat: oxidative stability and volatolomic profile

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The carcass is usually stored after slaughtering procedures to lead the muscle conversion to meat. This process is called aging and depends on several factors such as species, muscle type, packaging conditions, slaughter age. It usually improves eating quality (tenderness and juiciness) but also some sensorial patterns (taste, odour, and flavour) could be affected by the aging period, often depending on oxidative processes. During aging, there is a release of peptides, free amino acids, and free fatty acids due to proteolytic and lipolytic phenomena, leading to an increase in concentration and number of flavour compounds. The aim of the present work was to study the effect of two different aging methods, dry and vacuum, on horse meat oxidative stability and volatolomic profile after 14 aging days. Ten male 18-months IHDH horses were included in the trial. After 24 hours from slaughtering, *Longissimus lumborum* muscle of both half carcasses (between the 15th and 18th dorsal vertebra) was sampled. The two samples have been randomly assigned to vacuum or dry aging treatment, both lasting 14 days. Vacuum aging was done by storing samples after vacuum packaging at 4 °C, dry aging was done in an aging room set with 2 °C, 82% of humidity and 0.2 m/s of ventilation. After 14 days, TBARS, hydroperoxides (HY) and protein carbonyls (PC) concentration were analyzed. Besides, superoxide dismutase, catalase and glutathione peroxidase activity were carried out. Finally, samples were grilled and volatile organic compounds (VOC) profile was performed by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Data were analyzed using a one-way ANOVA, where ageing method was set as an independent effect and the horse a random effect. Enzyme activity resulted higher in vacuum aged meat ( $p < .01$ ), associated with lower TBARS, HY and PC production ( $p < .01$ ). Moreover, dry-aged meat showed higher VOCs production, with higher aldehydes concentration ( $p < .01$ ). Hexanal was the most represented VOC ( $27 \times 10^3$  vs.  $4562 \times 10^3$  AU for vacuum and dry, respectively). Generally, aldehydes have often also tallowy and meaty odors. Hexanal, commonly considered a meat oxidation marker of lipids, is characterized by high aromatic potential, providing freshly cut-grass and green aroma notes. Vacuum aging, associated with low film permeability, resulted in lower oxidation processes, reducing VOCs amount, but preserving a higher antioxidant enzymatic activity.

The aim of this study was to investigate the potential use of NIRS for the identification of different European autochthonous pig breeds from FT-NIRS spectra of fat and meat samples. Fresh intact samples ( $n = 267$ ) of subcutaneous fat and longissimus dorsi were collected from ten European local pig breeds: Bisara (BI), Black Slavonian (BS), Gascon (GA), Iberian (IB), Krskopolje (KR), Lithuanian Indigenous Wattle (LIW), Lithuanian White Old Type (LWOT), Majorcan Black (MB), Schwabisch-Hallisches Schwein (SHS) and Turopolje (TU). For each sample, two aliquots were scanned using Antaris II FT-NIRS (Thermo Fisher Scientific) considering the infrared region ( $3999$  to  $9999\text{ cm}^{-1}$ ). Spectra were standardized to a null mean and a unit sample variance. Mahalanobis distance was used to detect outliers (5 SD of the mean). Discriminant analysis of principal components (DAPC) was applied on spectra in the adegenet R package. External validation was used with 80% of samples in training (TRN) and 20% in testing (TST). Samples were randomly assigned in TRN-TST (with 10 replicates), restricted on all breeds having representatives in both sets. Results were averaged over the 10 replicates. The optimum number of principal components was selected using 10-fold cross-validation (30 replicates) in TRN, targeting the lowest mean squared error. In muscle samples, considering TST results, BI obtained the highest percentage of correct breed assignment (84%), followed by SHS (81%). Lower values (70 and 60%) were obtained for TU, GA, LWOT, KR, and IB; finally, the LIW, MB and BS muscles seem to be the most difficult to assign (from 51 to 35%). Using fat samples, both SHS and KR achieved ~93% identification success, followed by BI, LIW, and GA (from 88 to 83%). Other breeds, such as BS and MB, also performed higher success (79 and 73%, respectively). Intermediate accuracy was obtained for IB (68%), while the lowest successful identification was for LWOT (56%). Overall, results indicate the DAPC on FT-NIRS spectral data as a promising tool for local breed identification considering fresh intact samples, with fat being, on average, more efficient than *Longissimus dorsi*.

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O083

### Potential use of near-infrared spectra from meat and fat samples for the identification of autochthonous pig breeds

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## O084

### High-alpine forage milk characterization by DART-HRMS signatures

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Dairy products authentication is a challenging and complex experimental procedure. It has become meaningful for both food agencies and producers to be provided with a reliable traceability system that could allow authentication of a given dairy production chain based on metabolomics fingerprint. The study aimed to authenticate milk samples according to the main dietary roughage sources through direct analysis in real-time coupled to high-resolution mass spectrometry (DART-HRMS). Three experimental groups were assessed: lowland maize silage (LMS), lowland dried/ensiled grass forage (LGF) and highland (alpine) grazing/dried forage (HGF). The study involved 14 lowlands (North East of Italy) and 6 alpine (South-Tyrol) dairy farms. Breeds and productive performance were similar within the two lowland groups. Raw bulk milk samples were collected in each season ( $n=4$ ) and analysed for DART-HRMS metabolites by two extraction solvents per two ion modes. Dataset spectra were pre-processed and jointed by using a low-level data fusion approach. The train set (70% samples,  $n=61$ ) was submitted to a PLS-DA that allowed to identify of 25 most informative ions, which were submitted to hierarchical cluster analysis (HCA). Creatinine, methyl 2-furoate and dimethyl fumarate were the main metabolites detected in LMS milk. Nogramine and monoacylglycerols-20:2 (MAG 20:2) were found as a biomarker of the LGF feeding system. Lactate and few MAGs (16:0 and 18:0) were identified as biomarkers for the HGF samples. Further studies are needed to prove the suitability of these biomolecules as potential feeding biomarkers. The 25  $m/z$  values (ions) were used to perform a linear discriminant analysis (LDA) model on the training set that was validated in the independent test set (30% samples,  $n=25$ ) by using a blind tenfold cross-validation. The results of the predictive performance arranged in a confusion matrix highlighted a correct classification of the HGF samples (misclassification rate =0.00). An overlapping was recorded between the two lowland dietary theses (misclassification rate =0.55), even though no sample was misclassified as HGF milk. The reliability of DART-HRMS in tracing lowland milk samples might be influenced by feeding variability occurring during the 1-year experimental period. Summarizing, DART-HRMS data coupled with low-level data fusion, proved to be a rapid and powerful analytical strategy to discriminate raw bulk mountain milk compared to the lowland ones.

## SESSION 17 – MONITORING AND MITIGATING IMPACTS ON ENVIRONMENT – II

## O085

### A general framework for the assessment of environmental impact of food: an application to six healthy dietary patterns

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In this study, the environmental impact of six worldwide spread dietary patterns (Mediterranean diet, Palaeolithic diet, Flexitarian diet, Low Carb diet, Low Fat diet and Zone diet) is assessed in terms of use of natural resources (water and ecological footprint) and GHG emissions (carbon footprint). The assessment is based on an innovative standardized system-based framework for the analysis of environmental impacts of food systems, allowing to estimate the amount of food to be produced to meet a food consumption pattern. The methodological framework is based on a multidimensional approach that allows the assessment of environmental impacts. Consumption data are converted into quantities of raw food needed to satisfy food demand (considering food production and transformation processes). Each raw food is then associated with the environmental impact deriving from its area of production. All six diets have been chosen as they allow to explore the environmental impact deriving from a different proportion of macronutrients (carbohydrates, lipids, and proteins) or different sources of protein in the dietary pattern. For each pattern, an ideal week menu has been drawn up respecting both basic criteria of the theoretical approach on which the food pattern is based, and the Italian guidelines for a healthy and correct diet. The six diets were also compared with the real Italian food consumption, estimated based on the EFSA Consumption database using as common link the FoodEx 2 classification. The environmental impacts were assessed in terms of kg CO<sub>2</sub> equivalent emitted, litres of water used and m<sup>2</sup> needed to produce sufficient food to meet the average daily dietary income of each of the seven food regimes. The results are provided both in total terms and broken down by macronutrient or calories. A trend emerges from the analysis that links the proportion of macronutrients and protein sources with the number of natural resources used and the emissions produced. The results also underline the importance of a multidimensional approach to impact assessment: diets ranking is not the same according to the three footprints. The tool proves to be useful support for policymakers and institutions involved in promoting scientific research in the agri-food sector. It allows estimating the impact that policies in favour of the innovation of sustainable production practices,

both in terms of animal and vegetable production, can have on the whole food system.

## O086

### Comparison of different models to predict methane emission from milk fatty acids in dairy cows

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The identification of methods to estimated ruminant methane (CH<sub>4</sub>) emissions is the key to setting mitigation strategies. Many Authors have developed prediction equations for CH<sub>4</sub> emissions based on the milk fatty acid (FA) profile. Milk FA profile change with lactation stage and parity order. However, little effort was done to test if the estimates of CH<sub>4</sub> production (g/d), CH<sub>4</sub> yield (g/kg DMI), and CH<sub>4</sub> intensity (g/kg of fat and protein corrected milk; FPCM) produced by these equations are influenced by lactation stage and parity order.

The aim of this work was to compare the CH<sub>4</sub> values obtained with different literature equations and to study the influence of the lactation stage and parity order on these estimates. The FA profile of 1150 milk samples of Brown Swiss cows was determined by gas chromatography. Twenty-three equations were found in the literature, but only 11 were used because some minor FA were missing in the analytical dataset. These equations were proposed by: Visker 2014 (A); Williams 2014 (B); van Lingen 2014 (C); van Gastelen 2017 (D), and van Gastelen 2018 (E). The predicted CH<sub>4</sub> emissions resulting from the equations were mutually regressed and each dataset was statistically analyzed according to the lactation stage and the parity order. Depending on the equation used, the estimates of CH<sub>4</sub> were between  $341 \pm 106$  and  $401 \pm 5$  g/d, between  $20.0 \pm 2.2$  and  $22.7 \pm 1.5$  g/kg DMI and between  $14.2 \pm 1.8$  and  $18.1 \pm 2.8$  g/kg FPCM. Only a pair of equations produced highly correlated estimates of CH<sub>4</sub> intensity (B vs. E;  $y = 1.11x + 1.8$ ; g/kg FPCM;  $R^2 = 0.935$ ). The 3 equations (B, D, E), that estimate the CH<sub>4</sub> production, did not suggest any significant variation over the lactation. Contradictory trends were observed for the CH<sub>4</sub> yield over the lactation. All the equations that estimate CH<sub>4</sub> intensity suggested an increase during the lactation but with different trends: C and D an asymptotic trends; B and E an exponential trends. Parity order was found to influence the CH<sub>4</sub> production (D, E), CH<sub>4</sub> yield (A, D), and CH<sub>4</sub> intensity (B, E).

The estimation of CH<sub>4</sub> from the milk FA profile is a promising strategy. However, it was found that different equations produce results scarcely correlated between them. It was found that these estimates are scarcely influenced by the lactation stage and the parity order, suggesting a scarce coherence of prediction. Major

efforts should be done to increase the analytical detail of the milk FA profile and clarify the relevance of some minor FA.

## O087

### Kinetics of rumen methane production in *in vitro* system

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EU has recently resolved to reduce 36% methane (CH<sub>4</sub>) emissions by 2030 to mitigate greenhouse gases accumulation in the atmosphere and global warming. The fermentations that occur during the normal process of feed digestion in ruminants, contribute to about 30–40% to total anthropogenic CH<sub>4</sub> emissions. The present work aims to the study kinetics of CH<sub>4</sub> production by *in vitro* batch fermentation system. Four common ingredients for ruminants were used (barley meal, B, alfalfa hay, A, corn silage, C, soya bean hulls, S) and both total gas and CH<sub>4</sub> were measured continuously in four fermentation runs.

The fermentation apparatus is composed of eight 500 mL bottles (filled with filtered rumen fluid and buffer (ratio 1:2)) closed by a stirring device and connected to a MilliGascounter (Ritter Apparatebau GmbH & Co. KG). Ground and dry feed samples of 3.3 g of DM are added in each fermentation bottle, which is hermetically closed and immersed in a water bath at 39 °C for 48 h. Methane concentration is measured directly and continuously from the gas counter by an IR gas analyzer (RI.sens mono IR1, Ritter Apparatebau GmbH & Co. KG). Fermentation fluid was sampled at the end of the incubation period for the volatile fatty acid (VFA) analysis. Asymptotic gas yield differed from feeds (from 1836 to 1000 mL, from S to B,  $p < .01$ ) while CH<sub>4</sub> yield differed from 373 to 240 mL (from S to B, respectively,  $p < .01$ ). Total VFA were higher for B and S than A and C (57.6–58.5 vs. 50.1–53.0, mmol/L). The rate of total gas and CH<sub>4</sub> yield were quite similar among feeds while the lag phase was equal to zero from total gas and ranged from 0.2 to 0.9 h for methane. The proportion of CH<sub>4</sub> to total gas was not constant, and increased rapidly in the first 6–10 h of fermentation for all the feeds and reached a stable value of about 20–23% of total gas. Our calculations indicate that neglecting this phenomenon and assuming a constant proportion of methane to total gas causes an overestimation of total methane of about 10%. In conclusion, the CH<sub>4</sub> kinetics differ from that of total gas in the initial phase of fermentation, where the CH<sub>4</sub> concentration increases rapidly from zero to about 20%. In batch fermentation systems not equipped for continuous measurement, frequent gas sampling is required in the initial phase of the fermentation process to well approximate the actual methane yield.

O088

## Carbon footprint of fully and partially indoor dairy goat farming systems in Sardinia: a comparison between IPCC 2006 and IPCC 2019 prediction methods

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The aim of this work was to compare the carbon footprint (CF) of dairy goat farms estimated by the Tier 2 method of the 2006 Intergovernmental Panel on Climate Change (IPCC) with that estimated by using the Tier 2 IPCC 2019 refined method. A sample of 6 Sardinian (Italy) dairy goat farms was selected from 2 farming systems, identified as i) fully indoor (FI) with cosmopolitan specialized dairy breeds kept indoor and ii) partially indoor (PI), with mix breeds partially kept indoor and with access to pasture. Farms were surveyed, in a complete annual life cycle inventory (LCI) of cradle-to-gate farm production processes, from 1 October 2016 to 30 September 2017. The LCI included information on flock, animal diets, feed purchases, crops, farm stocks, and energy use audits. Data were analyzed by using the equation of Tier 2 of the IPCC (2006) and compared with those provided by the Tier 2 IPCC (2019). Reported values of CF were allocated 100% to milk yield. Total emissions were related to one kg of fat and protein corrected milk (FPCM). The FI and PI farming systems were, on average, 50 and 19 ha in size, with flocks of 265 and 192 female heads, respectively. Average milk production levels of the flocks were 761 and 576 kg FPCM head<sup>-1</sup> year<sup>-1</sup>, for FI and PI farming systems, respectively. Total emissions were higher in the FI (185226 and 187676 kg CO<sub>2</sub>eq) than in the PI (176394 and 179649 kg CO<sub>2</sub>eq) farming systems, with an estimation based on IPCC 2006 and 2019, respectively. In contrast, CF was lower in the FI (1.12 kg CO<sub>2</sub>eq kg FPCM<sup>-1</sup>) than in the PI (1.86 kg CO<sub>2</sub>eq kg FPCM<sup>-1</sup>) farming systems, with no difference evidenced between 2006 and 2019 IPCC methods. The contributions of each emission component on total CF were 47 and 53% from enteric methane, 13 and 7% from manure management, 13 and 13% from energy use, 23 and 22% from purchased feeds, 5 and 5% from on-farm feeds, in the estimation based on IPCC 2006 and 2019, respectively. In conclusion, the FI farming system exhibited lower values of emission intensity irrespective of the IPCC method used. However, compared to the IPCC 2006, the IPCC 2019 guidelines incorporate additional studies and proposed new coefficients for goat species which in the previous method were not included suggesting that the new inventory method could improve the estimation.

## SESSION 18 – BEHAVIOUR AND WELFARE

O089

## An innovative tool for assessing welfare of camels

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Whilst there are many tools for the assessment of welfare in livestock, there is none for camels. This study aimed therefore to pilot a method for assessing the welfare status of camels using animal-, resource- and management-based indicators at a camel market in Qatar. Adapting the AWIN protocol, data related to housing, feeding, health, and behaviour were collected at three levels: caretaker, herd, and animal. The Caretaker level was an interview exploring the caretaker's background, experience, and routine management practices. The Herd level was a check of the herd and of the place (i.e. box/pen) where camels were kept. At the Animal level, BCS, health, and behavioral parameters were recorded from 2 animals/pens, randomly selected. The number of animals/pens varied (average: 7, range: 1–37 animals) with a total population of 528 animals. The size of the pen was variable (26–256 m<sup>2</sup>), and consequently the space allowance varied from 2.5 to 34 m<sup>2</sup>/animal. The environmental temperature was high (average: 42 °C, range: 37–50 °C) and when in the paddock there was a shelter (86%) the camels moved into the shade (313/528 animals). In all paddock, there was a water point, but the water was often not available (22%), dirty (41%), or warm (max: 42.9 °C); the majority of the camels therefore drunk when clean and fresh water was offered (bucket test latency time: median = 8 sec, IQR = 3–40 sec). BCS varied and was rarely optimal (median = 2, IQR = 2–3). Most of the animals (89%,  $p < .001$ ) were free of movements (1% tied, 10% hobbled). However, many animals were not free from disease (38%), injuries (5%), scars (7%), and cauterization (38%). Skin diseases were the most common health problems (28%;  $p < .001$ ), followed by respiratory diseases (4%). The majority of the animals showed a good human-animal relationship (friendly, 48%, or neutral, 30%, approach;  $p < .001$ ), and no stereotypes were noted. However, some animals were aggressive (6%), when they were old, in pain (2%), or distressed (8%). The caretaker came mainly from Sudan (91%;  $p < .001$ ), with experience in camel handling often learned by father-son tradition (82%;  $p < .001$ ) and for many of them, animal welfare was 'treat the animals gently, feeding and watering them'. This was a preliminary study to pilot a tool to assess welfare in camels; further studies are needed to validate this tool in other camel farms worldwide.



O090

## Farm animals are long away from natural behavior: genetic and technical perspectives to improve welfare

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The concept of welfare applied to farm animals has undergone a remarkable evolution. The recent public debate on human-animal relationships is a consequence of the growing awareness of citizens that the use of animals for human purposes must include the obligation on the part of farmers to guarantee to their animals a high level possible of welfare. News perspective could be open for animal welfare reasoning around the concept of domestic, especially farm animals, as a human artefact. Therefore, it is important to understand how much a particular behavior of a farm animal is far from the natural one of its ancestors. This review is a contribution to better understand the role of genetics on the behavior of farm animals finalized to demonstrate that, as they are partially human artefacts, their welfare should be assessed taking into account the artificial environment where farm animals have been selected. The most important behavioral traits shaped by selection are docility juvenile behavior, fear and aggressivity towards humans, circadian rhythm, and environmental adaptation and resilience. Reduce the aggressivity of animals has a series of positive benefits for both animals and humans. An interesting case where the aggressivity was not reduced is the selection for the fighting aptitude in South America and European cattle breeds. One example is the not cruel traditional events of fighting among cows belonging to Aosta Chestnut and Aosta Black Pied, or Valdostana cattle breeds in North Italy. These behaviors were largely changed, from those of the wild ancestors, to meet humans' needs. This means that the naive approach to animal welfare that brings it back to a natural state is wrong and that welfare assessments must necessarily consider that the behavior of farm animals has been partially constructed by a human. Therefore, to accomplish sustainable animal welfare, the behavioral changes in some farm animals should be considered when planning and implementing farm animal welfare standards at the farm level. Animal scientists can contribute to these decisions by increasing the research about this topic to give information on how farmers can guarantee animal welfare in all breeding conditions at acceptable costs.

O091

## Measuring feeding synchronization for welfare assessment in dairy farms

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In social species, behavioural synchronisation is indicative of group cohesion. Feeding synchronisation is an anti-predator strategy and may yield several benefits, such as a reduction of competitiveness. Therefore, this indicator has been suggested for the evaluation of positive welfare. In this preliminary investigation, we tested a method for measuring feeding synchronisation in dairy cows. The synchrony during feeding was evaluated in 14 different pens ( $n = 12$ : lactating cows;  $n = 1$ : dry cows;  $n = 1$ : infirmary pen) from 9 dairy farms. Cows were bred in loose housing systems with cubicles and were fed TMR, except in two pens, where they received ventilated hay and fresh grass. Animals were observed for one hour (instantaneous scan sampling; 5-min intervals) after the morning feed delivery; the max and mean percentage of animals feeding simultaneously, and the percentage of scans with  $\geq 80\%$  of animals feeding together (which is considered as an acceptable synchrony level) were calculated. These variables were compared by one-way ANOVA depending on feeding place:cow (FP:C) ratio ( $\leq 1$  vs.  $> 1$ ; infirmary excluded). Results showed that the trend of synchronisation over time was similar in all pens, with a peak within 30 min after feed delivery. Differences were found depending on FP:C ratio for the max (FP:C  $\leq 1$ :  $45.12 \pm 23.46\%$ ; FP:C  $> 1$ :  $79.75 \pm 9.46\%$ ;  $p = .017$ ) and the mean (FP:C  $\leq 1$ :  $29.83 \pm 16.72\%$ ; FP:C  $> 1$ :  $57.37 \pm 8.67\%$ ;  $p = .011$ ) level of feeding synchronisation. The highest mean levels were found in pens where cows were fed grass or hay, probably because of the higher palatability of these feeds. Scans with  $\geq 80\%$  of cows feeding simultaneously were observed only in two farms with FP:C  $> 1$  and in one farm with FP:C = 1, with no statistical differences depending on FP:C, probably due to the low and unbalanced sample size. The infirmary pen (FP:C = 1.5) showed a very low level of synchronisation, confirming that animals in poor welfare conditions have a low feeding synchrony. In general, our results highlighted a medium-low level of synchronisation in the observed pens, suggesting the need for some interventions in order to improve cow welfare. The proposed scan sampling approach allowed to measure feeding synchronisation in dairy cows, and highlighted the role of management factors, such as FP:C and type of feed, to guarantee a positive welfare state of dairy cattle.



## O092

### Evaluation of inter-observer reliability of animal welfare indicators: which is the best index to use?

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In animal welfare studies, the use of the appropriate index when calculating inter-observer reliability can cause the inclusion or the exclusion of a promising indicator, which in turn has a strong impact on the robustness of a welfare assessment protocol. In this study, we compared the performance of the most popular agreement indexes (i.e. Scott's  $\pi$ , Cohen's  $k$ ,  $k_{\text{PABAK}}$ , Holsti's  $H$ , Krippendorff's  $\alpha$ , Hubert's  $\Gamma$ , Janson and Vegelius'  $J$ , Bangdiwala's  $B$ , Andrés and Marzo's  $\Delta$ , and Gwet's  $\gamma$  ( $AC_1$ ) to evaluate inter-observer reliability of welfare indicators in the case of dichotomous categorical (e.g. yes/no) animal-based indicators and the presence of two observers. The dataset was created using observations obtained from assessments conducted on nine dairy goat farms in Italy and Portugal using the AWIN protocol. Udder asymmetry was chosen as an example of a dichotomous categorical indicator; the concordance rate ranged from 75% to 100%. Our results show that Cohen's  $k$  (by far the most frequently used agreement index in animal-welfare studies), Scott's  $\pi$  and Krippendorff's  $\alpha$  were affected by a paradoxical behaviour: in some circumstances, where the concordance between observers was very high, they showed unacceptably low values. On the contrary, other less known and used agreement indexes, such as Bangdiwala's  $B$  and Gwet's  $\gamma$  ( $AC_1$ ), showed values very close to the concordance rate, independently from its value. For confidence intervals calculation, besides the most commonly used closed formulas of variance estimates, we used the bootstrap and exact bootstrap methods. Both methods turned out simpler when compared to the implementation of closed variance formulas and provided effective confidence intervals not only for Scott's  $\pi$  and

Cohen's  $k$ , as already reported in published literature, but for all the considered agreement indexes. Our results can be extended to any welfare assessment indicator, even in different species or contexts of application, when two independent observers assess dichotomous variables at the same time.

## O093

### Alternative farrowing systems for sows can affect litters performance?

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Within the intensive pig production systems, one of the major criticized welfare issues involves the housing in the confined systems in individual crates for sows at the farrowing and during lactation. This system is primarily used with the aim of reducing piglet crushing by providing greater control during the sow posture changes, but they have severe implications for sow welfare. However, considering that piglets' death mainly occurs during the first 24–72 hours postpartum, confining the sow beyond this period may not be necessary for the piglet's survival. The aim of this study was to investigate the effects of different housing systems at the farrowing on piglet mortality and growth besides to sow's welfare (behaviour and shoulders lesions) and performance (number of weaned piglets).

The study involved 21 sows, assigned to one of 4 farrowing systems: Conventional crate (CC) in which sow was kept in the crate for the entire lactation (28 d), and 3 alternative pens (APa, APb, APc) in which sows were confined only for 4 days after farrowing. The APs differed for pen size and position of the temporary farrowing crate (in the middle of the pen in APc or lateral near to a wall in APa, APb).

Different farrowing systems did not affect significantly the rate of piglet crushed (1,6), the number of weaned piglets (12,1) and the piglet average daily gain (233,6 g/d). Significant differences were instead observed when considering the time of occurrence of the crushing events with fatal outcomes. Piglet mortality due to crushing in the first 24 hours after farrowing was significantly higher in CC than in the APc (86% vs. 20% respectively). Even after 72 hours post-partum there was a significant difference only between CC and APc systems, being significantly lower in CC than in APc ( $p = .03$ ). Among APs systems, APc had therefore the lowest loss of crushed piglets when the cage is closed, the period when piglets crushing was significantly higher than after opening ( $p = .041$ ). However, after opening, the APc system resulted in the highest in crushing deaths ( $p = .008$ ).

This study suggests that allowing the sow in a pen instead of crate 4 days after farrowing doesn't increase the total number of crushed piglets as long as piglet growth and sow performance. In particular, one AP system (APc) has resulted in lower mortality during the more risky period 24h after farrowing, but further research is required in order to reduce crushing, especially after opening.

## O094

### Hopping displacements of rabbit kept in a collective housing system

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Restriction of movement expressed as the inability of performing three consecutive hops is considered a main welfare consequence in farmed rabbits, whereas few information on their hopping behaviour is available. The present study compared the hopping behaviour of 72 reproducing does of two genotypes (Grimaud vs. Hycole) housed in 18 collective parks (2.0 m × 1.0 m, 4 animals) without enrichment, or enriched with a platform, or with platform and pipes. After weaning of litters (33 d), the activity of does was video-recorded for 24 hours. Single, double, triple, and multiple consecutive hops were scored during 30 min per h. The data (total number of events and rate of total events during 12-h observation per park) were submitted to ANOVA by PROC GLM of SAS with genotype, pen enrichment and interactions as main effects and pen as an experimental unit.

On average, 985 hopping events per pen were observed during 12 h which corresponded to 20.5 hopping events per rabbit doe in 30 min. Single hops accounted for 56.1% of total hopping events; double hops stand at 19.6%, while triple hops and multiple hops at 12.7% and 11.6% respectively, without significant differences according to genotype or park enrichment.

Taking into account the location, hopping events observed on the floor were obviously higher in non-enriched pens compared to pens with platform or platform + pipes (100%, 72.0% and 83.2% of total events;  $p < .001$ ). The difference was significant for single (54.6%, 42.6% and 51.8% of total events;  $p = .06$ ), double (22.3%, 12.2% and 14.5%;  $p = .001$ ) and triple hops (12.2%, 9.21% and 9.88%;  $p = .10$ ). As for hops performed to move between the floor and the platform, their rate was significantly higher in pens containing only the platform compared to those with platform + pipes (16.6% vs. 9.90%;  $p < .001$ ) which was also associated with a higher rate of hopping events on the platform in the former pens compared to the latter (11.4% vs. 6.92%;  $p < .001$ ).

In conclusion, the hopping pattern was similar in the two genotypes. The presence of the pipes reduced the use of platforms, as

for the lower number of hopping events between the floor and the platform and on the platform. Most hopping events did not overcome two (75.7% of total events) or three consecutive hops (88.4% of total events). Further data are necessary at different time points to get more knowledge about the behaviour of rabbits under commercial conditions with special reference to hopping behaviours and motivations.

## SESSION 19 – FEED EFFICIENCY AND GROWTH PERFORMANCE – II

## O095

### Evaluation of *Lactobacillus plantarum* and *Lactobacillus reuteri* as feed additives for swine

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In swine farming, effective alternatives capable to reduce antibiotic consumption are needed to cope with the increasing concern of antibiotic resistance. In this perspective, functional feed additives, such as probiotics, are able to sustain the health status and reduce the risk of diseases development, that have become a fundamental tool to prevent pathological conditions in livestock. The aim of this study was to evaluate *Lactobacillus plantarum* and *Lactobacillus reuteri* *in vitro* for their functional characteristics and *in vivo* for their effect on animal performance and health. Firstly, *L. plantarum* 4.1 and *L. reuteri* 3X7, isolated from swine by Biotechnologie BT were genetically characterized by PCR reaction. Subsequently, their resistance to pH, temperature and digestive process were evaluated. Furthermore, the Lactobacilli mucosa adhesion ability was assessed on IPEC-J2 cell line. For the *in vivo* trial, 350 weaned piglets (28 ± 2d) were randomly divided into four experimental groups receiving basal diet respectively supplemented with: i) CTRL no supplementation; ii) PLA 2 × 10<sup>8</sup> CFU/g of *L. plantarum*; iii) REU 2 × 10<sup>8</sup> CFU/g of *L. reuteri*; iv) PROBIO 1 × 10<sup>8</sup> + 1 × 10<sup>8</sup> CFU/g for both bacterial strains. Growth performance and faecal consistency using a four-point scale (faecal score 0–3; considering diarrhoea ≥ 2) were recorded individually. Faecal samples were collected for the evaluation of main bacterial families, and blood serum aliquots were obtained for the assessment of metabolic parameters. *In vitro* characterization revealed a great resistance to a wide range of pH (3,4,5,7) for both species. At pH 2 a statistically significant reduction of bacterial growth was observed ( $p < .05$ ). Both species showed

good tolerance to a wide temperature range, while at 60 and 70 °C a statistically significant reduction of bacterial growth was observed ( $p < .05$ ). Both species survived well to all the steps of the digestion process. The LiCl treatment strongly inhibited the adhesion ability of *L. reuteri* ( $p < .001$ ), while it showed no significant effects for *L. plantarum* strain. Piglets supplemented with *Lactobacilli* significantly decreased the faecal score ( $p < .0001$ ) during the experimental period. *L. plantarum* and *L. reuteri* revealed interesting functional proprieties and health-improving effects as functional feed additives for weaned piglets.

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## O096

### Effects of dietary metabolizable energy content on productive performance of turkeys

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Understanding the metabolizable energy requirements of modern turkeys is a crucial step toward sustainable intensification of poultry production. Therefore, this study was carried out to evaluate the effects of feeding diets with different metabolizable energy content on growth performance and slaughter yields of female turkeys. A total of 1620 BUT Big 6 turkey females were divided into two experimental groups (9 replicates each): CON group receiving a commercial basal diet formulated according to the current recommendations, and REC group fed the same diet with reduced energy content during the last three feeding phases of the rearing cycle (–75, –175 and –125 kcal/kg, respectively from 61 to 76 d, 77 to 89 d, and 90 to 104 d). Turkeys were weighed pen-wise at placement (0 d), at each diet switch (14, 34, 60, 76, 89 d), and slaughter (104 d). Similarly, feed consumption was determined at the end of each feeding phase, whereas mortality was recorded daily. Accordingly, productive traits such as daily weight gain (DWG) and feed conversion ratio (FCR) were calculated. At processing, slaughter yields were determined on a group basis. As expected, CON and REC turkeys presented similar body weight and feed consumption after 60 d of trial. Compared to CON, the REC group showed higher body weight at 76 d (6476 vs. 6418 g/bird, respectively;  $p < .05$ ) as well as a tendency for higher DWG (126.9 vs. 123.9 g/bird/d, respectively;  $p = .06$ ) and lower FCR (2.374 vs. 2.422, respectively;  $p = .08$ ) from 61 to 76 d. Although no significant effect of the treatment was observed during the grower IV phase (77–89 d), REC turkeys reached

higher body weight at slaughter (104 d; 9598 vs. 9504 g/bird, respectively for REC and CON group;  $p < .05$ ) and exhibited lower FCR during the finisher phase (90–104 d; 3.350 vs. 3.491, respectively for REC and CON group;  $p < .05$ ). Considering the overall period of trial (0–104 d), the REC group showed higher DWG and lower FCR than the CON group (91.7 vs. 90.7 g/bird/d and 2.277 vs. 2.296, respectively;  $p < .05$ ). Finally, CON and REC turkeys presented comparable carcass and breast meat yields (74.8 vs. 74.5% and 32.8 vs. 32.0%, respectively). In conclusion, the metabolizable energy content reduction tested in the present study allowed to improve growth performance and feed efficiency in female turkeys, highlighting the need for a more accurate estimation of the actual energy requirements of modern turkey hybrids.

## O097

### Effect of genotype and feeding plan on growth performance, slaughter results and meat quality of growing rabbits

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The present study evaluated the effects of the genotype and the feeding plan, on growth, slaughter results and meat quality of growing rabbits.

A total of 336 crossbred rabbits (184 Grimaud, G, and 184 Hyla, H) were divided into 8 experimental groups (42 animals/group) according to a tri-factorial design (2 genotypes × 2 digestible energy (DE) levels × 2 crude protein (CP) levels) and reared from weaning to slaughter (33–76 d of age). Animals were fed with 4 diets obtained by the combination of 2 DE levels (HE; high DE: 10.9 MJ/kg vs. LE; low DE: 9.2 MJ/kg) and 2 CP levels (HP; high CP: 15.9% vs. LP; low CP: 14.4%) during the post-weaning period (33–64 d of age) and then received a unique commercial fattening diet (10.7 MJ DE/kg, 15.0% CP) until slaughter.

Compared to H rabbits, G rabbits showed lower live weights (LW) at the beginning of the trial (914 vs. 967 g;  $p < .001$ ), at 64 d of age (2537 vs. 2647 g;  $p < .001$ ) and at the end of the trial (3073 vs. 3147 g;  $p < .01$ ). In the whole trial, daily feed intake (DFI) was lower in G rabbits than in H rabbits (161 vs. 165 g/d;  $p < .05$ ), whereas daily weight gain (DWG) and feed conversion ratio (FCR) were similar. At slaughter, G rabbits showed a lower full gut incidence than H rabbits (16.2 vs. 17.6%;  $p < .001$ ), and a higher dressing out percentage (62.9 vs. 61.1%;  $p < .001$ ). The



increase of dietary DE concentration decreased ( $p < .001$ ) DFI (14%) and FCR (16%) in the post-weaning period. During fattening, the animals previously fed with LE diets showed higher DFI (+7%) and DWG (+15%), and improved FCR (5%) compared to those fed with HE diets ( $p < .001$ ). In the whole trial period, HE diets reduced ( $p < .001$ ) DFI (13%) and FCR (10%), without affecting the final LW. The reduction of dietary CP content did not affect growth performance and slaughter results. Meat quality traits (pH, colour indices and shear force) were not affected by the experimental factors.

In conclusion, the higher final LW reached by H rabbits may increase the profitability to the farmers, but the economic margin is reduced as compared to G rabbits due to the lower dressing-out percentage. The increase of dietary DE concentration is confirmed as a useful strategy to reduce feed costs and enhance the global efficiency of rabbit farms. The reduction of CP dietary content from 15.9% until 14.4% during post-weaning decreases the nitrogen excretion of rabbit farms and could reduce the feeding costs, depending on the market price of protein sources.

## O098

### Effects of different lipid sources and tannins on performances of high producing dairy cows

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It is well known that the degree of saturation in dietary lipids could influence ruminal metabolism and thus the performance in dairy cows. On the other hand, the addition of tannins is reported that could have positive effects on by-pass-proteins and modulation of ruminal fermentations. The aim of this experiment was to test two different lipid sources one saturated (hydrogenated fat – SF) and one unsaturated (extruded whole soybean – UF) and the addition of a tannins blend (T) on high producing dairy cows fed Parmigiano Reggiano type ration. 8 Italian Friesian cows were assigned to a Latin square design (14d adaptation, 7d sampling). Diets were balanced using NDS software, (CNCPS 6.55) to be isoenergetic and isoproteic: SFT+; SFT–; UFT+ and UFT–. Dry matter intake (DMI), daily water intake (WI), body weight, body condition score, rumination time (RT), reticular pH (r-pH) and milk yield (MY) were recorded. Rumen fluid VFA and ammonia, and milk composition were determined. Energy

corrected milk (ECM) was calculated. A mixed model procedure was used for data analysis; fat source and tannins presence and interactions were used as fixed effects, while animals and period as a random effect. Multiple comparisons were then performed by Student's *t*-test.

DMI (24.8 kg/d), WI (140 L/d), RT (521 min/d), BW (620 kg), BCS (2.25 pts) and total VFA production (98 mmol/L) resulted not strongly influenced by the treatments. r-pH resulted always safe excluding any risk of SARA (6.35 on average), however, the addition of T showed better pH stability, with fewer minutes below 5.8 (–20 min/d,  $p = .02$ ). UF diets reported higher MY (+1.55 kg/d,  $p < .01$ ) but lower milk components (–0.21% milk fat, –0.04% milk protein  $p < .01$ ), so ECM resulted equal (37.87 vs. 38.12 kg in SF and UF respectively,  $p = .38$ ). As expected, milk urea was positively modulated by T (–0.91 mg/dl,  $p < .01$ ), even if rumen ammonia resulted with no variations (5.02 vs. 4.71 mg/dl in T– and T+ respectively,  $p = .55$ ).

Obtained results showed that substitution of UF with SF produces an equalization of the production outputs with no risk of animal health and that tannins blend could improve nitrogen utilization and reticular pH.

## O099

### Evaluation of lactic acid bacteria as probiotics for gilthead sea bream (*Sparus aurata*): effects on growth and intestinal morphology, transcriptional response, and microbiota

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The administration of probiotics in fish has yielded positive results in numerous studies. The present research investigated the effects of dietary *Lactococcus lactis* subsp. *lactis* on growth performance, feed utilization, intestinal morphology, transcriptional response, and microbiota in gilthead sea bream (*Sparus aurata*). The trial was conducted with juveniles weighing 70–90 g, individually tagged and reared in nine 500-L tanks with 40 fish/tank. Fish were divided into 3 groups and were fed for 12 weeks with either a control (diet A) or experimental diets (diets B and C), supplemented with  $2.0E+09$  CFU/kg and  $5.0E+09$  CFU/kg dose of probiotic, respectively. The best growth performances were achieved by fish-fed diet C. Indeed, the final biomass of these animals was significantly higher than the control group (diet A), with intermediate values for fish fed diet B. Histological



analysis, performed using a semi-quantitative scoring system, confirmed that probiotic did not alter the macroscopic morphology of the intestine and did not cause inflammation.

A customized PCR array was designed to study the transcriptomic response. With regard to the probiotic effect, significant changes were found in the expression of interleukins (il10 and il12), and toll-like receptor 2 (tlr2) that resulted in upregulated in fish fed diet C as compared to the control group.

To analyze the microbiota of the feeds and the gut autochthonous microbial communities, the Illumina MiSeq platform and a metagenomics pipeline based on VSEARCH and RDP databases were used. Sea bream-fed diets containing probiotics showed a higher Firmicutes/Bacteroidetes ratio than control fish and this could be correlated to their better growth performances. Accordingly, the functional prediction showed that the microbiota of fish that received probiotics was more involved in the digestion and absorption of protein. The analysis of gut autochthonous bacteria indicated a clear modulation of the microbiota by the probiotic, even if there was no colonization of the *L. lactis* in the mucosa during the trial. This confirms that positive modification of fish gut microbiota can occur without probiotics colonization in the intestinal environment.

Our results highlight the interactions between diet and fish microbiota, suggesting that manipulating feeds to modulate the gut microbiome may represent a promising intervention to improve fish growth performances and digestive capacity.

## O100

### Effects of ensiling treatments on the fermentation profile of pulp and stem fractions after green biorefining of alfalfa

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The growing human population together with an increased consumption of animal protein per capita draws an increase in animal production and, consequently, in the request of protein for animal nutrition, but also an interest in sustainable and locally based farming systems, in particular within the organic agriculture area. In this context, forage crops can play a major role as they can produce a high amount of protein per hectare combined with favourable effects on soil restoration, carbon sequestration and environment preservation. Although the amino acid composition of their protein is comparable to soybean meal, their use in animal feeding for monogastric is limited by the high fibre content. Green biorefining techniques can allow the production

of fractions enriched in protein and with low fibre levels that can be profitably fed to pigs and poultry, whereas the residual fractions enriched in fibre can be valuably utilized for ruminant feeding. The high moisture content of these two latter co-products suggests their preservation as silages but its success could be impaired by their low sugars content. One lot of alfalfa leaf stripped stems (DM 30.69%; soluble sugars 2.81% on DM) and one of alfalfa pulp derived from press juice extraction (DM 29.75%; soluble sugars 3.57% on DM) were used in two separate, lab scales, experimental ensiling trials, carried out using the vacuum bags technique. The two products were ensiled as such (Control) or after addition of formic acid, lactic acid bacteria inoculum (LAB) and/or sugar-rich products, and for stems only also barley meal or sugar beet pulps. After 120 d from ensiling, pH, lactic acid, acetic, butyric, propionic acids, and ammonia were measured. The final pH for CTR stems and pulps were 4.65 and 4.93, respectively. The addition of molasses at 5% on a fresh basis, alone or in combination with LAB, significantly reduced pH (4.23 vs. 4.79,  $p < .05$ ), but the addition of LAB inoculum alone did not. A similar trend was evident also for ammonia nitrogen. For stems, also the addition of barley meal or sugar beet pulp reduced pH (4.18 vs. 4.65,  $p < .05$ ) but only barley increased the lactic acid content ( $p < .05$ ). The results show that both fibrous biorefinery co-products can be successfully ensiled also without any treatment and that sugar addition but not LAB inoculum alone did improve the silage quality, highlighting that the low sugar content of the products can be the most limiting factor for their fermentation.

## SESSION 20 – FEED EFFECTS ON NUTRITIONAL PROFILE ANIMAL PRODUCTS – I

### O101

#### Dietary olive pomace: effects on meat and back-fat quality traits in heavy pigs

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Olive pomace consists of peels, pulp, stone, water, and oil residues after oil extraction from fruit. Though its compositional features depend on the characteristics of the raw material and extraction system, it is rich in fat and antioxidant compounds, such as polyphenols and tocopherols. In this study, 20 Large White × Duroc pigs (initial BW 99.1 kg ± 7.83 SD) were used to investigate the effect of dietary dried olive pomace on animal performance, meat and backfat quality. During the 95-day finishing period, a control group (CTRL,  $n = 10$ ) was fed a

commercial concentrate while another group (SNS,  $n = 10$ ) received the same diet where 10% olive pomace partially and totally replaced corn and wheat bran, respectively. Diets were administered at a rate of 2.2 kg/head/day. The dietary treatment did not affect ( $p > .05$ ) growth performance. Specifically, the two groups had a comparable average daily gain (0.51 vs. 0.58 kg/d, for CTRL and SNS, respectively) and final body weight (144 vs. 158 kg, for CTRL and SNS, respectively). Similarly, the pH at 45 min and 24 h after slaughter did not differ ( $p > .05$ ) between groups. Conversely, SNS pigs had a greater backfat thickness (3.02 vs. 2.38 cm,  $p = .010$ ). Regarding meat colour parameters, dietary SNS increased  $a^*$  (11.9 vs. 10.8,  $p \leq .001$ ),  $b^*$  (4.01 vs. 3.10,  $p = .005$ ) and  $C^*$  (12.7 vs. 11.3,  $p \leq .001$ ), while reduce  $H^*$  (0.23 vs. 0.27,  $p = .043$ ). Dietary olive pomace did not alter ( $p > .05$ ) meat lipid oxidation (2-thiobarbituric acid reactive substances, TBARS), however, the percentage of Met-Myoglobin was higher in the CTRL meat ( $p = .002$ ). Regarding meat fatty acids, dietary olive pomace reduced ( $p \leq .05$ ) total PUFA (138.2 vs. 165.8 mg/kg meat). Interestingly, this reduction mostly involved n-6 fatty acids and could be responsible for the lower peroxidability index (PI) in SNS meat (190.9 vs. 237.20,  $p = .012$ ). Olive pomace administration affected ( $p \leq .05$ ) backfat fatty acids by reducing the concentration of MUFA and PUFA. Surprisingly, 18:1c9 was lower ( $p \leq .05$ ) in the SNS backfat. Moreover, 18:2c9c12, 18:3c9c12c15 and their elongation products were higher in the CTRL group, probably increasing the PI in CTRL meat ( $p = .001$ ). Concluding, dietary olive pomace did not affect the performance of finishing pigs and limited effects were reported on meat fatty acids, while it improved meat colour. Finally, SNS meat and backfat had lower PI, which may be an advantage considering that heavy pig meat is used for seasoned products fabrication.

## O102

### Linseed supplementation in the diet of fattening pigs: effect on the fatty acid profile of different meat cuts

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Dietary fat supplementation contributes to the fatty acids (FA) profile in pig fat and meat. Several works have demonstrated the beneficial effect of extruded linseed supplementation on FA composition, mainly for an increase of n-3 polyunsaturated FA (PUFA), particularly the alfa-linolenic acid (ALA). Pork is one of the very favourite types of meat, though rich in saturated FA,

which entails a certain health risk. Thus, a modification of the FA profile is necessary to increase the health properties.

The aim of the work was to evaluate the effect of extruded linseed supplementation on the FA profile in 5 different swine meat cuts, improving the nutraceutical properties.

A total of 60 pigs (Large White) were divided into two groups (30 animals per group) fed with two different diets: a control diet (C) and an experimental diet (L) with the addition of extruded linseed (8%). The pigs were housed in an experimental stable in 6 pens of 10 animals (3 pens per group). Each subject was included in the experiment at the age of 125 days (mean weight of  $66 \pm 7$  kg) and slaughtered at  $110 \pm 8$  kg on average. The cuts collected were: subcutaneous dorsal fat (S), bacon (B), Boston shoulder for salami (Bs), ham lean part (Hl) and ham fatty part (Hf). FA profile was obtained by GC-FID, whereas dry matter, fat, protein, carbohydrate and ash were measured by AOAC methods. Data were analysed by a linear model for each meat cut, considering the fixed effect of diet (C and L).

L diet was related to a significant decrease ( $p < .001$ ) of fat content in Hf (-6%) and B (-11%), while no differences were observed for the other cuts. The linseed supplementation increased the level ( $p < .001$ ) of n-3 PUFA (approx. 9-fold for all cuts), especially ALA which represented the main FA in linseed. The increase in n-3 PUFA led to a significant reduction ( $p < .001$ ) in the n-6/n-3 from 20 to 2.5, increasing the properties to prevent cardiovascular diseases. In the fatty cuts (S, B and Hf) of L group pigs, the level of n-3 PUFA exceeded the thresholds set by EFSA (2010) for obtaining the claim 'product rich in fatty acids Omega 3'. On the contrary, due to the low level of fat, the lean cuts (Hl and Bs) did not reach the threshold of n-3 PUFA required to obtain the claim. The results showed that a diet with 8% extruded linseed improved the quality of pork meat from a nutraceutical point of view. Indeed, the increase of n-3 PUFA allowed obtaining the claim, thus increasing the economic value of the product.

## O103

### Effect of dietary *Hermetia illucens* larvae meal on triglyceride composition of sea bream filets

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Administering *Hermetia illucens* meal (HIM) to marine finfish can be considered a strategy to increase aquaculture

sustainability. However, terrestrial insects contain little amounts of polyunsaturated fatty acids (PUFA) that could represent a limit in fish and human nutrition. Nevertheless, fatty acids distribution inside triglycerides (TGs) plays a pivotal role in fat digestion and absorption, due to the specificity of lipase activity on position sn-1 and sn-3. Since fatty acids in the sn-2 position are the first to be absorbed, our purpose was to analyze the FA profile of total lipids, triglycerides and their sn-2 position in fillets from gilthead sea bream (*Sparus aurata* L.) fed HIM. Three-hundred and sixty gilthead sea bream were randomly divided into 12 tanks with four isoenergetic, isolipidic and isoproteic diets. Briefly, HI larvae meals were included at 9.2% (HI9), 18.4% (HI18), and 27.6% (HI27) in order to substitute graded levels of the FM contained in a control diet (HI0) which amount 30%. After 120 days of rearing, 10 fish for each diet were slaughtered and the fillet FA profile was deepened. The total lipid content was not affected by the diet. Considering the total lipid FA profile, in HI18 and HI27 fillets C12:0, C14:0, C16:1n-7, C18:1n-9 and the total saturated FA (SFA) content significantly increased while PUFA<sub>n-3</sub> content lowered ( $p < .01$ ). The triglyceride contents in the C, H25, HI18 and HI27 fillets were  $2.65 \pm 0.24$ ,  $3.07 \pm 0.49$ ,  $3.28 \pm 0.20$ , and  $2.95 \pm 0.21$  g/100 g muscle, respectively. HI18 and HI27 fillets contained in their TGs higher level of C16:1n-9 and SFA ( $p < .05$ ) than C and HI9 fillets. In the same groups, a slight but significant decrease in PUFA<sub>n-3</sub> content was observed. Noticeably, the FA profile of the TG sn-2 position remained almost unaffected by the inclusion of HI in the diet. Indeed, only some SFAs, namely C12:0 and C14:0, significantly increased ( $p < .01$ ) in the HI27 group whilst PUFA<sub>n-3</sub> content did not vary. In conclusion, the effects observed on lipid FAs confirmed previous data on HI inclusion in aquafeeds for finfish. However, the changes appeared limited to the sn-1 and sn-3 position of TGs, thus highlighting the resilience of the sn-2 FA profile. Finally, this study evidenced that the inclusion of HI in the diet did not impair the presence of fatty acids important for human nutrition, as PUFA<sub>n-3</sub> in the sn-2 position of triglycerides, increasing the chances of being better assimilated and absorbed.

## O104

### Alternative protein sources in aquafeeds for rainbow trout: overview on fillet quality modifications

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Aquaculture has been facing the major challenge to answer the growing demand for fish to feed the world population, mitigating its pressure on finite natural resources. Since aquaculture highly depends on marine and vegetable materials as ingredients for aquafeeds, namely fishmeal (FM) and soybean meal (SBM),

many studies aimed to design a sustainable and eco-friendly fish production chain by cutting down FM and SBM incorporation rate in aquafeed. Novel ingredients, such as insect meals or poultry by-products, are the most promising strategies for the aquaculture sector. Nevertheless, several impairments of fish fillet quality, such as the decreased content in polyunsaturated fatty acid, are the main weaknesses which need to be minimised while proposing new ingredients for feed formulation.

The present trial aimed to compare the use of poultry by-products (P60) and *Hermetia illucens* larvae meal (H60), as single or in the blend (H10P50), to substitute plant protein in a vegetable-rich based diets (CV) for rainbow trout (*Oncorhynchus mykiss*). After 27 weeks of the feeding trial, 27 fish for each group were slaughtered and fillet yield, color (lightness, L\*; redness index, a\*; yellowness index, b\*), hardness, and fatty acid (FA) profile of the fish fillets were analysed. A consumer test with 80 people was assessed to evaluate liking and intention of re-consumption. Fillets yield and hardness were unaffected by the dietary treatment. Fillet L\* value was significantly higher in H10P50 than CV, being 49.08 and 46.95, respectively. P60 and H60 fillets showed intermediate L\* values. Diet did not affect a\* and b\* values. Noticeably, the formulated diets were able to mitigate the differences in fillet FA profiles, in particular, the addition of animal-derived protein did not increase saturated FAs and the n-3/n-6 ratio was equal to 1.4. Consumers' liked trout fillets regardless of the dietary treatment, however, the H60 group was described as more fibrous and less juicy than the others. Despite this, 78% of respondents declared their intention to re-consume the product.

In conclusion, considering fish fillet quality characteristics, the formulated diets were suitable and valuable alternatives to reduce plant-protein ingredients in feed for rainbow trout. Improving the n-3/n-6 ratio remains a priority to support human health that deserves further investigations.

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## O105

### Productive performance and meat characteristics of kids fed anthocyanes

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The purpose of the study was to evaluate the in vivo performance and the meat characteristics of suckling kids fed a red-orange



and lemon extract (RLE) rich in anthocyanins. The trial was carried out on 60 Saanen kids. After colostrum administration, animals were randomly assigned to a treatment group (Group A;  $n = 30$ ) that received RLE (90 mg/kg PV) as food oral additive, and a control group (Group B  $n = 30$ ) that received a standard diet. Animals were weighed every two days and slaughtered at 40 days. The RLE supplementation did not influence daily weight gain, carcass measurements and incidences of different anatomical regions. On the contrary, anthocyanins administration significantly improved meat oxidative profile seven days after slaughtering, as demonstrated by the reduced levels of TBARS ( $0.34 \pm 0.01$  vs.  $0.53 \pm 0.01$ ;  $p < .01$ ) and hydroperoxides ( $0.56 \pm 0.01$  vs.  $0.80 \pm 0.01$ ;  $p < .01$ ) in Group A and Group B, respectively. Moreover, the juiciness decreased in both groups during the time ( $p < .01$ ) and, at day 7, was significantly higher in Group RLE compared to Group CON ( $0.47 \pm 0.01$  vs.  $0.40 \pm 0.01$   $p < .01$ ). No differences were observed for most of the colour parameters, except on day 7 for yellowness ( $1.55 \pm 0.01$  vs.  $0.46 \pm 0.01$  in group A and group B, respectively;  $p < .01$ ). There was also a lower saturated ( $41.75 \pm 0.50$  vs.  $46.47 \pm 0.50$ ;  $p < .01$ ) and a higher monounsaturated and polyunsaturated fatty acids concentration ( $43.29 \pm 0.49$  vs.  $40.44 \pm 0.49$  and  $15.01 \pm 0.22$  vs.  $13.08 \pm 0.22$ ;  $p < .01$ ) in group A meat, that shows also lower atherogenic ( $0.64 \pm 0.02$  vs.  $0.77 \pm 0.02$ ;  $p < .01$ ) and thrombogenic indexes ( $0.88 \pm 0.02$  vs.  $1.05 \pm 0.02$ ;  $p < .01$ ) compared to group B. In conclusion, the addition of 90 mg/kg anthocyanins to kids' diet regimen did not affect in vivo growth performance and meat chemical composition. However, this inclusion improved juiciness and reduces colour deterioration and cooking loss, making kids' meat more attractive to consumers at first sight. The quality of the meat was also positively influenced, due to delaying lipid oxidation and to a better fatty acid profile, resulting in healthier meat for human consumption.

## O106

### Effect of dietary alpha-linolenic acid on meat quality of Italian poultry breed and crossbreed

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Humans and mammalian species are unable to synthesize enough Long-Chain Polyunsaturated fatty acids (LCP) of both n-3 and n-6 series then, they must be introduced with the diet. The health benefits of n-3 LCP and consequently the production of foods rich in n-3 PUFA is a great goal for agroindustry. Indeed, fish, which is the major source of n-3 LCP, cannot meet the worldwide demand for n-3; hence improving the n-3 LCP content of poultry meat is

considered a useful strategy to balance the n-6/n-3 ratio in human diet. Many authors demonstrated that local chicken strains efficiently desaturate the essential fatty acids (α-linolenic acid, ALA) into n-3 LCP (eicosapentaenoic-EPA; docosapentaenoic-DPA and docosahexaenoic-DHA acids) when they were administered with the diet. The aim of the study was to evaluate the effect of flaxseed-enriched diets on fatty acid profile of breast meat in different chicken strains, also comparing different ages. Four different strains, Robusta Maculata (RM), Bionda Piemontese (BP), RM × Sasso (RM × S), BP × Sasso (BP × S) and a commercial hybrid (Ross 308) were divided in two dietary groups (15 chickens/strains/diet/replicates): control and flax (10% of extruded flaxseed) until the reaching of the 45 (all birds), 81 (Ross) and 140 (pure strains and crossbreeds) days of age. Chickens were raised in different indoor pens and subjected to standard conditions of temperature (20–32 °C) and humidity (65–72%). The effect of age (within strain), strain, diet, and the interaction strain × diet were evaluated with a linear model. At slaughtering, breast meat was sampled and stored for fatty acids profile evaluations (by GC-FID). Main differences were observed between dietary groups respect to the strains. The n-3 LCP content were almost double in flax groups than control, furthermore the main differences were found in BP at older age (2-fold higher value respect to that at 45 days). Both pure strains (BP and RM) and crossbreeds (BP × S and RM × S) showed lower proportion of ALA respect to Ross ( $p < .05$ ); in addition, the n-3 LCP was also lower ( $p = .062$ ). However, evaluation of the conversion of ALA into n-3 LCP (estimated as n-3 LCP/ALA) with both the dietary plan showed significantly higher value in BP and BP × S compared to the Ross (0.30 and 0.28 vs. 0.25, respectively). Furthermore, Ross strain, probably for their higher fat deposit, efficiently stored n-3 LCP when dietary ALA was administered.

## SESSION 21 – PRECISION LIVESTOCK FARMING – I

## O107

### Pre-weaned dairy calf housing: preliminary results on microbial air contamination

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Within the dairy herds, little attention is paid to young replacement animals as calves. Most of the investments and farmers' time are absorbed by adult cows, without considering that young animals play a key role as their development affects the future of the farm. The aim of the study was to investigate calves'



management system, focusing on the microbial air contamination of the individual pens where calves are generally kept in their first weeks of life. The study involved 8 dairy farms, located in Lombardy. Microclimatic conditions and microbial air contamination were monitored using Hobo® data loggers and VWR® Microbiological Air Sampler Surface Air System® Super ISO. The effects of individual housing location and microclimatic parameters on microbial air contamination were tested using GLM procedure. The 8 farms involved in the study had  $186 \pm 145$  lactating cows with individual milk production of  $33 \pm 1.90$  kg/d; the number of calves born per year was 251 with a minimum of 55 and a maximum of 615. The total mortality rate before weaning was  $12.5 \pm 9.81\%$ , with a perinatal mortality of  $5.43 \pm 5.65\%$  (intrapartum and within 24 h after birth) and early mortality from 24 h to weaning of  $7.08 \pm 4.56\%$ . The average area of the individual pens was  $2.61 \pm 1.48$  m<sup>2</sup>. Microbial contamination of air in the individual housing was: Standard Plate Count (SPC)  $4.46 \pm 0.46$  log<sub>10</sub>MPN/m<sup>3</sup> of air; mold  $3.77 \pm 0.46$  log<sub>10</sub>MPN/m<sup>3</sup>; yeast  $3.33 \pm 0.77$  log<sub>10</sub>MPN/m<sup>3</sup>; Enterobacteriaceae  $2.65 \pm 0.68$  log<sub>10</sub>MPN/m<sup>3</sup>; *Escherichia coli*  $2.14 \pm 0.95$  log<sub>10</sub>MPN/m<sup>3</sup>. Five farms had values of SPC above the threshold of 4.18 log<sub>10</sub>MPN/m<sup>3</sup>, which is indicated by Zieger (2019) as a critical value. Pens location (indoor, outdoor, etc.) influenced the air *E. coli* count while the average day temperature had an effect on yeast ( $2.997$  vs.  $3.645$ ,  $p < .05$ ; for  $T_{max} < 17^\circ\text{C}$  and  $T_{max} > 17^\circ\text{C}$ , respectively) and mold ( $3.551$  vs.  $3.969$ ,  $p = .07$ ; for  $T_{max} < 17^\circ\text{C}$  and  $T_{max} > 17^\circ\text{C}$ , respectively) presence in the air samples. In conclusion, the evaluation of microbial air contamination in calves individual pens is a little investigated interesting aspect, linked to housing design and location. The relation between airborne microbiota environmental exposure and calves' health has to be deeply investigated in order to understand how the monitoring of microbial air contamination can be effective in putting in action appropriate corrective actions for preventing health problems in calves.

#### Acknowledgements

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## O108

### The left side flank as source of information for animal behaviour and welfare in dairy cow

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The development of devices and methods able to spot changes of behaviours and physiological parameters from normality in a timely manner is one of the aims of Precision Livestock Farming. With this vision, the aim of the present study was to develop a

model to identify animals' posture and predefined behaviours (moving, feeding, resting, ruminating and standing still) from data collected by a single tri-axial accelerometer located on the left side flank of dairy cows and evaluate its accuracy and precision. This spot was chosen because in ruminants, beyond behaviour, it potentially enables also the monitoring of rumen and turaco-abdominal contractions associated with breathing and involved in both urination and defecation. Twelve Italian Red-and-White lactating dairy cows were equipped with a tri-axial accelerometer located on the left side paralumbar fossa and were observed on average for  $136 \pm 29$  min per cow by two trained observers who continuously recorded animals' posture and behaviour as a reference. Acceleration data were grouped in time windows of 8s overlapping by 33%, for a total of 35133 rows. For each row, 32 different features were extracted and used by machine learning algorithms for the classification of posture and behaviour. To build up a predictive model, the dataset was split into training and testing datasets, characterized by 75 and 25% of the observations, respectively. Four algorithms were tested: Random Forest, K Nearest Neighbors, Extreme Boosting Algorithm (XGB) and Support Vector Machine. As regards, behaviour classification a Convolutional Neural Network model (CNN, a Deep Learning Model), made of 8 layers, was also tested. Among machine learning models XGB showed the best accuracy (0.99) and Cohen's kappa (0.99) in predicting posture, whereas Random Forest had the highest overall accuracy in predicting behaviours (0.76), showing a balanced accuracy from 0.96 for resting to 0.77 for moving. A higher rate of misclassification was found between feeding, moving and standing still. The Deep Learning model showed an overall accuracy in predicting behaviour of 0.92. Overall, the application of a single tri-axial accelerometer at the left side paralumbar fossa of mid-lactating dairy cows gave very accurate results regarding the prediction of posture and resting behaviour using machine learning models, whereas precision and accuracy for ruminating and feeding behaviours were greatly improved by the use of CNN.

## O109

### The livestock sector digitalization: indicators on livestock's farming digital performance

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Digitalisation plays a pivotal role in the development and modernization of livestock farming. Nowadays, the adoption of a new IT system is mandatory for the smart farm to achieve the sustainable development goals accordingly with the new EU Common

Agricultural Policy. Digital tools allow farmers to immediately react to any event and to adopt the best technical decision. The control of the food chain leads to improve food security (both in terms of traceability and re-traceability) but also to enlarge the profit for the farmers.

There are some basic requirements to make use of digital farm: access to the internet and cell phones, skill to use digital technologies, a culture that encourages digital livestock farming as a business for young people; these are important critical points for the applicability of the smart farming in Italy. In fact, only 3.8% of the 217,449 farms with SAU and livestock, use the internet to manage the farm and the farmers are 55 years old on average. Over the last decade, in Italy, the structure of the farms with animals changed, and a concentration of the small farms in the larger companies occurred (ISTAT, 2010 Agricultural Census); in this scenario, is important to detect what digital technologies have been introduced and what precision tools are used.

The aim of this study is to make a focus on the evolution of digitalisation in Italy and to detect the introduction of precision tools for the management of the livestock farms by using the national and regional Livestock survey (Reg. CE 1165/2008), carried on in June and in December of each year with a CAWI-CATI technique. For the first time, the survey of June 2020, included a question about the use of precision farming tools. Respondents were 6206 and only 25.1% of them declared they use a precision farming tool, the most used being for the management of the herd (46.5% of farms); 65.4% of the farms are located in the North of Italy and they have introduced two precision instruments (68% of farms). The survey of December 2020 included some additional questions about the introduction of computer systems such as the use of broadband, the use of a website or social pages, e-mail, the use of the cloud. This will allow us to define a set of indicators related to the livestock domain that will be able to characterize the innovation processes that are changing the way livestock farming is done, with a focus on the impacts and expectations for cattle livestock farms.

assess cows at health risk and welfare status of herds. A tool devoted to this purpose is the hematochemical profile that allows predicting of health risks, often anticipating clinical signs. The Piacenza Metabolic Profile (PMP) includes specific plasma parameters representative of energy-protein-mineral metabolisms, inflammatory status, liver activity, oxidative stress. This profiling in early lactating cows could be also integrated with a composed index representative of liver functionality (LFI, Liver Functionality Index), that combines concentrations of albumin, total cholesterol and bilirubin, considered proxies of the acute phase proteins synthesized by the liver. It measures the changes in concentrations between 3 and 28 days in milk, standardized for an optimal pattern of change, for the 3 parameters obtained from healthy cows at the same stage of lactation. This index allows the evaluation of the consequences of an inflammation occurring around calving. The NIR transmission spectra were acquired from plasma samples of 149 pluriparous dairy cows from 7 dairy herds and in different physiological phases (dry period, transition period, early and mid-lactation). Each sample was also analyzed using accepted reference clinical chemical methods carried out with ILAB 650. NIR measurements were performed in parallel with the reference analyses, and on the same plasma aliquot, with a Bruker MPA Multi-Purpose FT-NIR Analyzer. 200 microliters of each thawed sample were gently transferred in a single-use UVette®. FT-NIR spectra were collected at a spectral resolution of 4 cm<sup>-1</sup> over a wavelength range of 12,500–4000 cm<sup>-1</sup> with 32 scans per spectrum. The acquired spectra were processed by the Uscramble®X software (version 10.5.1) to develop calibration and prediction curves for each parameter by using the partial least square (PLS) fit method. For the parameters included in LFI, excellent prediction models were obtained for total cholesterol and albumin ( $R^2_{pred} = 0.996$ , RMSEP = 0.111;  $R^2_{pred} = 0.825$ , RMSEP = 1.124 respectively) while total bilirubin resulted less predictable ( $R^2_{pred} = 0.107$ , RMSEP = 1.175). Our results may open an interesting perspective towards more cost-effective monitoring of the animals' metabolic status, health and welfare conditions.

#### Acknowledgements

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## O110

### Near-infrared spectroscopy for the prediction of plasma parameters for liver functionality index in dairy cows

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It is becoming clear that the development of hematic clinical methods characterized by rapidity and cost-efficiency could be really useful. Promptly setting of the situation is necessary to

## SESSION 22 – FREE COMMUNICATIONS

O111

# Aneuploidy detection in Murrah buffalo MII oocytes by tri-colour FISH

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Aneuploidy is one of the main causes of fetal and embryonic mortality in mammals. The aneuploidy incidence in domestic ruminants has been little investigated and most of the studies are focused on bovine and limited to few chromosomes, whereas oocytes aneuploidy has not been investigated in buffalo so far. *In vitro*-matured Metaphase II (MII) oocytes with corresponding first polar bodies (PB) from Chinese river buffaloes (*Bubalus bubalis*, 2n = 50, XX) have been investigated in Murrah breed to provide a preliminary analysis of aneuploidy incidence.

Sample collection has been achieved at Guangxi Buffalo Research Institute (Nanning, China). A total of 60 cumulus-oocyte complexes (COCs) were collected through needle aspiration from ovaries of slaughtered buffaloes. After stereoscopic examination, COCs were washed in 1x PBS and transferred in 50µl maturation droplets made of TCM199 supplemented with 10%FBS, 0.5µg/ml FSH and 5µg/ml LH for 24h at 38.5 °C and 5% CO<sub>2</sub>. After maturation, COCs were washed in a hyaluronidase solution (1mg/ml) and exposed to hypotonic solutions (Na-Citrate 0.8% and KCl 0.5%). Oocytes were individually fixed on microscope slides by methanol/acetic acid fixative (1:1). Chromosome-specific painting probes (1q, 5q and Xcen) were prepared by chromosome micro-dissection and two DOP-PCR amplification (standard + direct labelling). Aneuploidy was screened by tri-colour Fluorescent In Situ Hybridization experiments using a Nikon E1000 microscope.

Ten oocytes out of 60 did not reach the MII stage, therefore the efficiency of the *in vitro* maturation process was 83%, whereas in 5 of them chromatin of the first PB was not found and they were excluded from the analysis. The final number of slides successfully analyzed was 45. One oocyte resulted in disomic for the Xcen (2.22%). This incidence is almost 10-fold higher than the average level of disomy for the X-chromosome observed in domestic cattle (0.25%) but agreement with the total aneuploidy level (2.25%) of *Bos taurus*. An unreduced diploid set of chromosomes was detected only in one oocyte (2.22%).

Further studies are necessary to extend the knowledge on aneuploidy in buffalo breeds to assess the impact on productive and reproductive efficiency in relation to climatic changes and environmental hazards. In addition, aneuploidy knowledge could be useful for improving the *in vitro* production of embryos destined to transfer.

O112

# Hermetia illucens reared on selenium enriched substrates for designing fortified insect meal

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Selenium is an essential mineral element for the life of animals. It is involved in the functioning of several enzyme systems and its deficiency in the diet can lead to the onset of different diseases such as white muscle disease in cattle and sheep, exudative diathesis in chicken and myopathy in horses. For this reason, selenium deficiency must be avoided. Biofortification represents a quick strategy to increase the amount of selenium always considering that deficiency doses and toxic ones are closely related. Several examples are known in the literature that deal with the biofortification of yeasts, but no studies are present on insects. The aims of this study were i) the evaluation of selenium bioaccumulation capacity of these insects in order to create fortified insect flours for specific feed purposes and ii) the investigation of selenium speciation applying an ICP-MS/HPLC-ICPMS method. The larvae were reared on three different substrates: i) Gainesville diet, used as control substrate (CTR), ii) *Ascomyllum nodosum* diet (AN30%), with 30% substitution of the alfalfa meal with brown algae and iii) selenium diet (Se), based on Gainesville diet fortified with 0.3 mg·kg<sup>-1</sup> of selenium in the form of sodium selenite. Eggs collected from a stable colony of BSF were placed for hatching on the three experimental substrates. All experiments were carried out under dark conditions, at 25 °C with 70% relative humidity. The mean total selenium content in the prepupae reared on Se enriched substrate (Se diet) was more than 5 times higher compared to BSF larvae reared on CTR and seaweed enriched substrates (AN30% diet). Considering the selenium speciation analyses, in all experimental groups, the main part of selenium content (>50%) was in the form of organic selenium. Furthermore, the main part of inorganic selenium was in the form of selenite with an average percentage of 38.6%. These results could indicate



that BSF larvae reared on specific/selected rearing substrates are able to convert inorganic Se to organic Se. The overall results show that both the seaweed and sodium selenite inclusion affect the BSF prepupae total Se and selenium species.

## O113

### Management strategies reduce antimicrobial use in Italian beef farms

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Animal production is a principal contributor to the increase of antimicrobial resistance, thus a more prudent use of antimicrobials (AMU) is required. The development of cost-effective strategies may help to reduce AMU in the livestock sector. The efficacy of biosecurity measures, such as the practice of quarantine, in preventing the introduction and spread of diseases on-farm are well known. Recent studies highlighted the role of these measures in minimising AMU, however, their effectiveness in beef production has been poorly studied. This study aimed to investigate the effect of quarantine in beef farms as a management strategy to reduce AMU. The study was carried out in 5 Italian specialised beef fattening farms. A total of 1206 Charolaise heads (576 males and 630 females) were included in the trial. Within the farm, roughly half of the animals were randomly assigned to the control group (NO-QUA) which followed the standard procedure of the fattening cycle without an initial quarantine, and the other half to the experimental group (QUA) which followed a 30-day-period of quarantine. Bodyweight (BW) at slaughter and information on the number of treatments administered per animal, amount (ml) of antimicrobial per treatment and reason for its administration were recorded. A treatment incidence of 100 (TI100) per animal was calculated. Data were analysed using the software SAS version 9.4. Approximately 57% and 43% of the total parenteral treatments ( $n = 675$ ) administered to the animals were reported for NO-QUA and QUA groups, respectively. However, a significant difference of TI100 and BW between groups was reported for males only, with NO-QUA having greater TI100 (3.76 vs. 3.46;  $p = .033$ ) and lower BW (713.4 vs. 723.7 kg;  $p = .033$ ) than the QUA group. The main reason for AMU was locomotion

disorders (58%) likely due to the high BW of Charolaise breed or inadequate flooring systems, followed by respiratory diseases (37%). Overall, significant differences between groups were observed in this study, suggesting that quarantine can be an effective management strategy to reduce AMU in beef production without compromising animal performance. This practice seems to be particularly efficient in males likely due to their higher susceptibility to diseases, thus other strategies should be investigated to reduce AMU in females too.

#### Acknowledgements

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## O114

### Effect of mixing animals in France on the use of antimicrobials in Italian beef fattening farms

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Since their discovery, antimicrobials have contributed to treat infectious diseases. However, several studies have highlighted an association between antimicrobial use (AMU) and the development of antimicrobial resistance, a threat to both human and animal health. Italy is the main European importer of young beef bulls and heifers from France. Before transportation to Italy, animals are collected by exporter companies from farms located across the country and mixed. The practice of mixing animals from different farms is known for its negative effects on animal health and welfare, such as the increased incidence of bovine respiratory disease. The aim of this study was to investigate the effect of the initial mixing of beef cattle in France on AMU in Italian fattening farms. The study was carried out using the information of 25 Italian specialised beef fattening farms associated with a producer organization of Veneto region (AZoVe, Cittadella, Italy), and included approximately 50,000 male and female animals of Charolaise and Limousine breeds imported from France from 2015 to 2018. In vivo performances and information on animal ID, a number of treatments administered per animal and amount of antimicrobial per treatment (ml) were available. A treatment incidence of 100 (TI100) per animal was calculated to quantify AMU. Each animal belonged to a batch defined as a group of individuals of the same breed and sex and similar entry body weight in the fattening farm. To investigate the effect of mixing



on AMU, each batch was assigned to 1 of 6 classes created according to the French province of origin. Specifically, class 1 contained batches of animals collected from 1 province only, and so on up to 6 provinces. Data were analysed using the software SAS 9.4. Overall, males were more treated than females (TI100: 4.15 vs. 3.56;  $p < .05$ ), and Limousine tended to receive more treatments than Charolaise (TI100: 4.06 vs. 3.62;  $p = .09$ ). The highest TI100 was observed in autumn and the lowest in spring (3.98 vs. 3.62;  $p < .05$ ). The practice of mixing animals from different provinces was significant in explaining the variation of AMU ( $p < .05$ ), with class 1 having lower TI100 compared to other classes; this suggests that reducing a priori the number of provinces where animals are originally collected may contribute to decreasing AMU in beef production.

#### Acknowledgements

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## O115

### Response of weaned piglet's gut microbiota to the dietary inclusion of different doses of extracted olive oil wastewater

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Weaning is a delicate phase in a piglet's life, owing to more stress and immaturity of the gastrointestinal tract. These factors favor intestinal dysbiosis, an alteration of the microbiota equilibrium, recently reported as a leading cause of post-weaning diarrhea. The intake of polyphenols could modulate the gut microbiota, promoting the growth of beneficial bacteria. The aim of this study was to understand the effect of olive oil wastewater (OOW) on growth performance and fecal microbiota profile post-weaned pigs. At  $7.40 \pm 1.22$  kg of body weight (BW) (mean  $\pm$  SD), 48 post-weaning piglets ( $25 \pm 1$  day of age) were randomly assigned within litters, sex and genotype for *Escherichia coli* F4 susceptibility (Mucine4) to three dietary treatments. The CON group (basal diet), OOW1 (basal diet +0.5% of OOW) and OOW3 (basal diet +2% of OOW). The growth performance and fecal score were daily monitored. From each subject, a fecal sample was collected on the day (d) 5, d10 and d19 post-weaning. The 16S rRNA V3-V4 amplicon was amplified and sequenced with the Illumina MiSeq platform. Results showed no effect on BW, however, ADFI and

ADG of OOW1 were higher ( $p = .02$ ,  $p = .06$ ) compared with CON from d8 to d19. The fecal score was lower in OOW3 ( $p = .03$ ) at d7 and d10 post-weaning. The inclusion of OOW in the diets significantly increased bacterial alpha diversity indices for the overall period of the study ( $p = .009$ ). Whereas analysis of individual days, alpha diversity was not influenced by treatment on day 5, moreover is visible an increase in alpha diversity for treatments OOW1 and OOW3 at d10 and d19. Overall, microbial composition was influenced by treatment ( $p = .002$ ), day of sampling ( $p = .001$ ) and genotype ( $p = .01$ ). In both OOW treatments there was a reduction on *Escherichia-Shigella*, (OOW1 vs. CON, P-adj  $< .001$ , Log2FC = -4.22; OOW3 vs. CON (P-adj = .01, Log2FC = -2.80). Whereas, greater abundance of *Campylobacter* was recorded in CON group (OOW1 vs. CON, P-adj  $< .001$ , Log2FC = -4.85; OOW3 vs. CON, P-adj  $< .001$  and Log2FC = -4.71). In conclusion, the OOW's supplementation in piglets' diet had a positive impact on ADFI and fecal score. Additionally, OOW reduced the relative abundance of some negative bacterial genera frequently associated with post-weaning diarrhea of piglets.

## O116

### LEO project: developing a national open data repository to support livestock biodiversity, health, sustainability, and welfare

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LEO (Livestock Environment Opendata) is an Opendata Platform for Animal husbandry, funded by Ministerial Decree no. 0020929 of 10/05/2019, within the National Rural Development Program 2014/2020, Sub-measure: 16.2, 'Support for pilot projects and the development of new products, practices, processes, and technologies'. The project will create a unitary database that merges existing and novel information on the environment, animal health and welfare, climate, productive and reproductive performances, etc. The ultimate goal is to enhance knowledge and help overcome livestock production's future challenges as sustainability, climate changes, safety, and biodiversity protection. The six-year project is organized into five actions: action 1, identifies and validates novel parameters; action 2 collects data at the national scale; action 3 performs big data QC; action 4 built the open linked data platform; and action 5 is devoted to broad dissemination. We expect to collect and store over 20 Terabytes of raw data that will be made available as Open Linked Data level 5. Up to now, LEO includes 18,194 livestock farms and is collecting more than 60 novel parameters on  $4.4 \times 10^6$  animals. Livestock Biodiversity in LEO counts 109 breeds, of which 103 are local and

autochthonous. In the first 36 months, LEO stored 183,680,724 data at an average rate of  $54 \times 106$  for laboratory data and  $35 \times 106$  for field data per year. Five external databases, i.e. BDN (National Animal Identification Data Bank), Siall (Official Performance recording database), Ist. Spallanzani (Reproductive and semen quality database), IFCQ (Parma and San Daniele Ham certification agency) and DQA (Mozzarella di Bufala certification Agency) are sharing their data with LEO in applicative cooperation mode. A multidisciplinary partnership ranging from Universities and Animal Health departments to breeder associations and computer engineering is collaborating actively to achieve the objective. The website at [www.Leo-italy.eu](http://www.Leo-italy.eu) reports up-to-date info about the project.

## SESSION 23 – GENOMICS OF LOCAL BREEDS

### O117

#### Genome-wide diversity of African and Eurasian fat-tailed sheep

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Genome-wide SNP arrays have been largely used not only to investigate the origin and extent of breed diversity but also to determine genomic regions that might explain important phenotypic traits. In this study, thanks to a large informal collaborative effort, we collected the most comprehensive dataset of African and Eurasian fat-tailed sheep breeds, with the aim of understanding the evolutionary scenarios behind the dispersal of the fat tail phenotype and identifying genes involved in this trait. The dataset comprises 162 sheep breeds (3013 individuals), including 67 fat-tailed and 95 thin-tailed sheep breeds from Eurasia and Africa. We performed the main population genetic analyses to assess the distribution of genetic diversity (genetic indices per population, multidimensional scaling plot and admixture analysis), while phylogenetic and comparative methods were used to assess genetic relationships among breeds and to evaluate a possible origin of the fat tail phenotype. The observed genetic structure

was in accordance with the geographic origin of most of the sheep breeds. The genomic components at lower  $K$  values reflect post-domestication processes that led to breeds differentiation in Europe, Asia and Africa with different levels of admixture indicating peculiar evolutionary histories. However, the fat-tailed sheep breeds did not appear to cluster in a specific genetic group. The trait is hence hypothesized to have evolved multiple times independently in different geographic areas, accompanied by extensive dispersal of the fat-tail genetic material. Our results provide novel insights into the genomic origin and diversification of fat-tailed sheep helping to add important information for possible conservation and genetic improvement strategies.

### O118

#### GWAS of fitness and productive traits in the local Valdostana breeds

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The advent of the genomic era has allowed for the creation and implementation of powerful analytic tools for animal breeding. Such genome-wide analyses have two purposes: shedding light on the molecular basis for traits of interest (both productive and fitness traits) and helping animal breeders to select individuals based on the information from their genome. Both features are extremely interesting in local breeds, which are often composed of a relatively low number of individuals with a unique genetic background. Considering the local Valdostana cattle breed (Aosta Chestnut and Aosta Black Pied strains) as a study subject, we aimed to run a Genome-wide Association Study (GWAS) to detect associations among genomic regions and both productive and fitness traits of interest.

We used a 150K SNP markers panel to profile the genotype of more than 900 individuals, in order to investigate somatic cells count, longevity, fertility as fitness traits, and test-day milk yield, and the morphological factorial scores for muscularity and udder volume as productive traits. We performed the GWAS with freely available programs within the BLUPF90 package. Specifically, we used the single-step ssGWAS approach, able to combine information of both individuals with known phenotype and/or genotype. Therefore, phenotypic information of a number of individuals ranging from 23470 to 37411 was joined to the available genotypes to run the association analyses. We then performed post-GWAS

analysis, to shed light on the molecular pathways or mechanisms underlining the variability of the trait. Firstly, we inspected the position of each SNP via UCSC Genome Browser and we determined if it was located within or close to a gene or another functional DNA element. Then, we examined the role of the element in any molecular pathway via databases such as Gene Ontology and KEGG. Finally, we evaluated how each specific pathway or molecular element could shape the phenotype of interest.

We detected some significant associations between SNPs and key fitness and productive trait. As an example, we found that SNPs situated within the MCM9 gene was linked to udder physiology. Polymorphisms in this gene have been found to be linked to estrous behaviour in cattle and several ovarian pathologies in humans. Our results underscore the power of GWAS and give insight into the genetic architecture of key traits in local breeds.

## O119

### Genome-wide search of distinctiveness selection signatures in Maremmana cattle compared to Italian Podolian-derived breeds

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Several Italian breeds such as Marchigiana, Romagnola, Chianina, Calvana, Podolica, Cinisara, Modicana and Maremmana belong to the so-called 'Podolian' group. The Maremmana is one of the most distinctive cattle breeds because it is raised in semi-feral conditions and it still displays many ancestral features such as horn shape and size, sexual dimorphism and longevity. The aim of this study was to map genomic regions that might explain the phenotypic distinctiveness observed in Maremmana cattle compared to the other Podolian-derived Italian breeds. Putative selection signatures in Maremmana were investigated using genotypes generated for 146 Maremmana and 174 individuals belonging to Podolian-derived Italian breeds using the Bovine SNP50K v2 BeadChip and combining three statistical approaches designed to quantify the excess of haplotype homozygosity either within (iHS) or among pairs of populations (Rsb and XP-EHH).

We used  $-\log_{10}(p\text{-value}) = 2.5$  as a threshold to define significant iHS, Rsb and XP-EHH candidate regions. Multidimensional scaling and neighbor-joining tree revealed a clear separation between Maremmana and the other Podolian-derived breeds. Overall, the three haplotype-based analyses revealed selection signatures distributed over 19 genomic regions. Of these, six regions spanning 87 known genes, were identified by at least two approaches. Within these regions, we found genomic signatures of selective sweeps spanning genes related to muscle development, growth and meat traits (SCIN, THSD7A, ETV1, UCHL1 and MYOD1) which reflects the different breeding schemes between Maremmana and the other Podolian-derived breeds. We also identified several genes possibly linked to Maremmana adaptation to the environment of the western central part of Italy, known to be hyperendemic for malaria and other tick-borne diseases. These include several chemokine (C-C motif) ligand genes crucially involved in both innate and adaptive immune responses to intracellular parasite infections and other genes playing key roles in pulmonary disease (HEATR9, MMP28, ASIC2) or strongly associated with malaria resistance/susceptibility (AP2B1). Information about the location of these regions can be used to detect variants that may underlie important traits of practical interest for the beef industry as well as to enhance our understanding of the genomic basis of the millenarian environmental adaptation of Maremmana to the semi-wild conditions of its rearing cradle.

## O120

### Whole-genome resequencing reveals specific genomic variants in Italian insular sheep breeds

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The development of high-throughput sequencing technologies revolutionized the way genetic variation studies are assessed. In livestock, whole-genome sequencing approaches with pooled DNA have recently developed into a powerful tool to identify genetic divergences among breeds. In this study, we applied pooled whole-genome sequencing to reveal differentiation and specific genomic variants in five Italian insular sheep breeds: Barbaresca (BAR), Comisana (COM), Pinziriti (PIN), Sarda (SAR) and Valle del Belice (VDB). Each pool consisted of 30 animals. These breeds



present differences in both morphology and production traits. In the Principal Component Analysis, the first dimension (C1) well-separated BAR from other dairy sheep breeds. As expected, relatively close relationships were observed among the dairy Sicilian sheep (COM, PIN, and VDB). A long genetic distance was found with the SAR breed. The average transitions to transversions ratio was 2.55 for all sheep breeds and similar to the values observed in other mammalian genomes, indicating relatively low potential random sequencing errors. We observed the largest number of single-nucleotide polymorphisms (SNPs) in SAR (9,434,843) and BAR (8,943,103), reflecting their genetic differentiation. In contrast, the COM showed the lowest number of SNPs (4,351,307). All breeds showed that about 9% of these SNPs were novel. There was approximately one variant every 260 base pairs (bp) for BAR, PIN, SAR and VDB, and one variant every 521 bp in COM. A total of 5,799,001 unique SNPs were identified among the five breeds, ranging from 419,211 (COM) to 1,817,926 (SAR). All the sheep breeds showed 4% of insertion and deletion compared to all variants discovered. SnpEff was used for functional annotation of identified autosomal SNPs based on the Ensemble sheep genome assembly Oar\_v3.1.99. Most SNPs were intergenic or intronic, with approximately 1% located in the remaining genic regions. These markers represent the most important subset of SNPs as probably associated with changes in protein sequences, structures and functions. An advantage of genotyping by sequencing with respect to medium or high-density SNPs arrays is that it allows detecting selection signatures over relatively short distances. The results provide an improved understanding of genetic diversity for these sheep and will facilitate the identification of genomic regions involved in the phenotypic variation among breeds.

## SESSION 24 – FEED EFFECTS ON NUTRITIONAL PROFILE OF ANIMAL PRODUCTS – II

### O121

#### Milk nutritional quality and rumen microbial community of Holstein-Friesian cows fed a diet supplemented with olive oil pomace

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Olive oil pomace (OOP) is a by-product derived from the olive milling process. Its disposal in the environment is complex and onerous but, in animal feeding, it may represent an interesting source of bioactive compounds for its chemical composition. Pomaces are rich in soluble polyphenols and functional fatty acids (FAs) as oleic acid. In literature, several authors reported positive effects of polyphenols in modulating rumen microbiota lowering methanogenesis and enriching milk with unsaturated FAs. Thus, 40 lactating Holstein-Friesian cows were randomly allotted in two groups (20 cows per group) and fed a basal diet (control, C) or the same diet supplemented with OOP (8 g/100g dry matter head and day, T). After two weeks of dietary adaptation, milk yield was daily recorded during the next 30 days. Milk samples were individually collected weekly (4 times of sampling: T7 d, T14 d, T21 d, T28 d) and analyzed for clotting parameters, chemical and nutritional characteristics, and FA profile. At the end of the trial, rumen liquor was collected for rumen microbial community analysis. No differences were found for milk chemical and nutritional parameters and ruminal bacterial taxonomic composition. Anyway, the difference in the relative abundance of specific bacterial taxa was observed and a significant delay was evaluated for the milk clotting time in the T group with respect to C. Concerning the fatty acid profile, saturated fatty acid concentration was lower in the milk from the T group than in C, while functional fatty acid, such as vaccenic (0.622a vs. 0.401b;  $p < .0001$ ), oleic (9.819a vs. 8.055b;  $p = .0043$ ), and conjugated linoleic acids were more concentrated in the milk from T group with respect to the one from C (0.226a vs. 0.116b  $p < .0001$ ). These findings highlight the effect of OOP on rumen microbiota metabolism.

### O122

#### Metagenomics of milk before, during and after summer transhumance to highland pasture in relation to human health and cheese making properties

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Summer transhumance is a traditional form of pastoralism to highland pasture in the dairy system of alpine regions and it is practiced all over the world. Positive influences can be obtained



for the environment, society, tourism, animal health and welfare. The aim of this study was to analyze the evolution of the milk microbiota before, during, and after summer transhumance to highland pasture in relation to possible effects on animal/human health and cheese-making properties. The study involved 12 healthy, multiparous, mid-lactation Brown Swiss cows reared in a farm located in the Trento province (Northern East Italy). The cows were monitored from June to October and divided into two groups: six cows were used as a control group and kept in the lowland permanent farm (PF); the other six cows were moved to a temporary alpine farm (ALP) from July to September. From each milk, samples were collected every four weeks for genomic DNA extraction and microbiota characterization using community 16S rRNA amplicon (V3–V4 region) based Illumina Miseq sequencing and QIIME2 (2018.2 version). The relative abundance was analyzed after a  $\log_{10}$  transformation and identified species were classified into two unfavorable categories: Spoilage and Pathogenic species, and two favorable categories: Probiotics and Dairy species. For the statistical analysis, we used a linear mixed model, where the combined Month  $\times$  Group (MG) effect was used as fixed and the cow effect as random. The results revealed a different behavior in the ALP group compared to the PF group during the 3 months of summer transhumance. The Spoilage bacteria tended to decrease in the alpine pasture (MG effect:  $p < .001$ ), with significant differences in terms of *Pseudomonas*, *Alicyclobacillus*, and *Clostridiales* ( $p < .001$ ,  $p < .001$ ,  $p < .05$  respectively). Pathogenic did not show large differences between the two groups; instead, the Probiotic category, which includes *Propionibacterium* and *Bifidobacterium*, showed a gradual increase in the ALP group ( $p < .001$ ). Dairy species also had a significant increase in the ALP group ( $p < .001$ ), especially *Lactococcus* and *Lactobacillus* (both  $p < .001$ ). All the microbiological changes disappeared when cows were moved back from alpine pasture to the permanent indoor farm. Summer transhumance to alpine pasture, therefore, has a favorable effect on the milk microbiota, with positive implications for both the cheese-making attitude of milk produced and its possible effect on human health.

## O123

### Dehydrated sulla forage in dairy ewes feeding: effects on cheese properties

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Feeding green sulla forage (GSF) (*Sulla Coronarium* L.), a short-lived perennial legume containing phenolic compounds, especially condensed tannins, showed to increase intake, milk yield and casein level in ruminants, and enhance fatty acid (FA) profile and antioxidant activity of dairy products. To obtain analogous favourable effects using conserved sulla forage, dehydration could be a valid alternative to the haymaking process which causes greater losses of biomass and its nutritional and bioactive compounds. The aim of this research was to evaluate the dairy products from ewes fed with pelleted dehydrated sulla forage (PDSF) in the diet by comparisons with those obtained using sulla hay (SH) and GSF based diets. Twenty primiparous (PR) and pluriparous (PL) milking Valle del Belice ewes were allocated homogeneously into 5 groups and fed 5 diets in a partial  $5 \times 2$  Latin square design with 2 periods. The diets differed for the forage component: SH ad libitum (SHL); 2 kg/d per ewe of PDSF and SH ad libitum (DSF); 2 kg/d of GSF and SH ad libitum (2GSF); 4 kg/d of GSF and SH ad libitum (4GSF); GSF ad libitum (GSFL). Concentrate feed was supplied to PR (0.8 kg/d) and PL (1.2 kg/d) ewes in the function of their different production needs. The results confirmed the superiority of diet in which GSF was the only forage source offered ad libitum, as occurs with grazing; in fact, the GSFL diet resulted in higher milk yield and casein content and enhanced in cheese the number of polyphenols and antioxidant vitamins A and E, the oxidative stability, and the level of health polyunsaturated FA (PUFA) such as rumenic, the main of conjugated linoleic acids (C18:2c9t11), and  $\alpha$ -linolenic (ALA, C18:3n-3). On the whole, the DSF diet showed better results than SHL and was comparable to the 4GSF diet for milk yield and casein level and, in cheese, for external colour and vitamin A and total PUFA contents; moreover, ALA was detected at the same high level in DSF and GSFL cheeses. Thus, dehydration seems to represent a valid opportunity to exploit the considerable potential of sulla forage, and maintain adequate production levels and nutritional and health properties of milk and cheese in periods of insufficient grazed green resources.

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## O124

### Use of different levels of hemp meal supplementation in diets of veal calves: effects on *in vivo* performances and metabolic profile

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During the last years, interest in hemp (*Cannabis sativa* L.) has increased due to its nutritionally beneficial effects on human and animal nutrition. In particular, hemp seeds and hempseed meal (HM), the ground product obtained by the cold mechanical extraction of oil, are good sources of crude protein, lipids and several bioactive compounds (i.e. phenolic compounds, bioactive peptides, etc.). This study aims to evaluate the effects of two levels of HM supplementation on the performance and health status of veal calves during a 184-d fattening trial. Forty-eight cross breed Belgian Blue calves (35 males, 13 females) were allocated in 12 pens, balanced according to sex and initial BW ( $62.0 \pm 4.5$  kg) and were assigned to three dietary treatments: 0% (T0), 3% (T3), and 6% (T6) of HM on the total ingested DM. The HM (CP =33.7%, lipids =10.4%, iron =179 ppm, on DM basis) was added and mixed during the milk preparation. All groups received 2 meals of milk per day following a specific plan (in total 304.9 kg DM/calf of milk replacer) and 2 meals of solid feed, based on flakes cereals (in total 183.8 kg DM/calf). T3 and T6 groups received an addition of 13.2 and 26.4 kg DM/calf of HM, respectively. Calves were weighed five times and blood samples were collected four times during the trial. All data were analysed by GLM of SAS. No respiratory or gastrointestinal diseases were observed in calves during the experimental period. No differences in values of average daily gain were observed among three experimental groups (on average 1323 g/d). During the experiment, the iron concentration in plasma was similar among treatments (on average 65 µg/dl) but decreased significantly ( $p < .05$ ) from the first to the following samplings (101, 48, 46, and 43 µg/dl, at 0, 42, 91, 133 d, respectively). The hemoglobin (HGB) concentration in plasma was on average 9.15 g/dl ( $p = .023$ ), showing a pattern similar to that of iron (10.23, 9.41, 8.65, and 8.29 g/dl, at 0, 42, 91, 133 d, respectively). Concluding, the addition of HM in the diet of veal calves did not affect the growth performances of animals. The health status was satisfactory without pathologies and preserving a good plasmatic concentration of iron and HGB. Final considerations about the effect of hemp meal will be obtained by analysing behavioural data, obtained by video recording system, and post-mortem parameters. In particular, the possible carry-over effect of n-3 fatty acids from feed to meat is expected.

## O125

### Effect of genotype and diet on carcass and meat quality traits of broiler chickens

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Soybean (*Glycine max* L.) is the main source of plant-derived protein in the diet of poultry and other farm animals. Its constant

rising price has led to increased production costs and reduced sector's profitability. One source of protein that can replace soybean to some extent in the poultry diet is pea (*Pisum sativum* L.). A study with 120 male broilers (60 Kabir Rosso Plus and 60 New Red) was conducted on a family farm to evaluate the effects of diet including soybean (S) or pea seeds (P). Diets were iso-energetic and isonitrogenous. Birds were housed by 6 in 20-floor pens, with 5 replications/experimental groups. At 83 days of age, all birds were weighed and 40 of them, randomly chosen (2 for each replication), were slaughtered. At slaughter, weights of the carcass, pectoral muscle (PM), thighs and wings were recorded, and the respective yields calculated. On the right PM, pH, color and water holding capacity (WHC) were measured at 24 h post mortem. The left PM was vacuum packaged and stored frozen ( $-20^{\circ}\text{C}$ ) until chemical analyses. Data were analyzed by two-way ANOVA, including diet and genotype with interactions. Neither live weight at slaughter nor slaughter performance (carcass and commercial cuts as weights and yields) as well as pH, color, WHC and total lipid and cholesterol content of PM were affected by diet. Taking into account the fatty acid profile of PM, diet did not affect ( $p > .05$ ) saturated fatty acids (SFA), n-3/n-6 and n-6/n-3 ratios and atherogenic and thrombogenic indices. P diet increased the content of monounsaturated fatty acids (30.09 vs. 27.92%, for P and S, respectively;  $p < .05$ ). Compared to the P diet, the S diet increased ( $p < .01$ ) polyunsaturated fatty acids (PUFA: 33.64 vs. 30.48%, respectively) and n-6 PUFA content (31.06 vs. 28.22%, respectively), and PUFA/SFA ratio (0.88 vs. 0.77, respectively). Regarding genotype, New Red showed higher ( $p < .05$ ) values of final live weight and carcass traits than Kabir. A significant interaction ( $p < .05$ ) was found between factors for PM weight and yield: Kabir showed greater values when fed soybean; New Red had higher values when fed pea seeds. Meat from New Red had a higher ( $p < .05$ ) pH value compared to Kabir. Genotype did not affect ( $p > .05$ ) color, total lipid, cholesterol content and fatty acid profile of PM. In conclusion, replacing soybean with pea seeds in the chicken diet could represent an interesting farm management strategy, especially in those agronomic realities where it is produced. As expected, chicken genotype affected slaughter traits: New Red chickens showed greater productive capacity.

## O126

### Novel by-products in goat nutrition: effect on milk yield and composition

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The reduction of feed competition between animals and humans using by-product and waste products in animal nutrition is one

of the main goals in livestock production systems. However, by-products arising from the agro-industrial sector need to be studied and tested to guarantee their healthiness. Spent coffee (SC) is a by-product obtained after the preparation of coffee beverages and it represents a source of carbohydrate, protein, and phenolic compounds. The aim of this study was to evaluate the inclusion of SC, a good source of polyphenols, in goat's diets on milk production and composition, including FA profile. Moreover, the plasma biochemical parameters of ewes were determined to assess the healthiness of the by-product. The experiment was carried out with 24 dairy goats divided into three homogeneous groups. The three groups varied only for the presence of the SC in the diet: a group (CON) received the control diet without supplementation, the second group (SC50) received 50 g/d/head of SC and the third group was fed with the control diet + 100 g/d/head of SC. The study lasted 5 weeks with 1 week the adaptation period. Milk yield was measured, and milk samples were collected weekly, blood samples were collected at the beginning and the end of the experimental trial. Linear and quadratic contrasts were used to evaluate the effect of by-product dose. SC did not affect milk yield ( $p = .67$ ) and milk fat and protein content ( $p = .90$ ). However, some of the milk fatty acid (FA) were influenced by the SC dose: some isomers of C18:1 and the CLA cis-9, trans-11 resulted influenced linearly. Some the FA groups, such as OCFA, OBCFA, BCFA and total CLA showed a linear increase, suggesting an influence of this by-product on rumen metabolism. Most of the biochemical parameters were not affected by SC treatments except for basophils and eosinophils that change quadratically ( $p < .05$ ), even if both fall in the normal range for goats. In conclusion, the SC in the dose until 100 g/d, could be used in goat's diet without negative effect on milk yield and composition and animal health status.

## SESSION 25 – PRECISION LIVESTOCK FARMING – II

### O127

#### Use of a portable NIR instrument as a rapid tool for cattle feeding control

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Avoid feed sorting and providing a homogeneous ratio is a crucial aspect of cattle feeding. We evaluated Near Infra-Red (NIR) as a tool for assessing TMR's homogeneity (Hi), animals' sorting (Si) and their relationship with mixer wagon (MW) and ration features. A dataset of 311 TMR samples was split into 55% testing and 45% validation sets. Samples were analyzed for chemical composition, particles size (PS) distribution through a 6-strata sieve and geometric mean length (GML). A portable NIR instrument was calibrated in a range of 902–1660 nm on the testing set by a partial least square (PLS) algorithm and validated

on the training set (v). An observational open cohort study of 19 dairy farms was conducted over two years. MW characteristics, TMR formulation, and milk yield were collected through a survey and TMR analysis was performed by NIR. The Hi (range 0–100%, 100 = perfect homogeneity) was calculated as the weighted sum of the standard deviation to mean ratios of TMR composition recorded along the feed alley at 16 points. In these 16 points, the Si (range 0–1, 0 = no selection) was calculated by comparing values of fresh distributed TMR with those collected after 2 h from feed distribution, by a  $t$ -test and a weighted coefficient correction. Parameters used for Hi and Si calculation were PS = 3.8 and 1.8 mm, pan, GML, crude protein (CP), aNDF, and starch. The median was used as a threshold for Hi and Si binary classification as inhomogeneous (Ibhi,  $Hi \leq 79\%$ ) or homogeneous (Hbhi), and as negligible selection (NSbsi  $\leq 0.30$ ) or evident selection (ESbsi). Logistic and linear models assessed the outcomes (Hi–Si) for predictors. ROC curves were drawn. In NIRs calibration, the coefficient of determination of validation ( $R^2$ ) was 0.95, 0.74, 0.93, and 0.82 for DM, CP, aNDF, and starch, respectively. The PS traits showed all  $R^2$  between 0.68 and 0.82. The logistic regression for binary Hi showed odds = 1.72 ( $p = .03$ ) and 0.39 ( $p = .06$ ), Akaike's information criterion (AIC) = 16.6 and 20.8, Area under the curve (AUC) = 0.89 and 0.79 for DM and aNDF, respectively. The Hi regressed for MX load fullness with intercept = 96.0, coefficient = -0.19, adjusted  $R^2 = 0.374$  ( $p = .02$ ). The logistic regression for binary Si showed odds = 2.57 ( $p = .06$ ), AIC = 19.5, AUC = 0.82 for aNDF. These findings confirm NIR as a reliable instrument for at farm TMR analysis and suggest a relation between aNDF and both Hi and Si and between DM and MX load with Hi.

### O128

#### Precision Buffalo Farming: preliminary results from the AGRIDIGIT project

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The precision farming principles are catching on all over agriculture sectors. Buffalo farming sounds still a step behind in this process, probably due to its low spread in the more advanced countries. The aim of this communication was to show some preliminary results streaming out of AGRIDIGIT project that includes a specific WP on smart buffalo farming, comparing buffalo behavior during the hottest and coldest months. An ear tag sensors system was used on 40 Mediterranean Buffaloes, farmed at Monterotondo (CREA). High activity (HA), activity (A), staying (S), eating (E), ruminating (R) and external temperature (T)



and humidity (H) were continuously recorded and analyzed, over the lactation (July to January). Data were analyzed, by a non-parametric regression, to check for common behavior patterns and then by the following linear model: (HA, A, S, E, R) = hour + month + hour \* month; which allows, for each independent variable, the reconstruction of the daily evolution as a function of the month. A strong pattern was found for each variable, with a trend to progressive variation, from the hottest to the coldest month. In July ( $T_{max} > 40^{\circ}\text{C}$ , mean  $25.8^{\circ}\text{C}$ ,  $67.2 < \text{THI} < 78.18$ ), animals concentrated their activity (early morning and late afternoon). At 5 am HA was 4.27 vs. 17.34 in January and July respectively and at 5 pm 11.41 vs. 25.95 min/hour ( $p < .01$ ). In winter range of activity was wider and trend flatter. Nevertheless, total movement (HA + A) was the same in the two opposite months ( $25.65 \pm 0.24$  vs.  $25.67 \pm 0.19$  min/hour in January and July). This may be due to the intensity of the HA peaks. Rumination was higher ( $p < .01$ ) in hot season ( $9.46 \pm 0.13$  min/hour in July), while eating had the same trend as moving. Probably when eating increases also high activity does, being animals involved in movement along with the manger and in competition fighting for position. In conclusion, buffalo seems sensitive to high temperature even though it is supposed more resistant than a cow, showing a modified behavioral pattern in comparison to mild (autumn) or cold periods. In particular, rumination increased and eating and movement concentrated. The cold period seems associated to more regular and distributed behavioral pattern letting suppose a certain comfort compared to hot, but we must consider that no critical temperatures were reached during observations ( $T_{min} -7^{\circ}\text{C}$ , average  $6.4^{\circ}\text{C}$ , in January). In mild season (data not reported) values were intermediate between the extremes.

## O129

### Precision livestock farming and beekeeping: development and testing of a prototypal smart hive

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With the exponential growth of the world population, there will be an increase in demand for products of animal and plant origin that will have to be met in a sustainable way due to the limited availability of land and resources. One of the solutions can be represented by the use of innovative monitoring and control approaches on production systems. The affirmation of these new technologies has given rise to precision agriculture and livestock farming, which is a way of managing agricultural activity by

monitoring and recording automatic measurements in real-time. Precision beekeeping is the monitoring of the hive to provide useful information to beekeepers such as the in-hive temperature (IHT) and humidity (IHH), hive weight (HW), and flight activity (FA). The aim of this work was the experimental validation of a prototypal precision beekeeping hive, named SMART HIVE\_1.0, which allows the monitoring of all these processes already listed. The temperature and humidity control system was validated with a comparison with reference measurements by a thermo-hygrometer; a correction model was developed that allows inferring the true IHT and IHH data through equations that had an  $r^2$  not less than 0.99. As far as the HW, some tests were carried out with increasing ballasts in the range 0.1 g–14.0 kg. It resulted that the electronic scale gave a 13% underestimated measure of the true HW, with a mean deviation of –3 kg. An HW correction model was thus obtained with  $r^2$  equal to 0.9998. The ongoing and outgoing bee flight accuracy was assessed by using a manual bee simulator and by using manual counting of bee flight recorded through a webcam. Both methods allowed us to assess that the flight sensor was unreliable being affected by a high asymmetrical bias unevenly distributed within the range of the flight activity video recorded that make impossible to find a simple correction model to be implemented at the hive and/or server level. Further efforts are needed to get a reliable flight counter that allows for a deep evaluation of the in/out balance of foraging bees, information that can make aware the beekeepers of some possible undesired loss of honey bees during the production season.

## SESSION 26 OMICS TECHNOLOGIES – I

## O130

### An alternative method to identify CSD alleles of the honey bees based on NGS data

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Focus of this study is the allelic variability of the complementary sex determination (CSD) gene, which regulates sex determination in honey bees: males are hemizygous for CSD, while females are heterozygous. Diploid males develop from fertilized eggs, which are homozygous for CSD; diploid males are eaten by worker bees in the larval stage. Therefore, there is a strong advantage for heterozygotes leading to a high number of distinct csd alleles. A hypervariable region (HVR), located in exons 6 and 7, is responsible for csd allelic variability. One hundred twenty-five worker colonies were sampled within summer 2017 and summer 2018



in twelve different Italian regions: Abruzzo, Emilia Romagna, Liguria, Lombardy, Marche, Piedmont, Sicily, Trentino, Tuscany, Apulia, Umbria and Veneto. In each region, beekeepers were chosen among those belonging to the 'Associazione Italiana Allevatori Api Regine (AIAAR)' and the 'Albo Nazionale Allevatori Api Italiane' associations. Genomes of the 125 worker colonies were sequenced through Illumina NextSeq with a 150 bp paired-end module. Sequences were mapped to the HAv3.1 *Apis mellifera* genome and the HVR of the CSD gene was manually inspected with the Integrative Genomics Viewer. The nucleotide sequences of both strands of this region were successfully reconstructed for 65 samples, and each strand was translated to obtain protein sequences for each allele of the heterozygous sequenced individuals. The resulting 130 sequences include variants of the HVR region which have not been described in literature, yet, contributing to characterize CSD allelic variability in Italian honey bee colonies.

#### Acknowledgements

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## O131

### Investigating the effects of diets enriched in PUFA and antioxidants on the gene expression networks and intramuscular fatty acid composition in porcine *Longissimus thoracis et lumborum* muscle

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Western diets are rich in n-6 and deficient in n-3 polyunsaturated fatty acids (PUFAs), resulting in a high n-6/n-3 ratio. Decreasing the n-6/n-3 ratio of meat products with n-3 feeding supplementations in livestock has been suggested as a viable strategy to increase meat nutritional value, but it also rises meat susceptibility to lipid oxidation. To date, novel antioxidants are available, and some of them can be obtained from by-products discarded by other food industries, such as grape skins. This study aimed at investigating in medium-heavy pigs the effect of high linolenic acid diets supplemented with synthetic or natural antioxidants on muscle fatty acid (FA) composition, and gene expression of *Longissimus thoracis et lumborum* (LTL) muscle. For this study, 48 growing-finishing pigs, balanced for sex and weight, were

assigned to 4 isoenergetic dietary treatments (12 pigs per treatment): barley, soya bean, (control, C); C with 5% linseed (L); L supplemented with vitamin E and selenium; L supplemented with grape skin and oregano extracts rich in polyphenols (L + natural antioxidants, LNA). Total RNA was extracted from LTL muscle and analysed with RNA sequencing. After sequence alignment, 10,169 genes were found expressed in the 48 samples and submitted for Weighted Gene Co-expression Network Analysis to WGCNA package in the R environment. The samples belonging to each treatment were analysed separately and associated with the amounts of FAs found in their LTL muscle. The linseed dietary inclusion significantly reduced the n-6/n-3 ratio and increased the n-3 PUFAs in LTL ( $p < .05$ ). These changes were also visible at the transcriptome level as the LNA diet caused great changes in the gene expression networks when compared to the gene co-expression structure identified in the LTL muscle of C pigs. In particular, the LNA supplementation stimulated the expression of genes involved in Type I interferon signaling pathway (Bonferroni  $p = 4.29E-10$ ) and Innate immunity (Bonferroni  $p = 1.17E-5$ ). This result agrees with the literature, which suggests that diets rich in n-3 and polyphenols may stimulate host immunity. On the other hand, the gene networks positively associated with the n-6/n-3 ratio in the C group were related to mitochondrial activity and cell energy metabolism. The obtained results support the evidence that supplementing pig diets with extruded linseed and polyphenols can positively influence the nutritional quality of meat products.

## O132

### Weighted Gene Co-expression Network Analysis identifies the main gene networks and biological pathways associated to chicken *Pectoralis major* myopathies

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High-throughput technologies and bioinformatics analysis represent both major tools to investigate gene expression patterns associated with pathological conditions in animal production. Growth-related myopathies affecting modern chickens (White Striping – WS; Wooden Breast – WB) have been deeply investigated in the past decade. Nevertheless, their precise etiology remains unclear. The present work aims at exploring the molecular mechanisms potentially involved in the onset of WS and WB, by identifying clusters of co-expressed genes associated with phenotypes that in our previous work resulted highly related to

WS and WB condition ( $p < .0001$ ): breast weight (BW), middle height (H2mm), width (Wmm), protein content (PC) and cooking loss (CL). To do this, we performed a Weighted Gene Co-expression Network Analysis (WGCNA) using the microarray profiles and the meat quality parameters of 12 broiler Pectoralis major muscles (6 normal vs. 6 affected by WS/WB) obtained in our previous study. Among the 212 modules identified by WGCNA, the red, darkred, midnightblue, paleturquoise4, turquoise, and yellow2 were found as the most significantly related to the considered traits. The darkred and midnightblue modules were positively related to BW, H2mm, and CL ( $r > 0.74$ ,  $p < .01$ ), while the red and paleturquoise4 were characterized by a negative correlation with the same phenotypes ( $r < -0.72$ ,  $p < .01$ ). The turquoise ( $r = -0.90$ ,  $p < .0001$ ) and the yellow2 ( $r = 0.91$ ,  $p < .0001$ ) were negatively related to PC and positively related to Wmm, respectively. The genes in these modules were submitted to DAVID online tool for functional annotation analysis. This analysis evidenced pathways involved in extracellular matrix (ECM) organization ( $p < .0001$ ), collagen metabolism ( $p = .04$ ), collagen fibril organization ( $p < .0001$ ), unfolded protein response ( $p = .01$ ), and ubiquitin-mediated proteolysis ( $p < .01$ ). The hub gene analysis highlighted several genes coding for ECM components as the most interconnected nodes in the gene network, with particular reference to collagen and laminin genes. Notably, the Collagen type IV alpha 1 (COL4A1) was found as the most significant hub gene, suggesting its possible implication in the development of WS and WB. In conclusion, the present work shows that abnormalities in ECM organization (e.g. collagen alterations) could be involved in the molecular mechanisms leading to the onset of growth-related myopathies in broilers.

## SESSION 27 QUANTITATIVE APPROACHES, INBREEDING AND CROSSBREEDING – I

### O133

#### Variance components estimation for milkability in Italian Holstein breed

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As the importance of workability is growing fast, because of the need to make a cost-efficient use of labour and machinery, we decided to update the statistical model on which our workability genetic index is based. The trait is binary, slow/not slow, and it is recorded by the milk recoding system twice a year. The frequency of the slow phenotype is very low (3%), making the estimation of the genetic parameters a little bit difficult. As the trait is categorical, a threshold animal model with repeated measures was used. After analyzing how the frequency of slowness was

affected by various other parameters, 8 fixed effects were selected: herd-year-season of classification, parity, number of observations per cow, milking frequency per day, month of calving, days in milk class (7 classes: 0–15, 15–30, 30–60, 60–100, 100–150, 150–200, 200+), age at first calving (as a covariate) and production of fat and protein the day of control (as a covariate). As random effects the animal, permanent environment and residuals were considered. After editing, our dataset is composed of 6,524,501 records, gathered on 2,673,976 animals with only 1 record per semester (2.44 records per animal on average): the pedigree goes up to 4 generations. After testing different models with a backward stepwise approach, we identified a final model without the number of observations per cow and milking frequency per day. For the variance components estimation we used the Gibbs sampler THRGIBBS1F90, with 110,000 iterations, a burn-in of 10,000 and a thinning rate of 10. The post-Gibbs analysis was carried out using the software POSTGIBBSF90. The estimated heritability is 0.26, with a Posterior Standard Deviation (PSD) of 0.01 and an effective sample size of 79 (>30 is preferred) for the genetic variance. Further improvements can be done when additional consistent information coming from milk flow sensors will be available.

### O134

#### The relationship between somatic cell score and udder type traits in Italian Mediterranean Buffaloes (*Bubalus bubalis*)

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Mammary infections in livestock are a major problem that damage animal health, resulting in significant economic losses in the dairy industry. Among the factors which can mitigate or increase the rate of intramammary infections, udder morphology plays a substantial role. An used phenotypic indicator of udder health is somatic cell count (SCC) which after being converted to somatic cell score – SCS is commonly used as a predictor in selection for mastitis resistance. The aim of this work was to investigate the relationship between SCS and udder traits in the Italian Mediterranean Buffalo (IMB). Production records (122.717) for milk yield (MY) and SCC plus six linear traits from 16.627 primiparous were provided by the Italian National Association of Buffalo Breeders. The type traits were a composite trait udder (UT) and six linear traits: fore udder attachment (FUA), rear udder width (RUW), udder depth (UD), teat

placement (TP), teat length (TL), teat position (TP). The relationship among udder traits and SCS was investigated fitting with a mixed model which included the following fixed effects: class of days in milk (30-days), year – month of milk-sampling and linear scores. Milk production (kg/d) was included as a linear covariate. Animal and herd-year-season of calving were fitted as random effect. Two separate models were used: M1 included only the composite UT, while M2 included all linear traits. Pearson's correlations between type traits and SCS were calculated. Analysis of variance suggested that SCS are significantly ( $p < .001$ ) affected by both udder morphology and milk yields. SCS increased across lactation, moving from 2.50 to 3.06. Least square means clearly indicated that having a morphologically correct udder contributes significantly to reducing SCS level in milk. Udders scorings high values for UT and UD showed a significantly lower level of SCS. The opposite was observed for TL, where an intermediate or low score suggested, as expected, a less amount of SCS. Overall, the correlations between SCS and morphological traits were low, ranging from  $-0.06$  UT to  $0.07$  TL. A selection focused on the udder and teat morphology can contribute to the reduction of SCC and then improve the milk production. These results can be useful to define reliable thresholds for SCC and select individuals that favor a decrease in its presence in milk considering also the morphology of udder.

#### Acknowledgements

The authors thank Dr. S. Biffani for his scientific support.

## O135

### Carcass value of purebred Holstein and crossbred cull cows from a 3 breed rotational scheme involving Viking Red, Montbéliarde and Holstein bulls

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The culled dairy cow is an important source of beef in Europe, but the carcass quality is expected to be highly variable because of heterogeneity in several characteristics, such as breed, age at culling and condition score. As the effect of crossbreeding on value of culled cows has been poorly investigated, the present study aimed to evaluate some carcass attributes of cull cows originated from a 3-breed rotational scheme involving Viking Red (VR), Montbéliarde (MO) and Holstein (HO) bulls compared to HO purebred cows. Data were collected on 1469 dairy cows kept in one specialized herd managed under the ProCross program. Cows (707 purebred HO and 762 crossbred – CR) were reared together and slaughtered from 2015 to 2019 in the same slaughterhouse. For each cow carcass weight, carcass price (€/kg),

fleshiness and fatness score according to EUROP classification (from 1, low, to 5, high) were collected. The total carcass value (€/head) was obtained multiplying carcass weight by carcass price. Before statistical analysis, data were classified for parity (5 classes, from 1 to  $\geq 5$ ), days in milk (5 classes of 100 d each) and genetic type (HO and CR cows). Cows that had undergone urgent slaughter ( $n = 76$ ) were not included in the analysis, which referred to 1393 cows ordinarily slaughtered. Carcass data were analysed with a mixed model which included parity, days in milk and genetic type as fixed effects, and the combined month and year date of slaughter as random effect. Carcass weight averaged  $295 \pm 63$  kg, and average price approached  $2.1 \pm 0.45$  €/kg. As a result, the average total carcass value was  $637 \pm 252$  €. Nearly 72% of carcasses were graded 1 (P) and almost 27% 2 (O) for fleshiness, being only 1% those graded 3 (R). Compared to those provided by purebred HO, carcasses from CR were nearly 8% heavier ( $p < .01$ ), and were graded higher ( $p < .01$ ) for fleshiness (+17%) and fatness score (+7%). As a consequence, carcasses from CR cows received a nearly 10% higher price than carcasses from purebred HO cows ( $p < .01$ ). Due to the greater carcass weight and price, on average, the value of carcasses from CR cows was nearly 100 € greater than that of carcasses from HO cows (+17%,  $p < .01$ ). Since cull cows represent a supplementary source of income for dairy farms, the superiority of crossbred cows in terms of the total value of cull cows should be taken into account in the evaluation of the effectiveness of crossbreeding schemes.

## O136

### Implication of collagen type IV in the onset of breast muscle disorders in broilers

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In the past decade, the high incidence level of growth-related disorders affecting the Pectoralis major muscles of broiler chickens belonging to fast-growing genotypes (i.e. White Striping, Wooden Breast and Spaghetti Meat) led to the development of several studies carried out to evaluate their impact on meat quality as well as to understand the main biological mechanisms and cellular processes involved in their onset and progression. Besides this, the foremost causative mechanism responsible for the sequence of events leading to their development has not been fully understood. However, a possible implication of collagen type



IV (COL4) in the pathogenesis of the growth-related abnormalities has been recently hypothesized. Thus, the present study aims at assessing the distribution of COL4 in Pectoralis major muscle through immunohistochemistry (IHC) and quantifying the amount of COL4 alpha 1 chain by Western Blot in a sample of 42-day-old broilers belonging to a medium- (MG;  $N=5$ ) and to a fast-growing (FG;  $N=5$ ) genotype, with only the last being affected by growth-related disorders (i.e. White Striping and Wooden Breast). Data were analyzed through the non-parametric Mann–Whitney  $U$  test considering the genotype (MG vs. FG) as the main effect. IHC evidenced a broad range of immunoreactivity in FG whose endomysial and perimysial connective tissue exhibited intensely stained bundles intermingled to faintly labelled ones. On the contrary, a more uniform immunoreactivity to COL4 alpha 1 chain was found in MG. Two bands having a molecular weight of about 75 and 130 kDa were detected through Western Blot. No significant differences were found concerning the 75 kDa-residue. On the other hand, a 3.5-fold increase in the relative intensity of the 130 kDa was found in FG compared with MG ( $p < .001$ ), mirrored by a significant increase in the total relative intensity of both bands (1.8-fold;  $p < .05$ ) in the same group. In consideration of the complex processes regulating COL4 biosynthesis, an impairment of the cellular mechanisms involved in the structural arrangement of the coded collagen proteins could play a pivotal role in the onset and progression of growth-related disorders in broilers.

## SESSION 28

This is a free session dedicated to short meetings of the ASPA councils, study commissions' encounters, and free meetings left for ASPA members.

## SESSION 29 ANIMAL EFFICIENCY – I

### O137

#### Technical-economic analysis of ventilation systems used to mitigate heat stress in dairy cows

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Due to global warming in the last years, the climate in temperate regions have been characterized by heat waves that negatively affect dairy herds in terms of welfare, productivity, and economic

losses. Among diverse heat mitigation strategies that farmers could implement, the use of cooling fans (CF) is the most frequent, even though the initial investment may be costly. This study tested the effects of heat stress (HS) and CF on dairy cows' milk yield, and whether CF guarantees an economic benefit for farmers. It was the first attempt to give an economic value to the welfare improvements by CF. The trial was carried out in 3 dairy farms over 3 years: 2 years before CF investment and 1 year later. Daily THI was calculated throughout the trial as a composite climatic-welfare index, representative of the HS condition of cows, and classified in: thermo-neutral ( $<72$ ), low risk (72–79), alert (80–84), and emergency ( $>84$ ). Milk yield was analyzed by farm with a mixed model that considered the fixed effects of THI, CF, and their interaction, with mature cow-equivalent milk yield as covariate and Bonferroni's adjustment. To assess the economic sustainability of the investment, the variation in milk income was assessed by subtracting the marginal costs from the marginal milk revenue. The additional costs consist of variable costs (VC) (extra feed, labour, electricity) supported by farmers during THI  $>72$  days, and fixed costs (investment and maintenance of CF). Interaction THI  $\times$  CF effect on milk yield was significant in all farms, resulting in an increased milk yield while using CF in alert ( $1.32 \pm 0.22$  kg) and emergency THI classes ( $1.21 \pm 0.37$  kg). Focusing on costs, energy accounted on average for 52% of VC, followed by feed (35%) and labour (13%) costs. This generated a great variability on economic benefits among farms and THI classes. Milk income being equal, all costs were hugely affected by some technical specifications of CF. Even the differences in CF management among farms did not allow a clear determination of the economical return of the investment. The relevance of energy costs and the variability of benefits highlight the need to consider the CF technical characteristics and other indicators of welfare and efficiency (i.e. milk quality traits and reproductive parameters) affected by CF. Although these preliminary results so far have been promising in terms of cows' productive performance, future cost-benefit analysis should involve further features.

### O138

#### Assessment of ruminal and fecal microbial biomass and protein content and their relationship with the digestion indicators

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The aim of the present trial was to measure the proportion of microbial biomass (MB) and microbial protein (MCP) in rumen fluid (RF) and faecal samples (FS) of dairy cattle and to study their relationship with the main digestion indicators. Samples



were collected from 30 high-producing fresh Holstein dairy cows raised in 6 farms and fed a hay-based total mixed ration (TMR). Diet was sampled in each farm where RF and FS were collected from 5 cows 3 h after the morning feeding. The RFs were collected through oesophageal probe and analysed for their volatile fatty acids, protozoa and enzyme content. The diets and FS were chemically analysed to determine their composition and nutrient digestibility. Both RF and FS were processed in order to determine their MB and MCP content. An aliquot of 20 g of FS was diluted at 1:20 ratio in a 0.9% sodium chloride solution and blended for 10 min. The RF samples and the diluted FS solution were divided each in two 200 mL bottles and frozen. The thawed bottles containing RF were sequentially centrifuged at  $500g \times 20'$ ,  $18,000g \times 20'$  and  $18,000g \times 25'$ . After the first centrifugation, samples were filtered through a 59  $\mu m$  and then through a 10  $\mu m$  mesh nylon filter. The diluted FS followed a similar process but were subjected to an intermediate centrifugation at  $500g \times 10'$  between the 2 filtrations and the last two centrifugations were limited at 15'. The supernatants were discharged obtaining 2 pellets. One was dried to obtain the weight for MB proportion calculation (%DM). The other was analysed for the determination of the MCP (Kjeldhal method). The MB were  $8.02 \pm 2.74\%$  and  $9.49 \pm 3.52\%$  DM respectively in RF and FS. In the same samples, the MCP content was  $45.2 \pm 7.54$  and  $30.4 \pm 11.4\%$  MB. Thus, MCP was  $3.68 \pm 1.51$  and  $3.03 \pm 1.16\%$  DM in the two type of samples. A negative correlation was found between the proportions of MB in RF and FS. The RF MB was negatively correlated with the dietary undigestible NDFom (uNDFom) and ruminal pH while it was positively correlated with potentially digestible NDF (pdNDF), estimated total-tract apparent NDF digestibility, propionic acid and dietary CP content. The FS MB showed a negative correlation with dietary pdNDF and fat content but it was positively related with dietary uNDFom, faecal amylase content, total protozoa and olotrichs counts. The MB and MCP determination in RF and FS are important to evaluate the digestion efficiency and appears to be related to several digestion indicators.

## O139

### Growth curve characteristics of dairy ewe-lambs of the Sarda breed from birth until start of mating age

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The objective of this research was the determination of the best non-linear growth model to describe growth patterns of ewe-lambs of the Sarda breed, fed with the aim to achieve the threshold values for weight at first mating as proposed by Brandano and Lanza (2001) for this breed. Data was collected over a period of

280 d from 58 Sarda ewe-lambs, born between January and February 2019, by using individual records of live weight from birth, until the start of mating age, which occurred in October of the same year, when the ewe lambs were  $270 \pm 10$  d old.

According to their body weight (BW) at mating, the ewe-lambs were equally divided in three groups: low (L;  $BW < 31.9$ ), medium (M;  $31.9 < BW \leq 33.9$ ) and high (H;  $BW > 33.9$ ) and the differences between them for growth curve parameters as well as BW and age at different phases of growth were analysed. Individual growth curves were fitted using four non-linear models: Brody, Logistic, Von Bertalanffy and Gompertz. The Brody model showed the best convergence criteria (higher  $R^2$ , lower MSE, Akaike and Bayesian Information Criteria) in all considered groups and its parameters diverged significantly from those of the other models. Values for asymptotic mature BW predicted for the H group were significantly higher than those predicted for the M and L group ( $43.1 \pm 4.5$ ,  $39.75 \pm 4.7$  and  $37.46 \pm 6.6$  kg, respectively;  $p < .001$ ), while the coefficients of the other parameters of Brody's model were identical in the three classes (proportion of asymptotic weight gained after birth:  $0.93 \pm 0.03$ ; daily weight change in relation to mature body weight:  $0.007 \pm 0.002$ ). Animals of the L group had significantly lower weights compared to the animals of the H group at birth ( $3.41 \pm 0.52$  and  $4.05 \pm 0.47$ , respectively;  $p < .001$ ), weaning ( $12.9 \pm 1.16$  and  $13.97 \pm 1.12$ , respectively;  $p < .01$ ), puberty ( $22.65 \pm 2.07$  and  $27.67 \pm 1.88$ , respectively;  $p < .001$ ) and mating ( $29.74 \pm 2.12$  and  $35.84 \pm 1.37$ , respectively;  $p < .001$ ). The values for the M group were intermediate and significantly different than those of the extreme groups at puberty and mating.

In conclusion, the results of this study showed that Brody model was the most suited for describing growth patterns of Sarda ewe-lambs and that it can be used to predict the future growth curve of this breed, if BW at birth and days of life are known. It also appeared that BW at birth strongly affected the BW at the other growth stages considered in this research.

## O140

### Evaluation of phytochemical dietary supplements on O138 *E. coli* challenged piglets

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In pig livestock, alternatives to in-feed antibiotics are urgently needed to promote health status and to control enteric infections. In particular, during the weaning period, the post Weaning Diarrhoea is a multifactorial gastrointestinal disease in which many pathogenic *Escherichia coli* strains play a pivotal role, is the most common indication for the antimicrobial prescription. Plant extracts have been widely studied as a natural source of functional compounds with antibacterial and antioxidant activities. The aim of this study was to evaluate the protective effect against *E. coli* experimental infection of innovative premix additives containing phytochemicals (caraway, lemon oil, clove, cinnamon, nutmeg, onion, pimento, orange peel, peppermint, chamomile) plus medium and short chain fatty acids in weaned piglets.

Thirty-two weaned piglets were randomly assigned into four experimental groups fed isoenergetic and isoproteic diets containing respectively. (i) Phytochemicals premix (PHY1;  $n=8$ ), (ii) phytochemicals premix and 2000 ppm of short-chain fatty acids and medium-chain fatty acids (PHY2;  $n=8$ ), (iii) containing the premix carrier (CTRL+,  $n=8$ ; and CTRL-,  $n=8$ ). The experiment lasted 14 days, after six days of dietary treatment (day-6), PHY1, PHY2 and CTRL+ were orally challenged with O138 *E. coli* F18+ strain ( $2 \times 10^9$  CFU). Animals were individually scored for health, consistency and colour of faeces. Individual faecal and blood samples were collected for the microbiological and biochemical analyses. Seven days after challenge sixteen animals ( $n=2/\text{trt}$ ) were euthanized and intestinal tissues were collected for the expression of the main genes related to the intestinal integrity, inflammation and health (IL-6, IL-10, COX1, COX2, LOX, GPX2, NQO1, occludin and claudin) by RT-PCR. PHY1 and PHY2 showed higher feed intake, average daily gain and feed efficiency ( $p=.04$ ;  $p<0.01$ ;  $p=.04$ , respectively) compared to controls. The faecal shedding of the challenge strain resulted lower in PHY1 and PHY2 ( $p=.04$ ) and PHY2 showed a lower incidence of diarrhoea compared to PHY1 and CTRL+. The molecular showed that the expression of occludin and claudin resulted lower for PHY2 compared to CTRL-group ( $p=.05$ ). Concluding, dietary phytochemicals supplementation could be an interesting alternative to antibiotic because they boosted the growth performance and alleviated the incidence of *E. coli* shedding in weaned piglets.

## O141

### Use of zeolite as zinc oxide replacement to control diarrhea in weaned pigs (from 8 to 20 kg): preliminary results on growth and microbiological profile of feces

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Research was carried out on inert clays as feed additives, especially due to their great adsorption capacity. Some studies have identified in clays a possible replacement to zinc oxide provided as antimicrobial to control diarrhea in weaned piglet. Clays were observed to reduce the incidence, severity, and duration of diarrhea, and to modify the microbial population in the gastrointestinal tract resulting in a more favorable microflora. This study aims at investigating the effect on live weight gain (LWG) and feces traits of zeolite inclusion as replacement of zinc oxide in feed for pig post-weaning. Immediately after weaning (28 days of age), 24 Large White piglets, balanced for sex, were individually housed. Three treatments ( $n=8$ ) were tested: Control group (C) fed a commercial diet added with zinc oxide; Z0.5 and Z1 groups fed replacing zinc oxide with 0.5% and 1% of zeolite, respectively. The daily feed amount was calculated as 5% of live weight. The following parameters were analyzed: live weight gain and fecal score (FS), *Lactobacillus* spp (LAB) and *Escherichia coli* populations and color. FS, ranging from 0 = normal to 3 = severe diarrhea, was assessed daily by 2 trained observers. On day 0, 4, 7, 14, 21, 28 of trial animals were weighted and feces were collected for microbiological analysis and instrumental color. Data were analyzed by ANOVA using SAS's GLM procedure. Initial LWG was similar among groups. At day 28, Z1 animals resulted significantly ( $p<.05$ ) lighter (19.03 kg) than C (19.89 kg) and Z0.5 animals (20.14 kg). For FS, groups scored similar (0.72, on average) at week 1 but differences ( $p<.05$ ) were observed subsequently: at week 2, FS of C immediately decreased (0.29), whereas Z0.5 and Z1 groups reached their minimum FS at week 3 (0.19) and 4 (0.44), respectively. Zeolite inclusion significantly affected both *E. coli* and LAB populations, with *E. coli* being higher in Z1 and the LAB lower in C. Moreover, from day 0 to 28, *E. coli* significantly decreased, and LAB increased, accordingly to FS, which suggested an enhancement of gut microflora. Concerning color parameters, C feces resulted lighter and more yellow than feces of Z0.5 and Z1 animals. Zinc oxide replacement with zeolite mainly affected LAB population

and feces' color: 1% of zeolite did not result to be effective in controlling *E. coli* population, thus leading to higher FS and LWG.

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### O142

#### Summer-Winter index: a visual tool to monitor dairy cow response to heat stress at farm level

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Dairy cow breeders are increasingly aware that climate management in their farms is one of the main factors for maintaining good production and profitability. Italy is facing frequent periods of extreme heat, where cows are frequently out of their thermal comfort zone.

Italian Breeders Association, within EU funded LEO project (Livestock Environmental Opendata, EU funded by the European agricultural fund for rural development – EAFRD), has adopted the Summer to Winter ratio (S:W ratio) index, a tool for assessing heat stress effect on cows, based on the ratio between summer (July–September) and winter (January–March) farm performances. The information presented in this paper is based on data recorded by Italian Breeders' Association, from Friesian breed farms from Lombard Pianura Padana, in the period between 2014 and 2020, with approximately 2500 farms. Data included productive parameters (FCPM, standardised milk yield to 4% of fat and 3.3% of protein, milk fat and protein contents and milk production at peak lactation), fertility traits (conception rate to all inseminations), and health traits (milk SCC). In addition, a comparison was made for the year 2020, where S:W ratio results in these farms was compared to a group of 100 farms in the region, where intensive cooling management was implemented.

The S:W ratio for all traits varied between years, being 2015 the lowest, most probably due to being the warmest year in the tested period. The S:W ratio for all traits was lower in adult cows, most probably due higher metabolic heat production by these cows, making them more sensitive to heat. The S:W ratios for productive and fertility traits were below 1.0, and of SCC higher from 1.0, representing the negative impact of heat stress on the cows. The S:W ratio was higher in the high producing herds, which can be related to better management practices used there. The S:W ratio for all the traits was significantly higher in the farms where intensive cooling was implemented, and this confirms and allows to quantify the potential improvement to be achieved by proper implementation of cooling means in this part of Italy.

The S:W ratio index can also be included as part of the strategy of welfare assessment, as it is capable to monitor how successful is cooling management used, in relieving heat stress from the cows.

### SESSION 30 OMICS TECHNOLOGIES – II

### O143

#### Epigenetic modifications in sports horses during training: analysis of genome-wide methylation as an adaptation phenomenon

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The Thoroughbred horse is universally recognized as the sport equine breed – with the advantage from bicentennial selection – making it the perfect environment for studying the adaptation to performance. Genomic and transcriptomic knowledge are at state of the art in equine species while the epigenome and its modifications in response to environmental stimuli, such as training, for example, is less studied. One of the major DNA epigenetic modifications is the 5' cytosine methylation, a biochemical modification essential in the mediation of biological processes and in shaping tissues phenotypic diversity. In addition, exercise has already been demonstrated to affect CpG islands methylation state, particularly in humans and mice.

In this work, we highlighted, with a genome-wide analysis of methylation, how the adaptation to training in the Thoroughbred impacts on the genome methylation patterns. The peripheral blood of twenty foals, subjected to the first workout season, under the same environmental conditions, were sampled at rest in a time course fashion, at the beginning of the training (T0), after 30 (T30) and 90 days (T90). The extracted DNA was analyzed with MSeEd (Methylation content sensitive enzyme ddRAD), an innovative reduced representation technique for a genome-wide methylation context analysis. From the observation of the 999 differently methylated genomic regions (DMRs) found in the different pairwise comparisons, we could detect that the CpG modifications are found in a large part of the genome and therefore referable to a physiological adaptation to training.

DMRs were crossed with the latest available annotation and Gene Ontology, as well as pathway analysis, was performed on the resulting functional elements producing meaningful enriched terms and pathways correlated to the cardiocirculatory function and the synaptic plasticity. Furthermore, we revealed different epigenetic signatures between the time course, suggesting that



there are genes that modify this state early and others that instead require more persistent stimuli.

**O144**

### OMIC characterization of cow, donkey and goat milk extracellular vesicles reveals their anti-inflammatory and immune-modulatory potential

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Other than being a valuable nutrition source, milk represents a sophisticated signaling system that delivers maternal messages. This property seems to be mostly mediated by signaling molecules enclosed in micro/nano-sized membrane-bound structures called Extracellular Vesicles (EVs). EVs act as signal mediators between distant cells and/or tissues, exerting biological effects as immune modulation, anti-inflammatory, anticancer, and pro-regenerative activity. Moreover, milk is a unique, scalable and reliable source of EVs. Our aim is to characterize the molecular content of cow, donkey and goat milk EVs (MEVs) through RNA and metabolites omic analysis in view of prospective applications as a nutraceutical in inflammatory conditions. RNA sequencing of MEVs resulting from two sampling of mass milk was carried out highlighting over 10,000 transcripts in each species. To compare MEV cargos, orthologous genes were selected and ranked by relative expression level. Within the 10% of the most expressed orthologous genes in all three species (1223), 110 were shared. Donkey and goat were the most similar species with 335 shared genes while cow had only 170 genes in common with donkey and 155 with goat. Functional analysis on the 110 core genes revealed as enriched GO terms 'translational initiation and regulation', 'protein refolding', 'cytoplasmic translation' and 'protein processing in endoplasmic reticulum' indicating a role in protein formation. Moreover, 'purine nucleoside triphosphate metabolic process', 'negative regulation to oxidative stress' and 'IL12-mediated signaling pathway' emerged, indicating potential involvement in innate and acquired immunity.

These terms were also confirmed in analysis on species pairwise shared genes where 'translation', 'peptide biosynthesis process', 'ribose phosphate biosynthesis process', 'ATP metabolic process', 'generation of metabolite and energy precursors' and 'cellular respiration', suggested also communication on energy metabolism. Concerning the most abundant genes for each species, donkey and

goat MEVs displayed additional terms relative to the immune system such as 'innate immune response-activating signal transduction' and amino acids metabolism. These results are in accordance, with some peculiarities, with our previous metabolomic analysis where common pathways among the three species involving metabolites with immunomodulating effects were identified, such as arginine, asparagine, glutathione and lysine.

**O145**

### SNPs and genes identification from muscle and backfat transcriptome of Italian Large White pigs with extreme and divergent fat deposition

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Subcutaneous and intramuscular fat deposition are important traits used for the production of typical Italian dry-cured hams and influence, respectively, the quality of carcasses and tights. This study aims to identify transcript variants expressed in skeletal muscle and backfat tissues, and related to fat deposition in pigs. To this aim, 2 groups of sib-tested pigs of the Italian Association of Pig Breeders (ANAS) were used: 20 Italian Large White (ILW) heavy pigs extreme and divergent for backfat thickness (BFT) and 12 for intramuscular fat content (IMF) in Semimembranosus muscle. For each phenotype, 2 subgroups of samples, high and low, were used. Raw sequences obtained by RNA-Seq were filtered using Trimmomatic and assembled using STAR to obtain the transcription profile of both tissues, and to perform variant calling using a GATK pipeline. Polymorphisms, both single nucleotide changes, and indels were detected and filtered. A total of 959 single nucleotide polymorphisms and indels were identified as possible candidate causative variants for BFT and 20,392 for IMF. The most promising 96 polymorphisms were selected for genotyping 190 ILW pigs, chosen from a population of 949 animals. After PLINK filtering, a mixed model in the R environment was used to perform an association study with BFT and IMF, considering litters and slaughter days as random effects. The genotype and sex of the animals were included as fixed effects. The association study highlighted 10 and 3 transcript



variants significantly associated ( $FDR < 0.1$ ) with fat deposition in backfat and muscle, respectively. Among the variants showing a significant additive effect (8 for BFT and 2 for IMF), one displayed an association with both traits. Some significant markers were located in the genes ACSS1, ALDH1, COASY, DLD, DHTKD1, and TPK1, which are molecular players involved in energy metabolisms or fat deposition or degradation. The presence of variants in the 3'UTR or introns of these genes suggests a possible regulative role of the identified mutations that could affect the mRNA stability and/or the functionality of the translated proteins. The new biomarkers associated with fat deposition identified in the present study can be useful for the implementation of genetic schemes aimed at selecting pigs with carcasses and thighs that possess a lean-to-fat proportion suitable to obtain high-quality pork products. Further studies, however, are needed to confirm the obtained results.

## O146

### Genomic and functional diversity of honey bee gut microbiota, after exposure to veterinary drugs or natural feed additives

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Honeybees are crucial to pollinate spontaneous wild plants and fruit trees and have an essential economic role in crop pollination and honey production. In the last years, the European honey bee colonies decreased. Several factors (e.g. global warming), may influence the health status of honeybees. Pathogens play an important role in honeybees health, and beekeepers may rely on antibiotic treatments. Antibiotics can significantly perturb the gut microbiota community, altering its metabolic functions. However, using antibiotics has not reduced the honeybee decline; therefore antibiotics for honeybee therapeutic use have withdrawn from the market since 2001. Thus, eco-friendly treatments should be efficient in pathogens control, while preserving honey bees health. This work tackles antibiotic and natural additives' effect on bee gut microbiota to unravel how the treatment influences its structure and functions. The gut microbiota of honeybees was studied using shotgun metagenomics, and 48 samples were sequenced using the Illumina HiSeq 2x300bp technology. Two experiments were prepared: (1) an Infield trial to test beneficial microorganisms and natural oils (Thymol + Probiotics, Tween80 and Control) and (2) a Laboratory trial to test antibiotics (Tylosin, Tetracycline, Sulfaquinoxaline, Antibiotic Control). Gut

content of 30 honeybees per replicate (3 replicates for each treatment) was picked, pooled and extracted at the beginning of the experiment and after 24 days. Reads were quality-filtered, assembled with SPAdes and microbial genes were predicted and annotated, using Prodigal and Diamond. These analyses allowed the taxonomic and functional profiling of all the sample treated with probiotics or antibiotics, reconstructed the antimicrobial resistance genes, and evaluated their SNPs. For example, the results show a reduction of *Lactobacillus* sp., *Bifidobacterium* sp. after antibiotic treatments, and mutations in genes coding for tetracycline resistance genes on *Gillimialla apicola* after tetracycline treatment. Eco-friendly treatments increased the abundances of *Gilliamella apis* and *Bifidobacterium* sp. Antibiotic treatments alter gut microbiota amino acids biosynthesis, showing an effect on microbiota gut biochemical function.

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## O147

### Rumen and hindgut microbiomes of dairy Italian Holstein Friesian heifers fed with camelina sativa cake

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Camelina represents a terrestrial vegetable and sustainable source of omega-3 PUFAs. These compounds can have interesting features both in rumen and hindgut environments, significantly contributing to animal health and production. The present study was aimed to understand how rumen and hindgut microbiota of dairy Italian Holstein Friesian heifers can be impacted by the inclusion of Camelina sativa cake in the diet. The trial was conducted at the tie stall dairy barn of the Experimental Farm of Animal Production Research and Teaching Centre of Lodi, University of Milan, Italy for 56 days. Sixteen Italian Holstein Friesian heifers 12 months old were randomly divided in two homogenous groups and subjected to 2 different treatments: CAME ( $n = 8$ ) receiving the basal diet supplemented with 800 g/head/day of camelina cake and CTR ( $n = 8$ ) receiving the basal diet supplemented with an isonitrogenous and isoenergetic soybean-based premix. Basal diet consisted of a dry TMR composed by alfalfa hay, durum wheat middlings, sugar cane molasses, corn meal, soybean hulls, rice bran, sunflower meal, and minerals

(15.15 PG, 42.63 NDF, 19.43 starch on DM basis). Performances were recorded weekly, feed intake daily. Rumen and fecal samples were collected at day 0, 28 and 56 of the trial and subjected to metabarcoding analysis. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were sequenced in two MiSeq (Illumina) runs with 2 × 250-base paired-end reads. Performance data were analyzed by PROC MIXED of SAS for repeated measures. The 16S rRNA gene analysis revealed significant differences in the alpha- and beta diversity of the rumen and hindgut microbiota between timepoints ( $p \leq 0.01$ ). After correcting for time effects, differences between treatments were significant for richness and diversity indices (Chao1, ACE, Fisher's alpha, Shannon, Simpson) in the rumen microbiota, and for evenness indices (Simpson\_E) in the rectum microbiota. As for specific taxa, the phylum Actinobacteria and the classes Coriobacteria and Deltaproteobacteria showed a significantly different between-treatment abundance ( $p \leq 0.05$ ) in the rectum microbiota, the order Mycoplasmatales ( $p \leq 0.01$ ) in the rumen microbiota. In conclusion, the cow rumen and rectum microbiotas showed a clear evolution over time, and some of their components seemed to significantly respond to the dietary supplementation with *Camelina sativa*.

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## O148

### Adaptations in neonatal calf hepatic transcriptome in response to enhanced post-ruminal maternal supply of methionine

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Maternal nutrient and metabolic stresses during pregnancy are important factors that can affect fetal and neonatal growth and development. The objective of the present work was to investigate if increasing the supply of methionine during late-pregnancy in Holstein cows affects hepatic transcriptome profiles, DNA methylation, one-carbon metabolism enzyme activities, metabolome and proteome profiles of nutrient-sensing pathways in the

offspring. Heifer calves were born from cows fed using a control diet (CON) or the control plus ethyl-cellulose rumen-protected methionine (MET) for the last 28 days prepartum and were fed and managed similarly after birth. Liver biopsies were harvested at 4 days of age from 6 calves per treatment for RNA sequencing, protein expression, enzyme activity, metabolomics and global DNA methylation analyses.

In summary, 568 genes were detected as differentially expressed (DEG) at FDR  $\leq 0.10$  threshold. Significant alterations of expression profiles were mainly detected in 'Glucose', 'Lipid', 'Glutathione', and 'Immune System' metabolisms. MET calves showed a higher global DNA methylation compared to CON calves and a greater ratio of p-AKT:AKT protein expression. Maternal supplementation with MET led to greater concentrations of Glycine, Adenosine, Serine, Taurine, Cystathionine, Glutamate, Fumarate, NAD, NADH. Whereas a lower hepatic activity for cystathionine  $\beta$ -synthase and 5-methyltetrahydrofolate homocysteine methyltransferase was detected in the MET calves.

Together the data supported the hypothesis that maternal supplementation with methionine during late-gestation programmed calf hepatic metabolism. Overall our findings indicated a greater global DNA methylation, and distinct hepatic transcriptome, proteome, and metabolome profiles after birth, particularly in the context of maintaining methionine homeostasis, glutathione, phosphatidylcholine and taurine synthesis, lipid and energy metabolism efficiency, relying at least in part on more insulin sensitivity. These alterations may result in better immunometabolic status of the offspring at birth, and confer the calf a physiologic advantage during a period of metabolic stress and suboptimal immunocompetence.

## SESSION 31 QUANTITATIVE APPROACHES, INBREEDING AND CROSSBREEDING – II

## O149

### Myostatin gene affects carcass and meat quality in Marchigiana beef cattle

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Over the past few decades, changes in lifestyle and food style of consumers have led to an increase in the demand for quality meat and meat products, achieved in a sustainable way. Animal genetics, feeding, and livestock practices mainly affect the quality performance of beef, as well as its nutritional properties. The study aims to analyze the effect of muscle hypertrophy, due to a point mutation in the Myostatin gene (MSTN), on slaughtering performance and meat quality of Marchigiana breed. The weight

gain performed in 7 months fattening period and slaughtering traits were recorded for 78 Marchigiana bulls. DNA was extracted from each meat sample and genotyped for the presence of the point mutation (g.874G > T) in MSTN exon 3 region by PCR-RFLP (wild type, mh + mh+; heterozygote, mh + mh-; homozygote, mh-mh-). Carcass weight and daily gain, pH at 45 min, and SEUROP classification were collected, and a sample cut (5th–6th thoracic vertebrae) of each left carcass side was dissected. Chemical (moisture, protein, fat and ash) and physical analyses (drip loss and colour by CIELab) were carried out on L. thoracis muscle according to official methodologies. Data were analysed with JMP software (ANOVA and ANCOVA, with appropriate covariates) to evaluate the effects of the MSTN mutation on the performance and quality traits. Out of 78 genotyped animals, 11 were heterozygotes, 67 wild types and none homozygote. An average carcass weight of the heterozygous greater than wild type animals (426.09 vs. 405.32 kg,  $p > .05$ ) was observed, as well as a better dressing percentage (62.55 vs. 60.96%,  $p > .05$ ), with more conformed carcasses (class E: 36.36%) and low grade of fat cover (class 2: 63.64 %). The low incidence of bone (9.44%), and a significant difference ( $p < .05$ ) on the amount of muscular and fatty tissue were observed in the sample cut dissection of mh + mh- animals. Regarding meat quality, few significant differences were found between the two groups: a lower fat content (2.01 vs. 3.04%,  $p < .05$ ) and a higher ashes content (1.26 vs. 1.15%,  $p < .05$ ) in mh + mh- bulls. Even if differences between the two groups in the tricolorimetric parameters were not detected, lightness was slightly higher in heterozygous bulls characterised by a higher muscle glycolytic activity than not hypertrophic animals as reported by several authors. Despite the small size of the studied sample, the present results confirm good performance of the heterozygous animals producing healthy and lean meat, which meets the modern consumer's needs.

## O150

### How post-collection storage time can affect the semen freezability of Mediterranean trout?

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Semen cryobank plays a valuable role in biodiversity preservation of fish species at risk of extinction. According to the Italian freshwater fish Red list, the Mediterranean trout, *Salmo cettii*, is listed

as critically endangered. In this regard, the 'Nat.Sal.Mo.' project aims to recover and conserve the native *S. cettii* populations of Molise rivers. The creation of a sperm cryobank is a project milestone, therefore the development of a semen cryopreservation protocol was an important goal to achieve. However, since the sampling sites in the project area are often not easily accessible, distant from each other and from the laboratory, the wide time that elapses between collection and processing of semen could negatively affect its freezability. In light of these considerations, two possible scenarios were developed to evaluate the effect of cool storage time intervals (1 h and 6 h) on both fresh and cryopreserved semen motility parameters and post-thawed fertilizing ability. Eggs and semen samples were collected by stripping. Each ejaculate ( $n = 10$ ) was split into two aliquots and stored on ice for 1 h and 6 h. After each time interval, the sperm was diluted into a final extender concentration of 0.15 M glucose and 7.5% methanol and loaded into 0.25 mL plastic straws, and a final sperm concentration of  $3.0 \times 10^9$  sperm/mL was obtained. After equilibration, the straws were frozen by exposure to liquid nitrogen (LN2) vapor 3 cm above the LN2 level for 5 min. The semen was thawed at 40 °C/5 s. Fresh and post-thawing sperm motility was evaluated by the CASA system. Fertilization trials were performed using three groups of eggs ( $N \approx 90$ ) inseminated with fresh sperm, and sperm frozen 1 and 6h post-collection. In fresh semen significant decreases ( $p < .05$ ) from 1 to 6 h of storage were recorded for total motility (93.7 vs. 57.3%), movement duration (36.1 vs. 28.6 s) and beat cross frequency (6.4 vs. 4.6 Hz). When the sperm was frozen, only the total motility was significantly reduced ( $p < .05$ ) from 1 to 6h (52.1% vs. 39.8%). No significant differences of fertilization rates (% eyed eggs) between the two storage times using frozen sperm (59.5% vs. 57.4%) were found. In conclusion, we showed that after 6 h of cool storage time post-collection, the post-thawing semen quality is preserved, and its fertilizing capacity is not compromised. However, the cool storage time significantly affects the freezability of fresh semen, eliminating the most cool-sensitive populations.

## O151

### Genetic parameters of predicted enteric methane emissions of Brown Swiss cattle at the population level in Italy

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The estimation of genetic parameters allows the establishment of genetic strategies to reduce the methane production impact



in dairy systems. The objective of this study was to estimate heritabilities ( $h^2$ ) and additive genetic correlations ( $rg$ ) of enteric methane emission (EME) traits predicted with milk FTIR-spectra (Direct-IR) and predicted indirectly using fatty acids (Indirect-IR). Data included the detailed milk composition of 42,462 Brown Swiss cows from 669 herds during 8 years. The traits included were daily milk yield (dMY, kg), fat (%), protein (%), the predicted milk fatty acids related to EME (FAs: C4, C16iso, C18:1t10, C18:1t11, C18:1c9, C18:2c9c12), methane yield per unit dry matter intake ( $CH_4$ -DMI, g/kg), daily methane production ( $dCH_4$ , g/d), and methane intensity per kg of corrected milk ( $CH_4$ -CM, g/kg), fresh cheese ( $CH_4$ -FC, g/kg), and cheese solids ( $CH_4$ -CS, g/kg). Univariate models were used to estimate  $h^2$  and bivariate models were used for the estimation of  $rg$  between traits. All models included the effects of parity (1–3), days in milk, and year-season interaction as fixed effects. The herd, additive genetic animal and permanent environmental effects were included as random. The program used for calculations was AIREMLF90. The  $h^2$  for dMY, fat and protein were 0.09, 0.26 and 0.40, respectively (SE <0.008) and for FAs it ranged from 0.03 to 0.13 (SE <0.004). The methane traits had similar  $h^2$  in Direct-IR and Indirect-IR methods, from 0.04 to 0.11 (SE <0.004). The  $rg$  between dMY, fat, protein and FAs ranged from -0.79 (fat – C18:1t11) to 0.55 (fat – protein). The  $rg$  between dMY, fat, protein and Direct-IR EME varied from -0.92 (dMY –  $CH_4$ -CM) to 0.47 (protein –  $CH_4$ -DMI), while the  $rg$  between dMY, fat, protein and Indirect-IR EME ranged from -0.53 (protein –  $CH_4$ -FC) to 0.73 (dMY –  $dCH_4$ ). The  $rg$  between Direct-IR EME and Indirect-IR EME were high (0.89–0.96) except for  $dCH_4$  with 0.30. The  $rg$  between FAs and Direct-IR EME had a wide interval of -0.77 (C18:2c9c12 –  $CH_4$ -DMI) and 0.95 (C18:1t10 –  $CH_4$ -CS). The  $rg$  between FAs and Indirect-IR EME varied from -0.88 (C18:2c9c12 –  $CH_4$ -DMI) to 0.45 (C16iso –  $CH_4$ -DMI). Results show the feasibility of using Direct-IR and indirect-IR EME in animal breeding programs, except for  $dCH_4$  because it is better estimated by multiplying dMY  $\times$   $CH_4$ -CM.

for instance, geographic isolation is an effective approach but it is not a common practice yet. In such a situation, pedigree is not complete because drone origin is missing and selection occurs only on the maternal path. Since 2015, 108 colonies from five sites of a honey bee breeding company from Lombardy have been evaluated for honey production (HP), hygienic behaviour (HB) and docility (DOC). A pedigree with information about breeding queens (BQ) and drone producing queens (DPQ) have also been recorded. The objective of this study is to present the results of a genetic evaluation developed using the aforementioned information. The original dataset included 494 records for HP, HB and DOC. A pedigree with 1267 individuals was also available, including information on drone origin. A total of 6 DPQ were used and traced back. Considering that in honey bees the observed performance is due to the contribution of both the worker bees and their queen, a direct and maternal genetic effect model was used to estimate variance components and breeding values. The model included the fixed effect of year of production, the random effect of the interaction of mating site and year of production, and the random genetic effects of the worker and the queen of the colony. The inverse additive relationship matrix was calculated by combining the classical inversion strategy of Henderson with the bee-specific properties of haploid drones. The random interaction of the mating site and year of production was included to mitigate the unbalanced distribution of DPQ across mating sites. The BLUPF90 programs were used to estimate variance components and to compute EBV fitting each trait separately. Heritability of the worker direct genetic effect ranged from 0.20 for DOC to 0.45 for HP. For all traits, the random non-genetic component accounted for more than 40% of the phenotypic variance. Residual variances accounted for about 10%. Heritability of maternal genetic effect ranged from 0.40 for HP to 0.65 for HB. The correlation between direct and maternal genetic effects was negative for all traits.

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## O152

### Genetic evaluation and variance component estimation in an Italian honey bee population

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In all domestic species, mating control is a key aspect of any breeding program. In *Apis mellifera* the problem is crucial due to the free mating of the virgin queen in its single nuptial flight and to the variable number of haploid drones. Controlled matings via,



## O153

### Genetic parameters for carcass weight at different age-classes in Italian Limousine beef cattle

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Genetic selection for carcass weight allows to maximize the profitability of meat-producing livestock species. The objective of this research was to estimate the genetic parameters for carcass weight at different age-classes in Limousine beef cattle breed. The experimental data set included information of 38,477 individuals, the progeny of 13,134 sires. Individuals were allocated to one of the eight classes depending on slaughter age: less than 12 (CL1); 12–14 (CL2); 15–16 (CL3); 17–18 (CL4); 19–20 (CL5); 21–22 (CL6); 23–24 (CL7); more than 24 (CL8) months. The mean (standard deviation) of carcass weights ranged from 240.34 (59.16) kg in CL1 to 381.17 (85.73) kg in CL7. A multi-trait sire model was used to estimate the heritability of carcass weight within each of the eight age classes, and the adjusted phenotypic and genetic correlations between them. The model included the fixed effects of sex, season, and year of slaughtering, and the random uncorrelated farm and slaughterhouse effects. Variance components were estimated by Bayesian methods via Gibbs sampling and posterior means and standard deviations have been provided by POSTGIBBSF90. Estimates of heritability for carcass weight were 0.28 for CL1, CL3 and CL4; 0.30 for CL2 and CL7; 0.27 for CL5; 0.26 for CL6; and 0.40 for CL8. Genetic correlations between carcass weights of different age classes were positive and ranged from 0.268 (CL1–CL4) to 0.895 (CL6–CL7). Phenotypic correlations varied from 0.218 (CL1–CL8) to 0.645 (CL6–CL7). Our results suggested that direct selection for carcass weight at different classes of age is possible allowing thus an alternative strategy for breeding.

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## O154

### Merinizzata Italiana sheep breed: genetic gain after 20 years of Performance Test

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The research was performed closely with Italian Sheep and Goats Breeder Association (ASSO.NA.PA.) in order to examine the trend of the selection plans applied to Italian sheep meat breeds over the years. More specifically, it was investigated whether the selective protocols and the resulting phenotypic indexes, used not regularly by farmers, have been effective and if they could be replaced by other most recent indexes. Therefore, after a period of interruption of the performance test from 2014 to 2017, in the occasion of the 38<sup>o</sup> Performance Test of the Merinizzata Italiana breed, a new BLUP-AM Multiple Trait index was developed. It was based on the weights reconstructed at 60 d, 90 d and 12 months of age with a model that takes into account the following fixed effects: cycle of affiliation, type of delivery and province of origin. The genealogical file contained 4506 animals (PEDIGREE VIEWER), 2272 of which with observations; in addition, some genealogical statistics with the same software were performed. On the morphological evaluations data an analysis of the principal components (PROC PRINCOMP, SAS) was applied. The results of the new genetic index recalculated for all the animals of the ASSO.NA.PA. historical dataset have revealed the positive effects of the selection performed over the years; in fact, also the results of the PCA on the base of the morphological traits, showed that all the rams spread out uniformly over the provinces of origin. Moreover, the study showed a rapid improvement of the genetic gain in the last 3 years after the adoption of the developed BLUP index. Actually, this work highlighted the importance to continue the use of Performance Test in Italian sheep meat breeds; the need to replace the morphological evaluation with linear evaluation, already studied and applicable on this breed; the need to continue to evaluate animals with BLUP-AM-MT genetic indexes, as genealogies have proven reliable. In conclusion, it is, therefore, appropriate to underline the importance of using a BLUP-AM index in selective protocols and to make it accessible to farmers in order to transform the progress obtained directly into an economic advantage. The experience has shown that, only if farmers find a proven return on their productions, they will commit themselves effectively to pursue selection plans.

## SESSION 32 COMPANION ANIMALS AND SOCIETY – I

O155

# Dietary supplementation with coconut oil in dogs with chronic enteropathies

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Chronic enteropathies (CE) are common disorders in dogs, with typical gastrointestinal signs (GIS) including diarrhoea, vomiting, loss of weight or appetite, or some combination of these clinical signs. Dietary therapy based on novel or hydrolysed proteins promotes a long-lasting state of remission in most of CE dogs. Due to their water solubility, medium-chain triglycerides (MCTs), fatty acid molecules with a chain length of 6–12 carbon atoms, can be absorbed through enterocytes by-passing the lymphatics and can be more efficiently utilised in dogs when fat malabsorption is suspected. Aim of this study was to evaluate the effects of a dietary treatment with a home-cooked diet (HCD) based on horse meat and potatoes, with or without supplementation of virgin coconut-oil (VCO) as a MCTs source, on clinical activity indices, faecal score (FS) and faecal fat composition in CE dogs. Twenty-one CE dogs were first fed a complete HCD (CP 39.1%, EE 8.0%, CF 1.4%, ash 6.3%; on DM basis) for 7 days and later received the same diet added with VCO (at 10% of ME) for 30 days. Faecal samples were collected before enrolment (day 0) and again after 7, 14 and 37 days of treatment. Faecal fatty acids were analysed by gas chromatography. Dogs were scored the canine chronic enteropathy activity index (CCECAI) at day 0 and 37. Data were analysed by a general linear model for characterization of faecal fat composition and non-parametric tests for FS and CCECAI (differences were considered statistically significant for  $p < .05$ ). The VCO did not lead to food refusal and neither induced adverse GIS in the dogs. Evaluation of FS revealed an improvement of faecal firmness ( $p = .001$ ) in scores between day 0 (median value 4/7) and day 37 (median value 2/7); moreover, dogs had a reduction in GIS following HCD + VCO treatment compared with baseline CCECAI score ( $p < .05$ ). VCO supplementation resulted in higher faecal excretion of capric, lauric and myristic (C10,  $p = .048$ ; C12,  $p = .040$ ; C14,  $p = .036$ ) at different time points: C10 was higher at day 14 compared to day 0 ( $p = .03$ ), while C12 and C14 tended to be higher at day 37 compared to day 7 ( $p = .058$  and  $p = .057$  respectively). These findings could indicate that in CE dogs intestinal passive absorption of MCTs with 10–14 carbon fatty acids could be impaired, even though dogs had shown an enhancement of clinical response after the dietary treatment.

O156

# Evaluation of body weight and body condition score in 64 European Shorthair adult cats

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In a study by Kienzle and Moik (2011), the 4 kg mean body weight (BW) usually attributed to lean (BCS 5/9) European Shorthair cats was questioned. A sexual dimorphism emerged, hence a different ideal mean BW for lean females and males was proposed. The aim of the present study was to assess BW and BCS in European Shorthair cats to establish the mean BW of males and females and to evaluate whether BCS is affected by lifestyle, feeding habits, and health status. 64 European Shorthair adult cats were weighed, scored (BCS 1–9 point scale) and divided into three groups: BCS <5, 5 and >5. Information on lifestyle (indoor or outdoor), health status (healthy or unhealthy), type of diet (homemade diet, commercial food or mixed), food weighing (performed or not) and snacks administration were recorded. The effect of these parameters on BCS was analysed using a non parametric approach. The correlation between BW and BCS was assessed using the Spearman's rank correlation. A  $p$ -value  $\leq 0.1$  was considered statistically significant. The study population included 30 females (28 neutered – 2 intact), 34 males (28 neutered – 6 intact), of which 54 indoor, 10 outdoor, 27 healthy, 37 unhealthy, 0 fed homemade diet, 56 fed commercial diet, 8 fed mixed diet, 8 fed weighed food and 56 fed non-weighed food, 57 received snacks and 7 did not. Twelve (19%) cats were scored BCS <5, 33 (51%) BCS 5 and 19 (30%) BCS >5. The mean BW in the BCS 5 group was 5.3 kg ( $\pm 0.6$ ) for males and 4.0 kg for females ( $\pm 0.7$ ). The mean BW in the BCS <5 group was 3.6 kg ( $\pm 0.7$ ) for males and 3.2 kg for females ( $\pm 0.4$ ). The mean BW in the BCS >5 group was 6.7 kg ( $\pm 1.2$ ) for males and 6.4 kg for females ( $\pm 1.5$ ). There was a positive correlation between cats' BW and BCS. BCS was significantly affected by sex (males > females,  $p < .0001$ ), by lifestyle (indoor > outdoor cats,  $p = .076$ ) and by health status (healthy > sick cats,  $p < .0001$ ). Feeding habits did not influence BCS. In this study population, it was shown that BCS is influenced by sex, health status and lifestyle. More information on the characteristics of food delivered in terms of energy content and nutrients profile should be collected in order to assess the influence of feeding habits on BCS. To achieve a more accurate cat's mean BW a further differentiation into sizes should be considered.

## O157

### Copper-associated liver disease in a Bergamasco Sheepdog: a case report

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Hepatic Cu accumulation in dogs may be the result of the impaired biliary excretion occurring in several liver diseases or a primary metabolic defect. An inherited Cu-associated hepatopathy has been documented in a variety of breeds, however, it is still unclear why most of them accumulate Cu consuming regular maintenance diets. Aim of the treatment is to reduce the excessive Cu content in the liver as well as to prevent further build-up, and it involves dietary changes and Cu-binding treatments. Case report. A 5-year-old, spayed female Bergamasco Sheepdog of 23.7 kg and BCS 2/9 was referred to the Veterinary Teaching Hospital of the University of Padova on 5 May 2020. The dog looked slightly icteric, and blood tests, urinalysis and abdominal ultrasonography showed: coagulation disorders, increased plasma enzymes, hyperbilirubinemia, marked bilirubinuria, hepatomegaly and a diffuse echogenicity variation. Therapy consisted of maropitant, lansoprazole, prednisolone, vitamin K, and tranexamic acid; after resolution of the coagulopathy, liver cytology was performed and Cu toxicosis was revealed by the rubeanic acid staining on May 18. Nutritional intervention was required as the dog showed hyporexia, severe weight loss, and chronic diarrhoea despite numerous dietary changes (all maintenance dry pet food-based); therefore, a home-prepared diet was formulated. Daily energy requirements (DER) were calculated using the ideal body weight (BW) and the formula  $110 \text{ kcal} \times \text{BW}^{0.75}$  recommended by FEDIAF, and all nutrient requirements were determined according to the same guidelines. A gradual increase in the energy intake (up to  $1.2 \times \text{DER}$ ) was applied over three weeks to promote weight gain. The diet was moderate-protein (60 g/Mcal) and moderate-fat (35 g/Mcal); the composition included non-meat, low Cu-containing protein sources (i.e. egg and ricotta cheese), a medium-chain triglyceride oil, omega-3 pearls and a multivitamin/mineral supplement specific for canine hepatic diets (Cu: 30 mg/kg, Zn: 5550 g/kg); as a result, maximum daily Cu intake was less than half of the recommended allowance (1.3 vs. 2.8 mg). In addition, the diet was supplemented with silymarin and therapy was complemented by ursodeoxycholic acid. After seven months (December 18), at latest check-up, the dog was in overall good health status and reached 34 kg BW (BCS 5/9); plasma enzymes drastically decreased (halved or more), bilirubin levels fell within range, and no bilirubinuria was present.

## O158

### Disorders of sexual development in 9 dogs: clinical, cytogenetics and molecular findings

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Reproductive health plays a key role in animal husbandry, in livestock it impacts mainly on the productive ability while in pets in addition to affecting veterinary expenses, it is also often a cause of apprehension in the owner of the affected subject. Among the reproductive problems of greatest interest there are undoubtedly the Disorders of Sexual Development (DSDs): congenital conditions in which chromosomal, gonadal or anatomical sex development are not coordinated each other. Currently, there is an increasing attention to these disorders because they are often diagnosed when the malformed organs develop pathologic conditions like strong inflammations or cancer lesions. Identifying causative mutations would allow the development of diagnostic tools for early identification of individuals who may develop these pathologies. Aim of this work is to describe clinical, cytogenetic and genetic aspects of nine dogs with different type of DSDs. All cases were submitted to clinical evaluation for the abnormal external genitalia conformation observed by the owner (enlarged clitoris, abnormal vulva opening, rudimentary penis, hypospadias or cryptorchidism), except for Case 5 that was examined for the sudden increase in volume of the abdomen. Gonads were surgically removed in 8 of the 9 cases and were found to be histologically testis in cases 1, 2, 3, 4, 5, 6 and 7 and ovo-testis in case 9. In Case 8, ultrasound examination allowed to observe ovo-testis like structures caudally to the kidneys. Cases 1, 2, 3 and 4 were classified as 78, XY DSD – cryptorchids. Case 5 showed the coexistence of testes and uterus and was classified as Persistence of Mullerian Duct Syndrome (PMDS). Cases 6 and 7 were classified as 78, XX testicular DSDs and Cases 8 and 9 as 78, XX ovo-testicular DSDs. Based on this classification Cases 1, 2, 3 and 4 were submitted to the analysis of MAMLD1, SRD5A2, and AR genes, Case 5 to the analysis of AMRH2 gene and Cases 6, 7, 8 and 9 to array-CGH analyses with a custom Agilent dog CGH microarray K133. Polymorphisms of the MAMLD1, SRD5A2, and AR genes observed in the four dogs analysed did not show an association with the observed ambiguities of the external genitalia, the analysis of AMRH2 showed the presence of the homozygote mutation 241C T exon 3 nt5 confirming the diagnosis of PMSD. Finally,



11 Copy Number Variations were found in the Cases 6, 7, 8 and 9 and one of them seems to be included in SOX9 promoter region that could therefore be the genetic cause of the DSD observed in these cases.

## SESSION 33 ANIMAL EFFICIENCY – II

### O159

#### Production and egg quality in brown hens kept in a cage-free system: effects of hen age and nest lighting

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The study evaluated the effects of hen age and nest lighting on egg production and quality, and oviposition pattern from 26 to 45 weeks of age in 1800 Lohmann Brown-Classic hens housed in 8 pens of an aviary system. From 17 to 26 weeks of age, half pens had the nest opened with the inner led light turned on 1.5 h before turning on the installation light (5:30); the other half had the nests closed until turning on the installation light and without any inner led light. Measurements were taken weekly and monthly.

As hen age increased, the oviposition rate ( $p < .001$ ) increased from 89.1% housed hens (on average of 26–34 weeks) to 92.8% (34–45 weeks). Both the oviposition rate of broken and dirty eggs decreased from the first period (5.13% and 7.65% on average, respectively) to the second period (2.28% and 4.21%) ( $p < .001$ ). The weight and width of the eggs increased and the shape index decreased as age increased ( $p < .001$ ). As for oviposition pattern from 26 to 45 weeks, eggs laid in the first hours (5:30–7:30) decreased (76.4–45.8% of eggs laid in 24 h), whereas eggs laid in the rest of the day increased ( $p < .001$ ). Moreover, eggs laid in the nest (as % eggs laid in each time interval) between 5:30 and 7:30 (77.9–86.4%) and between 7:30 and 9:30 (68.3–84.7%) increased from 26 to 45 weeks ( $p < .001$ ). A significant decrease of broken eggs (% eggs laid in each time interval) was also recorded for the eggs laid within 9:30 when the hen age increased. The use of nest lighting from 17 to 26 weeks increased oviposition rate in the following period (26–45 weeks) from 90.1% to 92.3% ( $p < .001$ ), decreased egg weight, width, and surface ( $p < .001$ ) as well as the rate of broken (4.22–3.43%;  $p < .001$ ) and dirty eggs (5.98–5.65%;  $p = .10$ ), whereas the rate of defective eggs was not

affected. The oviposition pattern (i.e. distribution of eggs laid in the different daily time intervals) did not change. In the first time interval (5:50–7:30), eggs laid in the nest were higher (83.2% vs. 80.2% eggs laid in the time interval;  $p < .01$ ) and broken eggs lower (4.83% vs. 7.01%;  $p < .01$ ) in the case of the presence of nest lighting.

Based on the above results, changes in the oviposition during the day according to hen age must be considered in cage-free systems to assure a correct use of nests. As for early nest lighting, further investigation is worth in view of the positive effects on egg production and quality, and on nest use.

### O160

#### In vitro protein degradability in fish: a tool to evaluate novel protein sources

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Sustainability issues drove the interest towards alternative protein-rich raw or processed feed materials to include in aquafeeds. Besides chemical composition, digestibility is a basic parameter to be considered. It is usually determined through in vivo methods. Anyway these novel ingredients are characterized by high variability due to their origin and the technological process applied. To overcome these issues, in vitro methods are widely used in the evaluation of the nutritional quality of feeds in land-animals but, recently, their application for aquafeeds has increased. These methods have been useful for ranking ingredients according to their potential digestibility, but also to establish the ability of different fish species to utilise them.

This study was aimed at estimating the nutritive value of a range of novel protein-rich ingredients to explore their suitability as protein sources in the diet for rainbow trout (*Oncorhynchus mykiss*): (i) poultry by product meal composed by chicken and turkey leftovers (PBM), (ii) *Hermetia illucens* pupae meal (HM), (iii) three dried biomasses of *Tetraselmis suecica* (TETRA), *Tisochrysis lutea* (TISO) and *Arthrospira platensis* (ART). The ingredients were characterized for their chemical composition, in vivo protein digestibility (ADC) and in vitro enzymatic protein hydrolysis based on an assay that used rainbow trout digestive enzyme extract. Protein degradation was monitored after 120 min by electrophoretic techniques using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to obtain a quantitative coefficient of protein degradation (CPD).

Protein ADCs were higher for PBM, HM and TISO (92.0–96.5%) than ART and TETRA (83.1–86.2%) ( $p < .05$ ). Despite CPD values



resulted always lower than ADCs ( $p < .05$ ), similar degradability values were attained for HM, PBM and T. lutea (72.4–75%) when compared *A. platensis* and *T. suecica* (65.5–67.7%) ( $p < .05$ ). When protein ADCs measured in vivo in trout were regressed against the corresponding in vitro CPD coefficients, a positive and a strong relationship was observed ( $r = 0.97$ ;  $p < .05$ ). In this study in vitro CPD was found to be highly predictive of in vivo protein ADCs and could be recommended as a preliminary tool for ranking or screening out ingredients for their suitability to be used as feed protein sources. Overall the results obtained fully support the use of HM, PBM and TISO in formulations for carnivorous fish species.

#### Acknowledgements

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## O161

### System dynamics applications in animal science and dairy farm management

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System dynamic (SD) is a modeling technique adopting a systemic perspective to improve complexity understanding and forecast future trends. Qualitative SD models include the description of causal links and multiple connections among elements of a system through causal loop diagrams. An important step is the identification of the main feedback loops from circular interactions among system elements. It determines the system structure and the endogenous dynamics driving the complex and dynamic system behavior over time. With quantitative modelling, the system conceptualization is made in Stocks and Flows diagrams (S&F) fitted by differential equations. Feedback loops can be reinforcing or balancing, generating exponential or asymptotic behaviour over time, respectively. The modelling process is particularly adapted to field experts being aided by graphic software and not requiring in-depth mathematical skills. This work aimed to present SD application in nutrition and farm management within the development of a dairy herd model able to capture the dynamic evolution of herd composition. The modeling steps included data gathering on farm. In particular, data from a farm raising  $1375 \pm 45.2$  heifers and  $1154 \pm 58$  milking cows having dry matter intake and milk yield equal to  $24.2 \pm 2.4$  and  $34.0 \pm 3.4$  kg/d, respectively, were collected in the period 2015–2019. The model, built on Vensim<sup>®</sup> (Ventana, Inc), was firstly conceptualized by mapping causal links among farm variables and then creating herd S&F using basic SD elements, such as

one stock and two stocks structures able to reproduce exponential growth and oscillating behaviours which can be used to show SD applications in the field. The complete model consisted of an aging chain of heifer stocks until first calving and the reproduction loop of cows with 4 basic stocks (early lactating, open, pregnant and dry cows). Calibration was performed on historical records of cattle categories and culling rates. Seasonal patterns of intake and pregnancy rates were also modeled. From model input, the model predicted with good accuracy the oscillating seasonal pattern of historical farm records of cattle consistency and milk deliveries until 2019. Cow consistency and milk deliveries were predicted with a RMSPE of 3.5% and 7.2% of observed values and good precision ( $r^2 = 0.68$  and  $0.72$ ) respectively. Economic and environmental sub-models are under development and will be discussed. SD can have broad applications in animal science.

## O162

### A survey on feeding economics in intensive dairy farms

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The main objective of this work was to identify aggregated indexes of dairy farm profitability using commonly used partial indicators that are routinely available in dairy cattle farms. These aggregate indexes may provide support for short-term decision-making processes and improve the economic efficiency of the cattle producing process. Data collected from face-to-face interviews with 90 dairy farmers located in the Po Valley and Sardinia were used to calculate 16 partial indicators of feeding and economic efficiency in dairy farms. These partial indicators described aspects related to feed efficiency, energy utilization, feed costs, milk to feed price ratio, income over feed costs, income equal feed cost, money corrected milk, and bargaining power for feed costs. Multivariate factor analysis (MFA) was then applied to the 16 partial indicators to extract latent structure. The results indicated that 5 factors explained 70% of the indicators' variability. Multicollinearity and redundancy of the partial indicators was reduced also combining them into a few aggregated and uncorrelated indexes that were related to technical and economic efficiency. Based on the loading scores of original indicators, 5 factors were extracted and labeled as 'economic efficiency', 'energy utilization', 'break-even point', 'milk to feed price', and 'bargaining power of the farm', in decreasing order of explained communality. The first 3 factors

shared 83% of the total communality. Feed efficiency was similarly associated with Factor 1 (53% loading) and Factor 2 (66% loading). Only Factor 4 was significantly affected by farm location. Milk production and herd size had significant effects on Factor 1 and Factor 2. This approach provided simple and effective descriptions of farm performance, related to feeding economics, based on a few uncorrelated indexes that were associated with specific aspects of management avoiding redundancy of multiple partial indicators. The proposed method allowed the evaluation, benchmarking, and ranking of dairy herd performance of individual farms and the comparison of multiple farms within a territory. This method might be useful for the development of well-defined and sustainable improvements in the efficiency of dairy farms.

## O163

### Performance of cull cows fed diets supplemented with hempseed cake

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Fattening of cull cows is not frequent because it is expensive, as these animals have low daily weight gain (ADG) and feed conversion. The interest in hemp (*Cannabis sativa* L.) cultivation is growing, and one by-product of the oil extraction is the hempseed cake, which has a protein with high biological value. Aim of this study was to assess the effect of dietary hempseed addition on performance of cull cows. Eighteen Italian Simmental cull cows were allocated into two groups, hay-based (HB, 10 animals) and corn silage-based (CS, 8 animals) diet. Within these groups, animals were randomly assigned to 2 dietary treatments, soybean meal (SM) or hempseed cake (HC, 5% DM basis), and in accordance to initial body weight,  $603 \pm 17.2$  kg, and body condition score,  $3.19 \pm 0.11$  points. Within groups, the diets were isocaloric and isonitrogenous. The trial lasted 4 months after an adaptation period of 1 month. Data were analysed considering groups (HB, CS) and dietary treatments (SM, HC) as block and fixed factor, respectively, with the exceptions of carcass conformation and fatness that were analysed using Fisher's exact test. ADG was calculated every 3 weeks and carcass traits were recorded at slaughter. The pH at 48h and meat quality after 14d of ageing (m. longissimus thoracis) were also considered. Including hempseed cake had not influenced ADG ( $0.929$  kg/d,  $p > .05$ ) and dressing percentage (48.8%,  $p > .05$ ). Considering carcass conformation, 56% and 44% of the carcasses belonging to HC were classified as fair (O) and good (R), respectively, percentages similar to those observed for SM, 33%, fair, and 66%, good ( $p > .05$ ). Considering

carcass fatness, 11%, 44% and 44% of the carcasses belonging to HC were classified as low (1), slight (2) and average (3), respectively, percentages similar to those observed for SM, 11%, low, 33%, slight, and 56%, average ( $p > .05$ ). Almost all the samples had a pH at 48h within the range of 5.40 to 5.59 that is considered as normal. HC had similar meat composition (e.g. ether extract,  $47.5 \pm 0.39$  g/kg,  $p > .05$ ), WBSF ( $36.1 \pm 1.28$  N) and cooking loss ( $26.9 \pm 0.87\%$ ) than SM. Including hempseed cake tended to increase L ( $33.4$  vs.  $32.0$ ,  $p = .08$ ) without affecting  $a^*$  ( $16.6 \pm 0.40$ ) and  $b^*$  ( $16.7 \pm 0.28$ ). Hempseed cake can replace soybean meal in the diet of cull cows without influencing in vivo performances, carcass traits and meat characteristics.

#### Acknowledgements

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## O164

### Bovine milk quality and apparent digestibility of diets as affected by diet characteristics and milking system

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The present study aimed to evaluate the effects of milking system (MS) and diet characteristics on milk quality and dietary total-tract apparent digestibility (ttaD) in dairy cows. Twenty-one Holstein dairy farms (ranging from 60 to 200 lactating cows) located in Northern Italy were involved in the trial. Nine farms fed to the cows a hay-based diet (HB) while 12 supplied a silage-based diet (SB). Among farms, 10 were equipped with robotic milking (RM) and 11 with a conventional milking parlours (CON). Farms were selected a priori in order to have both MS represented in both dietary type (DT). Diet and faeces were sampled 4 times in each farm every 30 days from June to September 2020. In particular, a representative sample of the total mixed ration was collected from the feed bunk, 2kg of faeces were collected from a 20% of the lactating cows and 500 mL of bulk milk representative of the previous 24 h were collected from the tank in order to assess the chemical composition. A subsample of both ration and faeces was used to evaluate the proportion of undigested NDF as marker to estimate the ttaD of nutrients (DM, NDF, ADF, ADL, cellulose, hemicellulose and ash). Fat and protein content, somatic cell count, mineral content (Ca, P, Na), milk

clotting ability (a30, RCT), pH, titrable acidity, and curd yield were determined on milk samples. Data were analysed using a linear mixed model; for milk traits the MS, month of sampling, DT and interaction between MS and month of sampling were used as fixed factors while the farm nested within MS was the random effect. As regards digestibility traits, the model included the fixed effects of DT, month of sampling, interaction between DT and month of sampling and MS, while the random effect was the farm nested within DT. Results show that milk composition, mineral content, clotting ability, pH and titrable acidity were not affected by the MS, but cows milked with CON had a greater DM and ash ttaD compared to cows milked with RM ( $p = .015$  and  $p = .029$ ). Cows fed with SB diet showed a higher milk fat ( $p = .006$ ), casein ( $p = .045$ ), and Ca content ( $p = .028$ ) compared to those fed with HB. The diet based on SB showed higher ttaD of DM compared to HB diet ( $p = .001$ ). Further analyses revealed that the interaction between MS and DT was significant for ttaD of cellulose and milk Ca content.

#### Acknowledgements

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### SESSION 34 OMICS TECHNOLOGIES – III

#### O165

#### Estrogen levels effect on mammary parenchyma explants from Holstein heifer calves fed an enhanced preweaning diet evaluated via RNA-sequencing

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To study the effect of estrogen on mammary gland development in young heifers, mammary parenchymal tissue (PAR) was collected at weaning (8 wk) from 3 Holstein heifer calves reared on an accelerated milk replacer (1.13 kg/d, 28% crude protein, 25% fat), and incubated for 6h at 3 estrogen concentrations: 0, 10, and 100 pg/mL, along with the no treatment control group. Total RNA was extracted and 12 libraries were sequenced on the Illumina HiSeq 4000 system, generating an average of 26 million reads of 100 nt in length per sample of which 93% were uniquely mapped and 77% assigned to genes. A linear model with estrogen dose as fixed and animal as random effect was fitted. Differentially

expressed genes (DEG) were declared at  $FDR \leq 0.10$ . The Dynamic Impact Approach (DIA) was used for pathway analyses to determine effect on biological pathways. DEG (428) were detected only for the comparison of 100 vs. 0 pg/mL, with 305 and 123 up and downregulated genes, respectively. Transcription factors (TF) significantly associated with our DEG list were predicted using ChEA3 tool. The results showed the differential expression of several genes involved with 'Estrogen signaling' pathway, notably ESRG, GNAQ, BCL2, CREB3L2, CYP1A1, TAB3, HSPB1, EDNRB, RPS6KB2, FOXO3, and PDPK1 genes. The functional analysis performed with DIA showed a global activation of all main KEGG categories and particularly of the pathways involved in 'Signal transduction' subcategory. This is compatible with a tissue stimulated to growth consistently and it is confirmed by the strong downregulation of 'Energy metabolism' subcategory. Overall, our results confirmed that the estrogen signaling is not limited by the canonical 'genomic' pathway, but its effect is mediated by multiple cytoplasmic signaling networks, notably 'Hippo', 'cGMP-PKG', 'PI3K-Akt', and 'Sphingolipid' signaling pathways. The TF enrichment analysis generated a list of 97 TFs significantly associated with our DEG list ( $FDR \leq 0.05$ ) and, among the others, predicted the activation of ESR1. Furthermore focusing on top-ten ranked TF, based on information retrieve in literature, many of them resulted to be very consistent with the estrogen treatment such as TEAD4, CREB, ZNF217, CTCF, MYOD1, GATA3, CEBPB, and TBP. Overall, results suggested a stimulation of growth and proliferation of PAR by exposure to estrogen at early stages of life in heifer calves fed an accelerated milk replacer.

#### O166

#### Genes related to fat metabolism in pigs and intramuscular fat content of pork

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Intramuscular fat (IMF), also called marbling, is an important meat quality trait of pork influenced by fat metabolism. IMF determines consumer quest and pork premiums. Strategies to optimize fat deposition for better marbling intensity are crucial. Genetics, age, sex, and environment, and dietary nutrients are key factors affecting the rate of lipid deposition and IMF in pigs. In this abstract, we give a brief communication on the most important



genes and epigenetic mechanisms central to fat metabolism and IMF deposition in pigs. Nowadays, the complex nature of the genomic and metabolic processes involved in gene expression has received significant attention from researchers. Results from genomic analyses have proven that the use of biomarkers and dietary markers are crucial fingerprints for accurately elucidating the genetic and nutritional regulation of fat deposition of pigs and IMF content of pork. Certain genes influence myogenesis and adipogenesis which determines marbling rate and fat deposition indirectly through MAPK (Mitogen-Activated Protein Kinase) cascades; a central signalling pathway regulating cell proliferation and differentiation. Interestingly, fat metabolism is also a MAPK-dependent transcription of various regulatory genes. Notably, FADS2 (Fatty Acid Desaturase 2), FASN (Fatty Acid Synthase), PPARG (Peroxisome Proliferator-Activated Receptor Gamma), SCD (Stearoyl-CoA Desaturase), H-FABP3 (Heart-Fatty Acid Binding Protein 3) and LEPR (Leptin Receptor) genes influence fatty acid metabolism and IMF content. FABP3 gene incorporates gene networks associated with IMF and affects fat traits in pigs independent of IMF. Identified SNPs (Single Nucleotide Polymorphisms) have revealed QTLs (quantitative trait loci) for active genes associated with fat metabolism and IMF deposition. These provide a possibility for marker-assisted selection for better fat deposition and marbling intensity. Further, the use of proteomics, transcriptomics, and muscle biochemistry has uncovered genes and biological markers associated with fat metabolism and IMF deposition in pigs. However, the nature of the correlation of such genes to dietary nutrient availability remains unclear. Therefore, matching nutriome (nutrient intake combination) with genotypes, and individual nutritional status of pigs can be used to optimize and modulate cellular metabolic functions and gene expressions related to fat metabolism and IMF content of pork.

## O167

### Proteomics to investigate tenderness biomarkers in Martina Franca donkey meat

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Tenderness strongly influences the consumer satisfaction and, consequently, the commercial value of the final product. Even if donkey meat is known to be healthy, due to greater content of proteins, vitamins and iron concentration, it is often characterized by low tenderness because animals are slaughtered at the end of their working life. In recent years, proteomics is applied in effort to achieve a better understanding of the mechanisms controlling tenderization processes. Therefore, this study aimed

to investigate the effect of aging on tenderness development and myofibrillar protein changes in 3 different donkey muscles. Longissimus lumborum (LL), semimembranosus (SM) and quadriceps femoris (QF) muscles, removed from 10 Martina Franca donkeys male carcasses, were divided into 5 sections, vacuum packaged, and stored at 2 °C for 1, 3, 6, 9, and 14 days of aging. At each aging time, Warner Bratzler shear force (WBSF), texture profile analysis, myofibril fragmentation index and changes of myofibrillar proteins with SDS-PAGE, Western blotting and Two-Dimensional Gel Electrophoresis (2DE) were estimated. Referring to instrumental tenderness, WBSF, hardness and chewiness parameters decreased during aging in all muscles, showing the lowest values ( $p < .01$ ) in LL muscle. Myofibril fragmentation index significantly increased ( $p < .001$ ) in LL muscles throughout aging time, while in QF muscle it increased ( $p < .01$ ) after 14 days of aging. Aging affected the intensities of several myofibrillar protein bands, particularly, band corresponding to intact actin showed a decrease ( $p < .05$ ) after 14 days of aging in all muscle analysed. Western blotting analysis revealed that desmin and troponin-T (TnT) proteins were affected by both muscle and aging effects. Desmin degradation was more intense in LL muscle showing the major intensity of degradation product after 14 days of aging compared to SM and QF muscles. An increase of degraded isoforms of TnT (30 and 28 kDa polypeptides) was found during aging in all muscles, however, in LL these bands showed a greater intensity starting from 1 day. During aging, 2DE image analysis results showed a significant increase of the total number of spots reaching the highest values in QF muscle at 14 days of aging ( $p < .01$ ). Data highlight that aging affects the meat tenderness and proteolysis with different intensities in each muscle and suggest that extensive post-mortem proteolysis occurred in QF muscle.

## O168

### Impact of acidogenic diets on rumen microbial community

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Modern farming systems could expose dairy cows to multiple stressors. Aim of this study was to investigate the evolution of microbial community composition in cows subjected to a dietary stress challenge. The challenge consisted in an abrupt change of ration from usual to a high grain diet (45.4:54.6 vs. 24.8:75.2 F:C). Rumen content was sampled from 12 animals two weeks before the administration of acidogenic diet (T0), on the first day of



administration (T1), two (T14) and four (T28) weeks after. Animals were equipped with collars to quantify rumination time, and amount and quality of milk produced was recorded daily. DNA was extracted and purified following a specific protocol designed for rumen fluid, then quantified and processed for sequencing. V3-V4 region of the 16S rRNA gene was amplified using 341F and 805R primers, then sequencing was performed on Illumina MiSeq according to the manufacturer's instructions. Raw sequences were processed using a pipeline combining PANDAseq, QIIME2 and DADA2. High-quality reads were clustered into high-resolution Operational Taxonomic Units (OTUs). Using taxonomic assignment, OTU tables were collapsed from phylum to genus level. Alpha diversity was computed using OTUs, Shannon index and Faith's diversity metrics. Beta diversity was estimated by computing UniFrac distances into Principal Coordinates Analysis (PCoA). Statistical analysis was performed using R studio 1.0.136. Significant differences in alpha or beta diversity were assessed by Wilcoxon test. Results obtained with the Beta diversity indicates that the abrupt change to acidogenic diet strongly impacted the microbial community during time. Interestingly, 4 animals underwent rumen hypomotility (RH), with a severe drop in rumination time (less than 200' per day), 5 showed milk fat – protein inversion (FD), while the other 3 displayed no particular health issues. Surprisingly, these differences were observed also in the microbial community composition. Fat depressed animal showed a significantly lower number of species compared to hypomotility ones (220 vs. 350 on average), and several genera showed similar differences: *Prevotella* was reduced in RH compared to FD (30% vs. 55%), while *Ruminococcus* increased (8% vs. 3%), as well as *Clostridiales* (9% vs. 4%). In conclusion, the abrupt change of diets induced the individual response of the animals, which could be significantly different among different subjects.

## O169

### MiRNAs cargo of milk exosomes are related to somatic cell count in dairy cows

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Bovine milk contains signaling molecules as short non-coding RNA, present inside and outside exosomes. The milk-derived exosomes' cargo can vary depending on several factors, especially those regarding the health status of the mammary gland. In the present study, we evaluated the composition of milk miRNA cargo of exosomes from cows classified according to milk total (SCC) and differential

(DSCC) somatic cell counts. A total of 34, 13 and 13 milk samples of healthy, at risk and subclinical or chronic mastitis cows, respectively, were collected during the same milking from a dairy herd. Healthy cows were in a group (G) with less than 200,000 SCC/mL and less than 69.3% of DSCC; bovines at risk were in a group called Y with less than 200,000 SCC/mL but more than 69.3% of DSCC. Subjects with a subclinical and chronic mastitis were in a group called R with a level of SCC higher than 200,000 SCC/mL and more than 69.3% of DSCC. Half of the samples were analyzed through NGS and the remaining were checked with qRT-PCR, in order to highlight the differences of miRNAs expressions in the 3 groups of animals. Three hundred thirty-four miRNAs were identified and assigned only to the *Bos taurus* species. After a filtering step, where miRNAs present in less than 20 samples were discharged, two hundred twenty-seven of them remained for the next steps of analysis. The statistical analysis was performed with the R package DESeq2, with comparisons between one group versus another. The comparison between cows classified with the letter G and the R group showed 4 differentially expressed miRNAs. The contrast of the Y group and the R group highlighted 8 miRNAs statistically different. The last one, the Y group versus the G group, resulted 17 significant miRNAs, the highest number. A Kruskal–Wallis nonparametric test was also performed to search for miRNAs that were differentially expressed ( $p < .05$ ) between the three groups of animals. A total of forty were differentially expressed based on the SCC and DSCC of the cows. Three miRNAs were selected for the qRT-PCR analysis of the samples not sequenced. Bta-miR-30a-5p and bta-miR-29c were significantly higher in healthy cows whilst bta-miR-223 resulted more expressed in bovines with chronic mastitis. miRNAs in exosomes are relevant signals involved in the regulation of immune response in the mammary gland.

## O170

### First assembled reference genome of white-clawed crayfish (*Austropotamobius pallipes italicus*)

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The white-clawed crayfish is a complex of native species (*Austropotamobius pallipes* and *Austropotamobius pallipes italicus*) that lives in lakes and freshwater streams in Italy and Europe. Unfortunately, in recent decades, the number of populations of freshwater crayfish has significantly reduced in relation to the increasing water pollution and rapid environmental changes. Currently, this species is classified as threatened by the International Union for Conservation of Nature (IUCN) and it has been included in Annexes II/V of the EU Habitat Directive. Despite the risk of extinction, the genomic information on this species is currently very limited.

Crayfish were sampled in Friuli Venezia Giulia (Italy). The body weights of crayfish were ranging from 18.2 to 25.48.6 g. Only one individual was used for the genome sequencing experiment. HMW genomic DNA was extracted from the crayfish tail muscle using the MagAttract HMW DNA kit (Qiagen, Hilden, Germany). The DNA library was sequenced at University of Udine using the Illumina HiSeq™ 2500 platform following the standard method. The genome sequences were assembled using the ALLPATHS software. To improve the quality of gene annotations, a *de novo* transcriptome assembly (RNAseq) was used. Based on the assembled genome sequences, a functional annotation analysis was performed using the high-throughput cloud Blast and InterproScan softwares, Gene Ontology mapping (GO) (analysis of signals of positive selection), Blast2GO Annotation and COG (Clusters of Orthologous). The number of sequences (contigs) was: 2.350.888, N50 lenght (bases): 668 and the average GC content: 43.61%. The Bloom filter false positive rate was very low: 0.627%. The obtained sequences were used to blast with UNIPROT and INTERPRO databases using BLASTx ( $e < 1e-5$ ). The putative genes were used to perform a gene ontology (GO) annotation with GO and GO slim analyses, and a total of 34,410 genes were identified and classified into the most represented categories: cellular processes (GO: 0032501), metabolic processes (GO: 0032502), binding (GO: 0005215) and catalytic activity (GO: 0008509). A total of 47,371 putative genes were classified into the COG functional categories: metabolism (42.4%), information, storage and processing (17.2%), cellular processes and signaling (7.8%). Overall, the draft assembly metrics and gene representation are consistent with other similar crayfish reference genome assemblies. The present work can help to improve and accelerate the discovery of new genes to be used in future genetic diversity and evolutionary studies.

indicate inbreeding from a recent past, whereas short regions from a long time ago. Besides the traditional studies about biodiversity, ROH have been also used for animal breeding purposes. The aim of this study was to evaluate the use of single-step genomic BLUP with a modified genomic relationship matrix, built using ROH information. SNPs were coded as follows: 0 = homozygote inside ROH; 1 = homozygote outside ROH; 2 = heterozygote; then, the genomic relationship matrix was built. A total of 80,417 test day records (milk yield, MY; fat yield, FY; protein yield, PY) from Italian Buffalo cows born from 1975 to 2009 was available. Genotypes at 49,164 loci (after quality control) were available for 498 animals (463 cows +35 sires). Pedigree-based BLUP and traditional ssGBLUP were also carried out to compare the new proposed method (name ssGROH). This method was repeated three times using different minimum length of ROH: ssGROH1 with 1 Mb as length, ssGROH4 with 4 Mb as length and ssGROH8 with 8 Mb as length. All methods were evaluated using 50 cows with both phenotypes and genotypes born in 2008 and 2009 as validation, by computing the (i) correlation between their adjusted phenotypes (in 2009) and (G)EBV (in 2007); (ii) the correlation between consecutive (G)EBVs (in 2007 and 2009). Among traits, MY showed the strongest correlations in all methods and validations. Average correlations between adjusted phenotypes and (G)EBV were  $0.49 \pm 0.04$  (BLUP),  $0.49 \pm 0.01$  (ssGBLUP),  $0.54 \pm 0.03$  (ssGROH1),  $0.54 \pm 0.02$  (ssGROH4),  $0.52 \pm 0.03$  (ssGROH8). The average correlations between subsequent rounds of evaluation (to measure stability of the method) were  $0.71 \pm 0.02$  (BLUP),  $0.82 \pm 0.01$  (ssGLUP),  $0.77 \pm 0.01$  (ssGROH1),  $0.77 \pm 0.01$  (ssGROH4) and  $0.76 \pm 0.01$  (ssGROH8). Results of the present study provide good insights on the use of ROH information within the ssGBLUP framework, possibly by integrating gene content and ROH information in a unique genomic relationship matrix.

## SESSION 35 GENETIC DIVERSITY

### O171

#### Using runs of homozygosity within single-step genomic BLUP

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Runs of homozygosity (ROH) are a widely studied tool applied to different branches of livestock genetics. The ROH are uninterrupted stretches of homozygotes loci without heterozygosity at the diploid state. These regions are likely to be present in offspring of related animals, i.e. inbred individuals. Long regions

### O172

#### Genomic inbreeding estimates with imputed genotypes in Italian Holstein dairy cattle

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Inbreeding coefficients can be calculated from genomic markers with different methods that provide varying estimates. Recent research was focused on genotypic data, while knowledge on imputed genomic data is limited. Hence, the objective of this study was to assess the effect of imputing single nucleotide polymorphisms (SNP) on different estimates of genomic inbreeding

coefficients (FG) in dairy cows. To this purpose, 95,540 Italian Holstein dairy cows with imputed SNP were analyzed. Cows were genotyped with 30 different SNP chips and imputed to 84,445 pre-selected SNP from the routine genomic evaluations of the Italian National Association of Holstein and Jersey Breeders (ANAFIJ). All SNP were used, being already filtered for call rate <95%, parent-offspring SNP mismatch >0.01, minor allele (<0.02) and genotype (<0.001) frequencies and extreme deviation from Hardy-Weinberg Equilibrium ( $p < .005$ ). To test the effect of imputation on FG, four medium-density (MD) SNP chips (each with >10k genotyped cows; GeneSeek Genomic Profiler3,4,MD and LabogenaMD) and one high-density (HD) SNP chip (641 genotyped cows; GeneSeek Genomic ProfilerHD-150K) were selected. Moreover, FG was estimated with seven standardized methods: (i) based on homozygosity excess (F and FHAT2), variance of the additively recoded genotypes (FHAT1) and correlation between uniting gametes (FHAT3), available in PLINK v1.9, (ii) VanRaden's genomic relationship matrix (GRM; FGRM1, FGRM2), estimated with an in-house Python script and (iii) runs of homozygosity (ROH) across the genome (FROH), calculated in detectRUNS v. 0.9.5 R package. Pedigree-based inbreeding coefficients were also estimated (FPED). Pearson ( $\rho$ ) and Spearman ( $r$ ) correlations of imputed vs. genotyped cases across SNP panels were calculated. Results are summarized as follows: (i) on average, in the HD SNP panel,  $\rho \approx r \approx 1$  in pairwise comparison (imputed vs. genotyped) across FG; in MD SNP panels,  $\rho < r$  and with higher variability across FG, with LabogenaMD showing the highest consistency ( $\rho = 0.85 [0.77, 0.97]$ ,  $r = 0.95 [0.92, 0.97]$ ), (ii) FROH was most consistent across SNP chip ( $\rho = 0.94 [0.79, 1]$ ,  $r = 0.97 [0.93, 1]$ ), (iii) FROH had the highest relationship with FPED ranging between  $\rho = 0.48\text{--}0.83$  and  $r = 0.54\text{--}0.84$ , (iv) non-linear trends across pairwise comparisons indicated that  $\rho$  might not well reflect the relationship between FG and FPED;  $r$  provides an alternative estimate when a monotonic relationship exists, and v) re-ranking across FG methods was observed.

## O173

### Inbreeding depression in small ruminants: from pedigree to genomic estimation

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Nowadays genomic data represent an increasingly important tool to exploit the traditional pedigree information in order to create better mating schemes and preserve biodiversity. In the Italian small ruminants populations the use of such information could improve the estimation of genetic population parameters leading to a better

management of the genetic background of the different flocks. In this work we investigate the relationship between different methods of inbreeding calculation from traditional pedigree information (FPED) and from 50k SNP chip genomic data (FROH) on the estimation of inbreeding depression effect on daily milk production.

We analyzed pedigree and genomic data from 980 individuals of Camosciata delle Alpi goat breed and 380 individuals of Massese sheep breed. Data were provided by the Italian association of small ruminants breeders (ASSONAPA) within the PSRN project Conservation, Health and Efficiency Empowerment of Small Ruminant (CHEESR). Editing of genomic data and calculation of FROH were performed with the PLINK software, while calculation of FPED was performed with the R package Optisel. The relationship between the two inbreeding indicators and daily milk production were calculated using two different GLM models.

The results showed a similar mean value of FROH in the two populations sampled, 6.6% in Massese and 5.4% in Camosciata, but the mean FPED value in Massese (3.9%) was more than double than in Camosciata (1.5%). As expected, the correlation between FPED and FROH was much lower in Camosciata (0.19) than in Massese (0.39). In Camosciata, 30.6% of FPED values equal to zero were unreliable (they exceeded the UIC 95% of the intercept). In terms of inbreeding depression effect (%) on daily milk production, daily milk production decreased by  $-0.017 (+ -0.005)$  in Camosciata and  $-0.015 (+ -0.018)$  in Massese using FROH and by  $-0.005 (+ -0.005)$  and  $-0.049 (+ -0.03)$ , respectively, using FPED. What emerges from these analyses is that, in populations with low average inbreeding, there is a similar inbreeding depression based on FROH estimate, but the completeness and validity of the pedigree recordings greatly affect the values of FPED. Based on that, the reliability of the pedigree information has to be fully evaluated in the estimation of the inbreeding depression effect.

## O174

### SNP-based characterization of Montecristo feral goats in a Mediterranean perspective

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The Montecristo wild goat is an endangered free-ranging goat population inhabiting the homonymous island in the Tuscan Archipelago. The origin of this feral population is still debated:



some authors suggest that goats were introduced during Neolithic times while others date their first occurrence between the 6th and 13th century of the Common Era when Montecristo island was occupied by a monk community. In this study, we assessed genetic diversity and population structure of two different nuclei of Montecristo goats, one sampled on the island, the other from an ex-situ conservation project on the Italian mainland. Furthermore, to investigate the origin and evolutionary relationships of Montecristo goats we assembled a 50K SNPs dataset including 44 breeds from the Mediterranean basin. Observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity were calculated to estimate diversity levels, while gene flow, population structure and relationships were assessed through multiple approaches, i.e. runs of homozygosity (ROHs), Principal Component Analysis (PCA), Neighbour-network reconstruction, Treemix software analysis.

Values of  $H_o$  and  $H_e$  for the insular Montecristo population were the lowest recorded in the dataset, highlighting reduced genetic diversity, while the nucleus from the mainland displayed a less severe reduction. Results from PCA, Neighbour-network and population structure analysis clearly separated the insular population from all other breeds, and further highlighted a remarkable distance between the two Montecristo nuclei. Also, Treemix software analysis pinpointed possible genetic contributions received by the two Montecristo goat populations from different sources. Lastly, ROHs indicated the occurrence of an ancient bottleneck or founder effect in the insular population, and of recent inbreeding in the continental nucleus.

Taken together, our results indicate that Montecristo goats faced several demographic fluctuations combined with admixture events over time, and also emphasized the marked genetic differentiation between the goats sampled on the island and those from the ex-situ nucleus. This evidence can represent a starting point for the implementation of a marker-assisted monitoring and conservation plans for the autochthonous feral goat of Montecristo.

#### Acknowledgements

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## O175

### A comparative analysis of pedigree- and genomic-derived inbreeding parameters in Italian heavy pig breeds over the last decades

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Inbreeding is an important parameter that is monitored in selection and conservation programs in livestock, as an increased inbreeding

rate leads to inbreeding depression and to an increased frequency of deleterious alleles. Traditionally, the inbreeding coefficient is calculated using pedigree information and it is defined as FPED. Single nucleotide polymorphism (SNP) genotyping panels allow to estimate genomic inbreeding by identifying runs of homozygosity (ROH) that are long chromosome regions in which all adjacent SNPs are homozygous. Genomic inbreeding (FROH) is calculated as the fraction of the autosomal genome covered by ROH. The breeding program of Italian pig breeds started about 30 years ago and pedigree data are recorded at a depth that reaches more than 20 generations. In this work, we retrospectively analysed FROH over this period in Italian Large White (ILW), Italian Duroc (ID) and Italian Landrace (IL) heavy pig breeds and compared this parameter with FPED. A total of 3055 ILW pigs, 758 ID pigs and 1940 IL pigs born over the last 25 years were genotyped with the Illumina PorcineSNP60 BeadChip and with Illumina GGP Porcine HD arrays. Quality filtering and ROH identification were obtained with PLINK version 1.9. Animals were grouped by year of birth and groups with a minimum of 10 animals were considered. The averaged FROH and FPED were computed for each group and for each breed. FROH was higher in ID (the highest averaged FPED in this breed was 0.12) with respect to the other two breeds (0.07 in ILW and 0.06 in IL) and was constant over all analysed time windows. In the considered period, the two increasing trends of FROH and FPED were significantly different in ILW and in ID breeds with a higher rate for the FPED mean values. In IL, the increasing inbreeding rate of FPED was stronger with respect to FROH, even if not significantly different. The results indicated that mean FROH in all three breeds over the years was more stable than FPED. FROH can be an effective parameter that could substitute FPED in breeding and conservation plans.

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## O176

### The impact of inbreeding in the Italian Holstein breed

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The Italian Holstein breed has been exposed to intense selection for milk production traits over the last 50 years, leading to an average of over 10,000 kg of milk produced per cow per lactation.



However, intense directional selection might increase inbreeding and loss of genetic variability. High inbreeding levels can in turn cause an excess of deleterious variants and reduced biological fitness. Thus, the objectives of this study were to (i) estimate the level of inbreeding in 27,735 Italian Holstein dairy cows from pedigree and imputed genotype data in the form of ~85k single nucleotide polymorphisms (SNP), and (ii) investigate the effect of inbreeding on 305-day milk (MY; kg), fat (FY; kg), and protein yield (PY; kg).

Pedigree-based inbreeding coefficients (FPED) were defined as the probability that an individual has two identical alleles by descent and were computed using the OptiSel R package. Genomic inbreeding coefficients were derived by means of runs of homozygosity assessment (ROH). The ROH segments were detected using the DetectRUNS package in R and defined as follows: at least 15 SNPs in a run, a minimum length of a run equal to 1 Mb, a maximum distance between consecutive SNPs in a 500 kb window, a lower density limit of 1 SNP per 100 kb and allowing for a maximum of one missing and one heterozygous SNP in a run. The genomic inbreeding coefficients (FROH) were calculated as the proportion of individual genome size covered by ROH. The presence of inbreeding depression was evaluated by regressing phenotype on FPED and FROH using a linear mixed model in ASReml 4.1. The following terms were included in the model: herd-year of calving (2242 classes), parity (3 classes) and month of calving (12 classes). Overall, FROH (mean FROH =  $0.16 \pm 0.03$ ) was higher than FPED (mean FPED =  $0.07 \pm 0.02$ , complete generation equivalent = 10.59), with a correlation above 0.60. Inbreeding depression was observed in MY, FY and PY for both inbreeding estimates ( $p < .01$ ). A 1% increase in FROH and FPED was associated with a decrease in MY of about 44 and 61 kg ( $p < .01$ ), respectively. The same trend was found when evaluating FY and PY ( $p < .01$ ), with an FY decrease of 1.31 kg and of 2.45 kg and of 1.41 kg and 2.0 kg for PY, based on FROH and FPED, respectively. Ongoing research is investigating the role of inbreeding in other economically important production and fertility traits in Holstein dairy cattle.

## SESSION 36 COMPANION ANIMALS AND SOCIETY – II

O177

### Genomic evaluation of the Italian shepherd dogs

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Dogs have provided great help to shepherds since ancient times, either by protecting or driving the flock. These distinct roles led to the separation of shepherd dogs in livestock guardians (LG) and herding dogs (HD). The constant selection conducted by shepherds and breeders to improve the skills of their dogs originated several breeds, with distinct morphology and behaviour. Given the importance of pastoralism, it is not surprising that Italy counts many local shepherd dog breeds spread across the peninsula. In this study, we investigate these breeds from a genomic perspective and identify selection signatures differentiating between LG and HD. Canine 230K SNPChip, updated to CanFam3.1, was used to genotype 134 shepherd dogs. The LG group consisted of 52 dogs of 4 breeds (Maremma sheepdog, Mannara dog, Pastore della Sila, and Fonnì's dog), whereas the HD group consisted of 82 dogs from 5 breeds (Pastore d'Oropa, Pastore apuano, Bergamasco shepherd, Pastore della Lessinia e del Lagorai, and Lupino del Gigante). Commonly used thresholds for quality control were applied (QC) and breed sample sizes were balanced. We compared LG and HD with Wright's fixation index (FST) and single-SNP cross-population extended haplotype homozygosity (XP-EHH). The top 1% markers for FST and XP-EHH values were considered as relevant.

The filtering stage retained 120 836 SNPs and 99 dogs (42 LG and 59 HD). The top 1% of FST (0.24–0.54) and the top 1% of XPEHH (2.72–4.97) had 65 SNPs in common, mapping on 49 different genes. The most intense signals located on chromosomes 6, 9, and 21. A gene-level analysis showed that 8 genes are associated with human eye disorders similar to those that Border collie and its related breeds are prone to develop. Other 8 genes play a role in the nervous system development or functionality. Finally, 20 genes are implicated in behaviour regulation and dog domestication. In this study, we reveal genomic traces of the differentiation of Italian LG and HD dog breeds for the first time. The identified genes are related to potentially relevant pathways throughout the selection and the differentiation of these breeds and deserve further investigation to confirm their role in this process.

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O178

### Dietary *Ascophyllum nodosum* and *Bacillus subtilis* C-3102 influence faecal fermentation patterns in dogs with chronic enteropathies

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Chronic enteropathy (CE) identifies a group of idiopathic inflammatory diseases of the dog characterised by persistent or recurrent gastro-intestinal (GI) signs. Provocative evidence shows an association between CEs and intestinal dysbiosis, therefore microbial modulation agents may feature in the management of these disorders. The study's aim was to assess the effects of the seaweed *Ascophyllum nodosum* (AN), rich in fermentable fibres and bioactive compounds, with or without the probiotic strain *Bacillus subtilis* C-3102, on clinical and metabolic outcomes in dogs diagnosed with CE. Fourteen CE patients not responding to dietary manipulations were selected from 115 sheltered dogs with chronic GI signs via an exclusion diagnostic protocol. The dogs, which had received the same maintenance, gastrointestinal-type control (CTR) food for a minimum of three months, were enrolled in a 90-day longitudinal, non-comparative investigation based on the serial administration of three different diets: (1) a hydrolysed protein (HP) diet; (2) 4.0% (as fed) AN supplemented HP (HPA) diet, and (3) HPA food fortified with  $125 \times 10^9$  *B. subtilis* C-3102 spores/10 kg body weight (HPAB diet). Canine inflammatory bowel disease activity index (CIBDAI), along with stool sampling, were performed prior to the initiation of the study and at the end of each 30-day treatment phase. Faecal concentrations of short chain fatty acids (i.e. formic acid, acetic acid, propionic acid and butyric acid), acetone, lactic acid, branched chain fatty acids (i.e. isovaleric acid, isobutyric acid and valeric acid), phenolic and indolic compounds (i.e. skatole, indole and phenol) were measured by liquid chromatography. Overall, 9 animals completed the study. No differences were found regarding CIBDAI scores in any of the pairwise comparisons among treatment phases. Concentrations of acetic acid were higher ( $p < .05$ ) in dogs that were fed HPA compared to CTR diet, and quantities of isovaleric acid and isobutyric acid were greater ( $p < .05$ ) in HPA as against HP food. A tendency for higher amounts of faecal butyrate was found for the HPAB dietary treatment ( $p = 0.06$ ). Comprehensively, while showing positive effects on faecal fermentative end-products, diet fortification with AN and/or *Bacillus subtilis* C-3102 failed to improve clinical conditions in CE dogs. As this investigation was likely underpowered, larger studies are needed to confirm the present findings.

**O179**

## Effect of different diets on metabolic profile of healthy adult dogs

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Traditionally, kibble diets are formulated with a high proportion (30–50%) of cereals associated with protein and fat of animal origin and oil. Recently, some pet-food industries commercialized diets named grain-free, formulated without cereals, and considered more adequate to carnivore diets. However, in literature some concerns were reported regarding risk related to the use of these diets. The study aimed to evaluate the effect of a grain-free diet on the hematological parameters of healthy dogs. In a dog kennel located in the province of Naples, 30 adult dogs (age:  $4 \pm 1.20$  years, weight:  $20.79 \pm 6.38$  kg, BCS:  $3.96 \pm 0.95$ , on 5 points scale) were divided into two groups. Two diets (cereal-based CB vs. grain-free GF) were alternatively administered to each group in a ratio of 130 kcal ME/kg metabolic weight. Then main nutritional characteristics of diets are the follow: CP 81 vs. 91 g/Mcal; EE 38 vs. 46 g/Mcal; NFE 123 vs. 69 g/Mcal for CB vs. GF, respectively. Each experimental phase lasted 8 weeks (3 of adaptation +5 of treatment). At the beginning of the trial and the end of each experimental phase, blood samples were collected at 8,00 a.m. on fasting dogs to determine the blood count and main biochemical parameters. The results obtained after the administration of each diet were statistically analyzed (ANOVA) to compare the diet effect. All dogs showed the blood count and biochemical values within the ranges considered physiological. The dietary treatment did not affect blood profile except for MCHC, which was significantly ( $p < .01$ ) higher when dogs fed GF diet (34.44 vs. 35.09 g/dl, respectively). This result is according to literature which evidenced higher haemoglobin levels with higher protein concentration in the diet. Regarding the biochemical parameters, significant differences were observed for Creatinine (80.68 vs. 72.61  $\mu\text{mol/l}$ ,  $p < .05$ ), BUN (6.81 vs. 5.74 mmol/l,  $p < .01$ ), AST (40.00 vs. 33.87 U/l,  $p < .05$ ), fructosamine (204 vs. 187  $\mu\text{mol/l}$ ,  $p < .05$ ), lipase (133 vs. 67 U/l;  $p < .05$ ) and triglycerides (0.88 vs. 0.59 mmol/l;  $p < .01$ ) in CB and GF diets, respectively. The observed differences seem to indicate that the different proportions of energy nutrients affected the carbohydrates, protein, and lipid metabolism. Also, the serum level (mmol/L) of potassium (4.92 vs. 4.55) and magnesium (0.90 vs. 0.81) were significantly higher ( $p < .01$ ) when dogs fed CB diet compared to GF one.

## O180

### Network-based gut microbiome analysis in dogs

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A growing number of studies in the last decade described the microbial population in different niches of the organism, and especially the gut microbiome, thank High-throughput DNA sequencing techniques, that are easily accessible to researchers. Furthermore, network analysis allows the characterization of bacteria with indirect associations with outcomes such as diseases, diet and sex of the host, via their association with other taxa. The present study was the follow up of a previous one, where we reported data collected from several in house experiments carried out in healthy dogs. The dataset represented 132 dogs with 334 faecal samples collected serially during dietary intervention studies. The animals were divided according to diet (commercial extruded diet, 171; commercial moist diet, 83; home-made diet, 30; BASE<sup>TM</sup> diet, 56) and sex (whole males, 78; whole females, 145; neutered males, 89; spayed females, 28). The procedure of samples collection, storage, DNA extraction and sequencing, bioinformatic and statistical analysis followed a defined pipeline. The extracted DNA was prepared for the sequencing of the V3 and V4 regions of the 16 rRNA gene with a MiSeq (illumina, San Diego, CA, USA) in a 2×300 paired-end mode. Raw sequences were processed using the bioinformatic program QIIME 2 and annotated to greengene database. Here, the results of the 16S rRNA sequencing of the V3 and V4 regions, was used to systematically analyze the structure of the gut microbiome of dogs fed with the above-mentioned different diets. The correlation network analysis was performed calculating pairwise relationship between taxa with the SparCC (Sparse Correlations for Compositional data) algorithm, which was designed to solve the problem of spurious correlations given by the compositional nature of microbiome data. Firstly, we identified candidate bacteria highly abundant in the microbial community, and secondly, we looked at taxa with direct and indirect associations with the factors diet and sex, that were the same consideration in the previous study. In conclusion, this study demonstrated an approach to interpret the network structure of the gut microbiome on dogs categorized by diet and sex, giving a better explanation of the interactions between bacteria that resulted in a clustering of the dogs based on environmental or genetic influences. In particular, this study paved the bases to understand how the gut community of bacteria was interconnected and operated in relation to dietary composition and sex.

## O181

### Renal petfood for dogs and cats: fatty acids profile

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Nutritional intervention is a keystone in the treatment of dogs and cats affected by chronic kidney disease (CKD). Dietary lipids, in particular omega 3 fatty acids, provide many benefits to CKD patients. Lipid content increases energy density and palatability of the diets, and the omega 3 fatty acids such as EPA and DHA could exert renoprotective effects. The present study aimed to evaluate the fatty acid profile in 30 samples of pet food (canine: 9 dry and 7 wet; feline: 7 dry and 7 wet) destined to patients affected by CKD. Total lipids were extracted according to the Folch method and fatty acids were methyl esterified according to Christie. FAMES were analysed by a TRACE<sup>TM</sup> 1300 gas chromatograph equipped with an FID detector. Data were then analysed using IBM SPSS Statistics V.25 software. Data are expressed as LSMEAN ± SEM. The mean lipid content of canine CKD pet food varied from 44.4 ± 1.8 g/Mcal in dry diets to 55.5 ± 3.4 g/Mcal in wet diets; while in feline products, they were 44.3 ± 2.1 g/Mcal and 76.7 ± 9.8 g/Mcal in dry and wet diets, respectively. Similar lipid content was present in dry diets of both species. Lipid requirements have been satisfied by the high content of fatty acids in wet food, which positively also affected the palatability. A total of 16 fatty acids, including 5 saturated fatty acids (SFA), 4 monounsaturated fatty acids (MUFA) and 7 polyunsaturated fatty acids (PUFA) were identified and expressed as g/100g of total FA. PUFA ranged from 25.0 ± 1.7% to 35.1 ± 4.2% in canine dry and wet diets, and from 24.9 ± 2.6 % to 28.6 ± 3.8% in feline dry and wet diets. In the canine pet food, the amount of EPA and DHA was 0.7 ± 0.2% and 0.7 ± 0.2% respectively in dry diets, while in wet diet the content was 0.5 ± 0.2% and 0.7 ± 0.2 % respectively. Similar concentration of EPA and DHA was present in dry (EPA 0.7 ± 0.2% and DHA 0.7 ± 0.2%) and wet pet food (EPA 0.8 ± 0.2% and DHA 0.7 ± 0.2%) formulated for feline patients. The present study contributed to characterize the fatty acid content of some of the most popular therapeutic kidney diets present in the Italian market. As expected, the data assessed a high variability in the fatty acid composition of the renal pet food, due to the different dosage and ingredients used in the selected renal products. Further studies are required to better define the optimal dosage of omega 3 fatty acid in CKD products formulated for dogs and cats.



## ANIMAL WELFARE AND RESILIENCE – P001

## P001

**Genetic parameters of temperament traits in Marchigiana, Chianina and Romagnola young bulls**

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Temperament has been defined as the reactivity or fear response to human's interaction or novel environments and it is a welfare indicator. Temperament is a complex phenotype often evaluated by measuring behavioural traits affected by genetic, environmental factors, and their interaction. This study aimed to estimate genetic parameters for temperament collected in Marchigiana (M), Chianina (C) and Romagnola (R) bulls under performance test (PT). Data of 445 bulls were used (159 of M, 142 of C and 144 of R) each one accounting for 12 traits recorded during 4 phases of PT. The 1<sup>st</sup> phase (1p) was at the end of quarantine and 4 temperament traits were recorded by scoring animals' reaction at the entrance into the paddock, the technician approach, touches, and the return into the pen. The 2nd and 3rd (2p and 3p), coincident with first and last weighing provided to score reaction at the entrance, staying and exit to the weighing cage. At the 4th phase (4p) behaviour during body measurements and halter attachment were obtained. All traits were scored by a single operator using a 1–4 scale system, e.g. from submissive to indocile. A general greater frequency of class 4 (indocile) on almost all traits was observed in R as compared to the other two breeds. In 1p the entrance into the paddock led to a 16.7% of incidence for class 4 in R, while M and C showed 1.9% and 4.9%, respectively. In 2p the young bulls from R still reported a higher incidence of class 4 than the other two breeds. Differences between breeds could be linked also to the breeding system at the farm, considering that R bulls had a higher incidence of loose housing and pasture system than other breeds. The GLM showed the significance of 3 main non-genetic factors, that is breed, breeding system and contemporary group. All effects included in the final model used to estimate genetic parameters under a single-trait animal model-REML accounting for 9289 animals in the pedigree file. Heritability estimates resulted in variable among traits analysed, ranging from very low (0.04) to high values (0.57) with a tendency to be reduced in subsequent phases, probably due to animal settling to environmental condition. Indeed, in the 1p heritability ranged from 0.05 to 0.57, in the 2p from 0.04 to 0.44, in the 3p from 0.16 to 0.34, and in the 4p from 0.20 to 0.23. We conclude that temperament of young bulls measured during the PT shows some genetic components although affected over time by rearing environment.

## P002

**Does the presence of weeping teats affect behaviour, health and productivity of dairy goats?**

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Goat's udder occasionally presents a congenital abnormality called 'weeping teats' that causes micro-lacerations in the mammary epithelial tissue, with subsequent milk leakage. As a precaution, animals with this pathology are excluded from herds. However, little is known about the consequences of this abnormality on goats' welfare and health. The present investigation was carried out to evaluate the effect of the presence of weeping teats on production, discomfort behaviour during milking and udder health in dairy goats. In order to highlight possible signs of discomfort or pain, we carried out behavioural observations during milking on 23 Alpine goats (11 with weeping teats [WP], 12 controls with normal teats [C]) at the beginning, in the middle and at the end of lactation. Additionally, four milk samples were collected from each teat of each animal and submitted to microbiological analysis and somatic cell count (SCC). Productive data were provided by the local farmers' association (APA). No differences between groups were observed for discomfort behaviours (detach or attempts to detach the milking cluster, urinating, defecating, kicks, attempts to escape), or for other behaviours that may indicate positive emotional state (tail up) or excitement (tail wagging). The duration of milking was longer in C than in WP goats, with significant differences at the beginning of lactation (C: 142.78 ± 13.04; WP: 98.63 ± 5.04 sec;  $p < .05$ ; Kruskal–Wallis test). However, these differences are probably due to individual variation, rather than to the presence of weeping teats or to the production level, given the fact that milk production did not statistically differ between groups (C: 682.00 ± 26.59; WP: 715.82 ± 31.78 kg/head/year; n.s.; Kruskal–Wallis test). No contagious microorganisms were found in milk samples from both groups. However, WP goats showed a higher proportion of teats positive to microorganisms (mainly *Staphylococcus* spp) (C: 15.6%; WP: 46.9%;  $\chi^2 = 5.8909$ ;  $p = .015$ ).

Our results suggest that the presence of weeping teats does not affect productivity and does not cause pain or discomfort to dairy goats. However, some concerns arise for udder's health, but further studies on a larger sample are required to confirm these preliminary findings.



## P003

### Reference intervals for plasma parameters in high-welfare multiparous dairy cows in different physiological phases

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Assessing multiple plasma analytes at the herd level and comparing their values to suitable reference intervals (RI) could potentially reflect the proper adaptation of dairy cows to the different phases of the lactation cycle. This approach is pivotal during the transition to calving when sudden changes in cow's metabolism could increase their likelihood in developing metabolic diseases. This study was aimed in defining new RI for a wide range of plasma biomarkers in multiparous Holstein cows at their transition to calving, selecting reference individuals among healthy subject raised in high welfare conditions. The welfare status of the herds was evaluated according to the computerized Integrated Diagnostic System Welfare model. Eleven high-welfare farms (HWF; welfare score >70%) located in northern Italy were selected and their herds used to recruit 361 clinically healthy cows undergoing the dry (from -30 to -10 days from real calving; DFC), the postpartum (from 3 to 7 DFC) and the fresh phases (from 28 to 45 DFC). Cows having plasma levels of beta hydroxybutyrate (BHB) higher than 1.2 mmol/L and calcium lower than 2 mmol/L were retrospectively excluded as affected by subclinical diseases (SCD), and a subset of 261 cows was included in the final database. Data of plasma analytes underwent ANOVA testing using physiological phases as predictors. Individual effect of each phase was assessed using a pairwise *t*-test. A bootstrap approach was used to define the RI for each blood analyte within physiological phases having a pairwise *t*-test  $p \leq 0.05$ . Concentrations of cholesterol, nonesterified fatty acids, creatinine, ceruloplasmin, total protein and globulin differed during all the phases considered, while the concentration of thiol groups did not. During the dry phase, the Packed cell volume and the plasma concentration of urea were equal to during the postpartum phase, and those of glucose, BHB, haptoglobin, myeloperoxidase, reactive oxygen metabolites, advanced oxidation of protein product and Ca were equal to during the fresh phase. During the postpartum phase, fructosamine and P concentrations were equal to during the fresh phase. This study updated the variability affecting plasma biomarkers during the transition to calving of healthy multiparous Holstein dairy cows raised in high welfare conditions and can be used to assess the ability of herds to cope with the stressful conditions in the transition period.

#### Acknowledgements

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## P004

### Associations between udder health traits and milk mineral profile in Holstein Friesian cattle

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Mastitis is one of the most widespread disease in dairy cattle with great economic impact due to milk production losses and treatments costs. It causes alterations in the concentration of milk components including minerals which have a role in the maintenance of osmotic equilibrium of cells and, as salts, in the stability of the structure of casein micelle. Somatic cell count (SCC) is the most common indicator of mastitis but recently a novel parameter, i.e. the differential somatic cell count (DSCC) which represents the percentage of polymorphonuclear neutrophils (PMN) combined with lymphocytes, has been proposed coupled with SCC for providing more information about the dynamics of udder health. Aim of this study was to investigate the associations between somatic cell score (SCS) and DSCC, and the milk concentration of 32 macrominerals (Ca, P, Mg, Na, K, S) and microminerals (Fe, Cu, Mn, Zn, Se, Co, As, Pb, Cd, Hg, Al, Cr, Mo, Ag, B, Ba, Be, Li, Ni, Sb, Si, Sn, Sr, Ti, Te, V) in a cohort of 260 Holstein Friesian cows in two herds. Milk mineral contents were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Data were analyzed with a linear mixed model including days in milk, parity, SCS and DSCC (discretized in quartiles) as fixed effects and herd-date as random effect. Minerals with a concentration below the limit of quantification were excluded from the statistical analyses. Results showed that SCS was associated with an increasing trend in Fe content ( $p < .10$ ). The DSCC was associated with a decrease in Na ( $p < .05$ ), an increase in Fe ( $p < .01$ ) and an increasing trend in Al ( $p < .10$ ). Results, although preliminary, indicate that DSCC was associated with alterations in milk mineral profile and that such variation is not always in the same direction as the SCC. As the project is still ongoing, increasing the sample size will provide further results that might help to draw more robust conclusions.

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## P005

### Effects of a selective dry-cow therapy approach on milk, inflammation, and metabolic profile during the dry-period

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Pressure towards the reduction in antibiotic usage in livestock is increasing and the dry-off could represent a phase to be improved. The objective of this study was to evaluate the effects of selective dry cow therapy in cows with different somatic cell count (SCC) at dry-off. Twenty-three Holstein dairy cows without microbial intramammary infections were enrolled in the study. Eight cows with SCC over 200,000 n/mL were abruptly dried-off with antibiotic treatment and internal teat sealant (HI-AB; parity  $2.8 \pm 0.7$ ,  $424 \pm 58$  days in milk); seven cows with SCC under the threshold of 200,000 n/mL were treated with antibiotic plus teat sealant (LO-AB; parity  $2.7 \pm 1.5$ ,  $366 \pm 87$  days in milk) and eight cows with SCC under the threshold of 200,000 n/mL only with teat sealant (LO-TS; parity  $3.0 \pm 0.9$ ,  $366 \pm 79$  days in milk). Milk yield at dry-off was  $11.5 \pm 8.7$  kg/day in HI-AB,  $19.6 \pm 6.8$  kg/day in LO-AB, and  $15.1 \pm 7.2$  kg/day in LO-TS. Foremilk and blood samples were collected from 10 days before dry-off to 28 days after calving. Milk yield and composition were recorded, and rumination time was monitored. Data were analyzed with SAS software.

HI-AB cows produced more than LO-AB ( $44.3$  vs.  $41.0$  kg/d) in the first two months after calving but had a lower average yield in the whole lactation ( $34.4$  vs.  $36.6$  kg/d). The SCC did not differ in the first 28 days in milk but HI-AB cows had lower milk solids (fat, protein, and casein). Considering the entire lactation HI-AB had higher milk yield. HI-AB cows had an existing inflammatory condition in the weeks before the dry-off, as suggested by lower plasma cholesterol and higher myeloperoxidase, and mobilized more fat reserves at the onset of lactation (higher NEFA and BHB). Rumination time was also lower in HI-AB cows, which mainly calved in summer due to allocation criteria.

LO-AB and LO-TS had similar performance in the previous lactation and produced a similar amount of milk in the next lactation. In the next lactation, despite similar SCC in the first 28 days in milk, LO-TS had a numerically higher average SCC due to a slightly higher mastitis prevalence (2 out of 7 in LO-AB and 4 out of 8 cow in LO-TS). Rumination time, metabolic and inflammatory conditions were not different between these two groups.

The antibiotic treatment in HI-AB cows likely limited the inflammation to the udder, without relevant negative impacts on health and performance in the dry period and next lactation. Internal teat sealant could be used without antibiotic therapy in cows with

low SCC, with minor effects on udder health and performance in the subsequent lactation.

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## P006

### Use of pomegranate extracts in bovine oxidative stress mitigation

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The present study tested the hypothesis that tannin-rich pomegranate (PMG) natural extracts can help mitigate the excessive ROS production in bovine aortic endothelial cells (BAEC), thus reducing lipid peroxidation damage. BAEC, used as in vitro model of oxidative stress, were collected from the aorta of healthy Holstein dairy cows as described by Sordillo et al. (1998). Cells were plated at a concentration of  $4 \times 10^4$  cells/mL in a 96-well and allowed to culture for 24 h before experimentation. The BAECs were treated with natural PMG extract at  $80 \mu\text{g/mL}$  and  $25 \text{ ng/mL}$  of LPS for the evaluation of cell viability, ROS production and cellular apoptosis/death. The PMG extract was also investigated for isoprostanes (IsoP) formation; for this purpose,  $5 \text{ mM}$  of 2,2'-azobis(2-amidinopropane)-dihydrochloride (AAPH) was used to mimic the excessive free radical ROS exposure as occurs in during oxidative stress condition. The free radicals produced by AAPH treatment are able to attack cell phospholipids membrane, producing IsoPs with a harmful effect on dairy cattle. Intracellular ROS production and cell viability assays were carried out with fluorescent and luminescent assays; apoptosis/death cells were determined by flow cytometry using co-staining with YOPRO-1α and propidium iodide, whereas the IsoPs were quantified using liquid chromatography associated with mass spectrometry.

Cell viability, cell apoptosis and death did not differ between control and PMG treatment with or without LPS. The antioxidant activity of PMG induced a reduction over 60% of ROS compared to LPS controls in BAEC culture. The IsoPs produced by BAECs after exposure to PMG natural extract and AAPH derived from arachidonic acid: 5-iPF<sub>2</sub>α, 8-iso-15R-PGF<sub>2</sub>α, 8-iso-PGF<sub>2</sub>α, 8-12-iso-iPF<sub>2</sub>α, 8-iso-PGA1, and 8-iso-PGA2. As expected, the

detected IsoPs showed an increasing trend in cells stimulated with AAPH compared with untreated cells. IsoPs 8-iso-PGA1 and 8-iso-PGA2 turned out to be the most abundant markers of oxidative stress in cultured BAEC. However, the mentioned IsoPs were influenced by PMG treatment, showing lower concentrations after AAPH exposure than AAPH control probably due to its ability to neutralize ROS generated from AAPH with a protective role against lipid peroxidation damage. In conclusion, the PMG could be administered as a natural feed supplement to improve bovine antioxidant defenses as a novel strategy to reduce oxidative stress and disease susceptibility in dairy cattle.

## P007

### Influence of the main dietary roughage sources on the health status of Italian Friesian lactating dairy cows

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The research was carried out on 10 specialized dairy farms located in a lowland area (Veneto region). They reared Italian Friesian cows (129 lactating dairy cows per farm on average) and, over one year, they fed them by a TMR based on either maize silage (MS, 4 farms), a mix of ensiled/dried forage (MF, 4 farms) or permanent meadow dried forage (DF, 2 farms) as main roughage source. All the farms had similar housing conditions, such as cubicles and concrete pavements in the manger corridor, without access to external paddocks. Every three months, each farm was visited to assess the animals' health status. Over the experimental period, data on high and low BCS, loose faeces, cleanliness (udder and hind quarter) and lameness were reported as % of affected cows on the total assessed cows for each dietary thesis. Regarding the pathologies (mastitis, respiratory and metabolic diseases), data were reported as % of each condition on the total pathological events recorded for each dietary thesis. Chi-square test was used to assess the dietary effect. The dietary roughage thesis did not affect the BCS of the animals, probably because all the herds received TMRs that were balanced in terms of net energy and nutrients to covered the animals' productive requirements. The dietary thesis based on MS roughage source showed the highest number of cows presenting loose faeces (16.5 vs. 11.5 vs. 4.9%;  $p < .05$ ). Therefore, the MS-cows had both dirtier udders (24.4 vs. 12.0 vs. 3.7%;  $p < .05$ ) and hind quarters (43.7 vs. 15.5 vs. 3.7%;  $p < .05$ ), even though these results may be influenced also by the management of the litter. Furthermore, the MS diet seemed to be related also to a higher incidence of lameness and metabolic diseases, comparing to the other dietary theses (lameness: 7.8 vs. 3.7 vs. 2.5%,  $p < .05$ ; metabolic diseases: 6.6 vs. 2.4

vs. 0.0%,  $p < .05$ ). Meanwhile, across the theses, there were no differences in terms of respiratory episodes (0.3% on average) and mastitis (39.2% on average). An attempt to explain the outcomes of this 1-year on-field study is that the prolonged use of MS may have induced subacute ruminal acidosis conditions more frequently than the HAY thesis and, therefore, caused the higher rate of animals with loose faeces and lameness, even though these outcomes may be further confirmed. Moreover, there is also the possibility that, by receiving maize silage, the cows had ingested aflatoxins, which often lead to pathological conditions such as laminitis.

## P008

### Heat stress in Italian Brown Swiss: effect of the interaction of genotype by environment using reaction norm analysis

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This study aimed at testing the genetic capacity against heat stress of Italian Brown Swiss breed using a random regression analysis for fat and protein percentage, protein and fat yield and fat corrected milk yield, energy corrected milk and cheese yield at 24h. A cow a breed can have a different performance in response to different environmental condition that can occur during its life depending on the genotype-environment interaction ( $G \times E$ ). If considerable  $G \times E$  is present, separate breeding programs for different environments can be advisable or can help the farmers to take different managerial actions such as the choice of a given bull also based on heat stress tolerance. The inclusion of bioclimatic index like temperature-humidity index (THI) as factor in the genetic parameter estimation is complex because heat stress threshold is extremely variable in the short period making the partition of test day records in discrete classes (production environments), according to the heat stress condition, difficult. The trend of heat stress condition typically follows a continuous, but not linear behaviour. The use of reaction norm models fitted through random regression are a powerful tool to identify and quantify  $G \times E$ ; they are a particular form of mixed-effect models in which individual phenotypes are modelled as a continuous function of a covariate. The additive variance component is analysed considering the intercept and slope as different random components allowing the estimation of the neutrality and the heat stress additive component as a multi-trait analysis.



In this study, a dataset of 200,000 test day records from 23,000 cows in the period 2008–2019, treating each parity as a separated and correlated trait, has been used. Results showed a moderately negative genetic correlation ( $-0.29$ ) between intercept and slope, ranging from a minimum of  $-0.69$  (parity 3 for Fat corrected milk) to a maximum of  $0.13$  (for cheese yield at 24 h), indicating that production is antagonistic to heat tolerance. For protein and fat yield, the genetic correlation was lower in parity 2 respect to parity 1 and 3, probably due to a different production level of the cows. Interestingly, the permanent environment correlation was higher for parity 3 (mean  $0.03$ ) and lower for parity 1 ( $-0.24$ ) indicating that herd condition is critical in younger animals.

## P009

### Effect of lactic acid bacteria addition or protozoa modulation on the in vitro rumen aflatoxin B1 decontamination

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The ingested Aflatoxin B1 (AFB1) by lactating animals is partially excreted in the milk and this is a serious problem of food safety due to high toxicity and carcinogenic effect. The rumen fermentation partially detoxifies AFB1 and there is a practical interest in the adoption of dietary strategies in dairy cows breeding to further increasing this rumen capacity. This study evaluates the effects of (i) the addition of pure lactic acid bacteria (LAB) inoculants and (ii) the protozoa modulation on fermentative profile and AFB1 recovery. Two in vitro experiments used the same in vitro fermentation apparatus (eight 500 mL bottles immersed in a water bath at 39 °C for 24 h, filled with filtered rumen fluid and buffer (ratio 1:2)) and the same feed substrate (3300 mg of corn silage containing 20 µg AFB1/kg). At the end of the incubation period, the fermentation fluid was collected and sampled for protozoa, volatile fatty acid, and ammonia. The remaining fluid was centrifuged (4600g for 20') and the AFB1 content of supernatant and pellet were analyzed by an HPLC technique.

In Exp 1, three pure bacteria strains (*L. plantarum*, *L. rhamnosus* and *L. mucosae*) were selected and added, autoclaved (121 °C × 15') or not, in a concentration of  $1 \times 10^8$  CFU/mL. After 24 h of incubation, the concentration of AFB1 was evaluated. The levels of AFB1 did not differ between the control and the treated samples, both in pure and autoclaved LAB. The addition of *L. plantarum* only increased the proportion of propionate in fermentation fluid (15.5 vs. 14.5–14.8%,  $p < .05$ ). Presumably, the rumen

environment and the interaction with other microorganisms may affect the AFB1 binding capacity of LAB tested.

In Exp 2 the protozoa population was changed by the addition of *Stevia rebaudiana* Bertoni extract (S) or Camphor oil (CO) at a dose of 1.5% of incubated DM. The protozoa count in fermenters added with S were lower than those added with CO (213 vs.  $293 \times 10^3$  Cell/mL,  $p < .01$ ), while the AFB1 residue after 24 h fermentation was higher for S than CO (56.0 vs. 42.9 ng,  $p < .05$ ) then the reduction of total protozoa generates higher AFB1 levels. This phenomenon could be explained by an interaction between protozoa and other rumen microorganisms.

## P010

### Application of a genomic tool for heifers selection in Italian Holstein cattle

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Recently, genetic improvement of animal health is attracting more attention from both animal sciences and farming. The introduction of genomic methods and tools contributed to enhancing insights into the genetic control of diseases, thus improving animal welfare and resilience. Aim of this study was to evaluate disease-related genomic indexes in animals reared in farms that can be considered virtuous as for the farming practices in comparison with less virtuous farms. A total of 10 farms were selected based on the average Somatic Cell Count (SCC), to obtain a 1:1 ratio between virtuous (V) and less-virtuous (LV) farms. Mean SCC ( $\times 1000$ ) of the two groups ( $\pm$ SD) was  $165.2 \pm 365.4$  and  $289.8 \pm 532.1$  (V and LV, respectively). A *t*-test was performed to evaluate the difference between SCC means ( $t = 4.5574$ ,  $df = 1039.4$ ,  $p$ -value  $< 0.001$ ). From the selected farms, 117 heifers were randomly sampled and genotyped using the Zoetis Clarifide chip (61 in V and 56 in LV farms, respectively). Genomic indexes were calculated on the abovementioned animals. The means of the Somatic Cell Score (SCS, calculated as  $\log_2(\text{SCC}/100,000) + 3$ ) index in the two groups were  $2.92 \pm 0.14$  and  $2.97 \pm 0.14$  (V and LV, respectively). Low values for this index indicate a more favorable SCC. Although not statistically significant (*t*-test:  $t = -1.8946$ ,  $df = 114.6$ ,  $p$ -value  $= 0.06$ ), a trend could be identified. Supporting this trend, 56.5% of the animals with an SCS index lower than the sample average (i.e. lower SCC) were reared in V farms, while 64.5% of the animals with an SCS index higher than the sample average were reared in LV farms. Out of the 5 V farms, 4 had at least one animal in the top ten ranked by SCS index. In particular,



the farm with the higher number of animals in the top ten was the first in terms of lower SCC mean. From these preliminary results it is possible to notice that the absence of a rigorous selection on health-related traits almost nullifies the positive effects of good management and animal care. It should be noticed that only heifers were tested. Thus, future farm data will be required to find out if any genetics effect can increase the positive results of the V farms (considering that at least two of these farms are currently under mastitis resistance selection). Breeders should be encouraged to improve their breeding plans to involve health-related traits, to further increase their animals' welfare, and to support the efforts they put into animal management.

## P011

### How the stage of gestation for moving at the group housing can affect sow's reproductive performance?

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Confinement in individual stalls of pregnant sows is a controversial welfare issue, as it precludes the freedom of movement, social interaction and expression of other natural behaviours. The legislation on the protection of pigs in the farm environment (2008/120/EC) allows to use the individual gestation stalls for 4 weeks after service, but there is an increasing request by the public opinion to reduce and/or ban their use. However, high levels of aggression are commonly observed in newly formed groups after mixing, pose a concern about the impact of mixing sows in early gestation on their performances. The aim of this study was to determine the impact of mixing sows in group pen on day 3 from insemination (PEN3) instead of 28 (PEN28) evaluating reproductive performance and litter size. At total of 326 sows were used in the study, divided in 234 weaned sows of different parity (from 2 to 11), 60 gilts and 32 sows that had reproductive failure before. The Group pens hosted 20 sows, had a concrete floor and a space allowance of 2.25 m<sup>2</sup>. Data collected allowed to calculate: pregnancy rate (proportion of sows inseminated that results pregnant at 28 d by a real-time ultrasound examination), farrowing rate (proportion of sows inseminated that farrowed) and litter size (total born, born alive, stillborn, mummified piglets).

Data were processed by chi-square test for the analysis of reproductive performance and by Mann-Whitney test for the analysis of litter size. No treatment effects were observed neither on reproductive parameters (pregnancy rate: 85% PEN3 vs. 80%

PEN28; farrowing rate: 85% PEN3 vs. 75% PEN28) nor on litter size (total born: 13.4 PEN3 vs. 13.3 PEN28; born alive: 12.1 PEN3 vs. 11.8 PEN28, mummified: 0.5 PEN3 vs. 0.5 PEN28) even considering all categories of animal included in study (weaned sows, gilts and repeated breeder sows).

Results of the study suggest that the practice of mixing sows into groups early post-insemination could be done without adversely affecting herd productivity. However, more aspects must be investigated before to reach a definitive conclusion such as the effects of day of mixing also on welfare indicators such as sow behaviour (conflict and aggression), injuries and physiological indicator of stress.

## P012

### What consumers perceive from geographical information and welfare of lambs?

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Recently, the geographical origin of food is considered one of the key information which influences the consumers' acceptance. Lamb meat is considered a local and typical food with high quality attributes for the consumers, being characterized by shorter transport and as consequence, a good welfare condition. The aims of the present work were the (i) exploitation of the role of information about geographical origin, transport duration, and welfare condition of lambs for consumers and their actual liking, (ii) and the evaluation of meat quality. Thirty Merinos-derived male lambs were subjected to two different transport distance, fifteen lambs were subjected to a short transport duration (STR), approximately around 1 h, starting from local farm to the slaughterhouse located at Foggia. A second group of lambs was subjected to a long transport (LTR), around 22 h going through 1250 km. At slaughterhouse, welfare issues (percentage of active animals, ambulatory animals, injuries, lameness, dead), were monitored by veterinarian. On longissimus dorsi lumborum (LDL) muscle was assessed the colour, pH, mechanical properties and the chemical composition. A preliminary focus group was carried out to evaluate the interest of consumers regarding the consumption of lamb meat from different geographical origins and with different welfare conditions. Furthermore, a number of 120 consumers were recruited in order to set up the consumer test. Nutritional, textural and quantitative descriptive sensory data were processed

by ANOVA using the GLM procedure of SAS. The MIXED procedure was used for analysed data from consumer panel test. In order to evaluate the effect of information on the consumer's acceptability, the difference between perceived liking score and expected liking score (P-E) as well as differences between informed and perceived liking scores (I-P) and informed and expected liking scores (I-E) were calculated. Data demonstrated that both short and long transport did not affect the meat quality indicators such as pH, colour, mechanical properties and chemical composition. The preliminary results from the focus group showed that the item geographical origin and welfare received the highest score although not significantly different from taste. Furthermore, consumers were affected by the information concerning short transport, local origin of lamb and good welfare moving their actual liking in the direction of expectancy.

### P013

#### Differential somatic cell count in buffalo species: preliminary results

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Differential somatic cell count (DSCC) has recently been developed providing an uprising indicator of mastitis: it refers to the proportions of individual cell populations such as lymphocytes, macrophages, and PMN in milk that play an important role in inflammatory responses within the mammary gland. The DSCC monitoring could increase the sensitivities in udder health management, therefore the reliability needs to be investigated. Aim of the present study was to investigate the potential reliability of DSCC evaluated by flow cytometry by comparing with a semi-quantitative cell count on a cytological smear in buffalo milk samples. The trial involved 32 Italian Mediterranean water buffaloes homogeneous for days in milk (DIM) and parity ( $119.25 \pm 6.86$  and  $3.84 \pm 0.24$  respectively) over a period of two months. Once a month, individual milk samples were obtained on two different sampling times (T1 and T2). The DSCC was analyzed by Milkoscan FT6000 (Foss Electric, Hillerød, Denmark) flow cytometer and by manual cytology. One hundred cells of each cytological smear were counted meander-shaped and differentiated into lymphocytes, macrophages and PMN.

Data on SCC, DSCC, macrophages, lymphocytes, neutrophils were analyzed by ANOVA for repeated measures with diagnostic approach as the fixed factor. Day of sampling was the repeated measure. Multiple linear regression was performed (stepwise procedure) in order to evaluate the relationship between DSCC, DIM and parity, and DSCC measured by manual cytology and flow

cytometer in order to investigate machinery reliability. Manual cytology confirmed to have a higher ( $p < .01$ ) sensitiveness compared to flow cytometer, showing DSCC values of  $77.36 \pm 1.01$  vs.  $55.82 \pm 1.96$  for total count. Likewise, at T1 and T2, DSCC values were  $79.66 \pm 1.33$  and  $75.06 \pm 1.44$  vs.  $50.85 \pm 2.70$  and  $54.79 \pm 2.83$  respectively for manual cytology and flow cytometer. Macrophages and lymphocytes were influenced negatively by parity ( $R^2 = 0.69$ ) and DIM ( $R^2 = 0.63$ ) respectively. The DSCC did not show any correlation with such parameters.

Our findings confirmed that the relationship between DSCC and DIM or parity is not yet fully resolved. Manual cytology confirmed to be the most sensitive diagnostic approach, pointing out the need for further studies in order to include flow cytometer in udder health management.

### P014

#### Health and immunological parameters of susceptible and resistant cows exposed to a rich grain diet

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The objective of this study was to investigate in high producing cows with different subacute ruminal acidosis (SARA) susceptibility, the modifications in health and immunological parameters. SARA susceptibility was obtained by exposing cows to stress the rumen environment by introducing a rich grain diet. Twenty-four lactating Holstein cows divided into three consecutive blocks were exposed to two different feeding/housing periods: period one (PreChallenge-PC): standard diet (S, forage concentrate ratio 45:55), free-stall housing (FS); period two (Challenge-CH): rich grain diet (RG, forage concentrate ratio 25:75), tie-stall (TS). Both periods lasted for 4wks and the change was abrupt. Blood was sampled 8 times: one right before the beginning of CH (0d, PC period), and 7 times in CH period to evaluate the acute (1,2,3 d), mid acute (7,14 d), and chronic (21,28 d) response. Hematologic, biochemical, and immunological parameters were determined. Rumination time (RT) was measured continuously along all the time by collar, and during CH cows showed a dramatic drop in RT ( $-114$  min/d). However, not all the cows expressed the same RT drop and we were able to differentiate two groups: resistant cows ( $n = 15$ , HR,  $-65$  min/d) and susceptible cows ( $n = 9$ , LR,  $-134$  min/d). Mixed model procedure was used for data analysis; time point, susceptibility group and interactions were used as fixed effects, animal as random effect, and

sampling as repeated structure. Obtained results showed on LR group lower content of hemoglobin ( $-0.35\text{g}\%$ ,  $p = .03$ ), hematocrit ( $-0.91\%$ ,  $p = .02$ ), erythrocytes ( $-161\text{n}^\circ/\mu\text{m}^3$ ,  $p = .05$ ), and higher values of MPV ( $+0.81\text{ fL}$ ,  $p < .01$ ). Moreover, LR group showed a decrease of RDW ( $-0.40\%$ ,  $p < .01$ ) and cholesterol ( $-1.1\text{ mmol/L}$ ,  $p < .01$ ) on chronic phase and up and down variations of lymphocytes ( $p < .01$ ). Modifications on red blood cells could be related to less regeneration capacity on the other hand variations on lymphocytes content point to lower ability to maintain high the defense system. Lower cholesterol is often associated with the worst health and metabolic condition. These data highlight that there are different responses in dairy cows fed rich grain ration, dividing the population into two groups with high and low susceptibility. Moreover, these results show different levels of biochemical and immunological parameters justifying the different responses on RT we observed.

## P015

### Preliminary laboratory trials on the efficacy of hop alpha iso-acids on the control of the honey bee (*Apis mellifera* L.) ectoparasite *Varroa destructor*

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In recent years, interest in alpha and beta acids of hops has increased, especially as regards their acaricidal effect. In particular, formulations of beta acids of hop were found to be highly effective on the ectoparasitic varroa mite (VM) (*Varroa destructor*), with a low impact on the honey bees (*Apis mellifera*). The experiment that we carried out aimed at verifying the tolerability by the bees of tetra and hexahydro alpha acids (HAA), in a 1:1 ratio, and their possible acaricidal effect. In four consecutive trials in a controlled environment, young (1–3 weeks) caged bees were submitted to an experimental scheme including (i) 6% oxalic acid (OA) in a 50:50 sugar solution as positive control; (ii) 50:50 water-sugar solution as a negative control; (iii) five levels of HAA 0.1%, 0.3%, 0.6%, 1%, 2% in 50:50 sugar solution. Four replicates for each treatment were set up. The bees have been caught on three bee hives with different infestation levels by VM and were transported in the shortest time possible to the laboratory where they have been acclimated in an incubator (34 °C; 70% humidity). After 24-h, 1.6 µL of treatment per bee was spread on the bees through 100 µL micropipette. For the next 5 days, caged bees were regularly checked and the consumption of water and sugar solution, VM death and dead bees were recorded daily. At the end of the trials, the total number of VM load per bee has been computed. Four repetitions of this experiment were carried out. The first two experiments were carried out by taking bees from

two hives with a lower varroa infestation (69.5 VM average fall in 48 h). For the second two experiments, the bees were taken from hives in which we had an high varroa infestation (389.5 varroa average fall in 48 h). It was found that the level of HHA showing the highest mortality rate on varroa was the 0.3%, with a mortality rate of 57%, HHA at 1% and 0.6% were also found to cause somewhat high mortality VM rates, respectively 43% and 47% ( $p < .001$ ). In all three levels of HHA, MV mortality was found to be higher than the OA positive control of 6% (mortality of 30%). However, bee mortality was also higher, especially for HHA at 0.6% (32%), compared to oxalic acid, which stood at 14% ( $p < .001$ ). From these evidences, the treatments that have had the best results (highest VM mortality with the lowest impact on the bees) will be further tested in the field.

## P016

### Practical perspectives of using the Bee Varroa Scanner (BeeVS), as an automated tool for the *Varroa destructor* parasite load monitoring in honey bee (*Apis mellifera*) colonies

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Monitoring of the varroa mite (VM) (*Varroa destructor*) infestation level can help beekeepers in managing honey bee (*Apis mellifera*) hives. With the advent of artificial intelligence-based technologies, the procedure can be faster and more accurate. In this field, the Bee Varroa Scanner (BeeVS, Apisfero APS) is a diagnostic tool for the automatized counting of VM fallen on adhesive sheets installed beneath the screened bottom of the hive through which VM specimen naturally fall down. The purpose of this work was to verify the reliability and practicality of use of the BeeVS system and to help provide further data on the real effectiveness of the tool. On a total of 60 sheets two manual counts by different operators and two automatized counts through the BeeVS were performed. The sheets had previously been placed for 48 h under the hives of the PRIMA PLANT-B apiary at the University of Tuscia. In some cases, it was observed that the manual counting differed greatly from the automatic one performed through the BeeVS. In fact, 91% of the counts made manually were found to be incorrect. As a consequence, the mean and median values of the counts carried out with the BeeVS resulted to be higher than the mean and median of the counts carried out manually (+25% and +32% respectively). In addition, the manual count proved to be less reliable, especially as far as the highest VM values counted, being affected by the



examiner. The BeeVS eliminates the human factor in the accounting process, consequently reduces the variability of the results by standardizing the data collection. For this reason, the BeesVS could be suitable for monitoring actions, especially on a large scale.

## P017

### Application of Fourier transform infrared spectroscopy for determination of immunoglobulins in sheep colostrum

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The quality of colostrum correlates directly with the amount of IgG it contains. A rapid method for assessing colostrum quality is needed to ensure enough immunoglobulin and to avoid failure of passive transfer of immunity. In dairy industry, Fourier transform infrared (FT-IR) is commonly used to measure chemical constituents of milk es. fat, total proteins, and is widely employed for rapid screen for abnormalities.

In this study a total of 39 colostrum samples were collected at the different post-partum time from two sheep dairy farms in the Catanzaro district. Immunoglobulin G content was determined directly by radial immunodiffusion (RID) and Total protein (TP) content was evaluated by Milkoscan FT+. The two estimations were correlated. The concentration of immunoglobulin ranged from 5.60 to 156.32 g/L and Total protein (TP) ranged from 6.32 to 22.88%. In our study, Milkoscan and RID results were highly and significantly correlated, and regression analysis showed a strong relationship between Ig concentration provided by RID and TP provided by Milkoscan FT+ ( $R^2 = 0.88$ ). Specificity and sensitivity were determined for RID compared with a cut point of 50 mg/mL IgG. In conclusion, the study indicates that the indirect method may be a reliable tool for the estimation of the total Ig concentration in sheep colostrum and a cut-off level of 13 % TP seems sufficient to assure adequate IgG concentration in colostrum.

## P018

### Effects of *Spirulina platensis* supplementation on performances and metabolic status of post-weaning calves

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Microalgae supplementation has been investigated as potential source of bioactive compounds able to promote health and production performances of livestock. *Spirulina platensis* is a microscopic filamentous alga, rich in polyunsaturated fatty acids, phycocyanin and phenolic compounds, considered a natural antioxidant and an immunostimulant with less side effects than synthetic antioxidants. This work aimed to evaluate the effects of the *Spirulina platensis* supplementation on intake, growth and metabolic profile in post-weaning calves. Eighteen calves at age of  $76.9 \pm 4.2$  days and weight of  $97.80 \pm 8.6$  and fed a basic dry mixed diet. Calves were split in two groups of 9 calves each, Control (C) and *Spirulina* (SP). Each calf was daily supplemented with 10 g Soybean meal or 10 g/d of dried *Spirulina* for 40 days. Groups C and SP were housed in 3 pens of 3 calves allowing 3 replicates per treatment. *Spirulina* did not significantly affect weight gain (1.22 kg/d per head) or dry matter intake vs. control diet. Otherwise, selection of physically effective fiber was significantly higher in SP vs. C. ( $p < .05$ ). *Spirulina* modulated hemoglobin production and calves fed SP showed significant increases for MCHC (Mean Cell Hemoglobin Concentration) over time ( $p < .01$ , average values 34.43 vs. 33.94 g/dl  $\pm 0.10$ ) and for MCV (Mean Cell Volume) and MCH (Mean Cell Hemoglobin) ( $p < .001$ ). SP affected lipid metabolism being Cholesterol (61.85 vs. 69.71 mg/dl  $\pm 2.14$ ;  $p < .01$ ) and Bilirubin (0.06 vs. 0.09 mg/dl  $\pm 0.01$ ;  $p < .05$ ). SP also induced lower values of CPK (Creatine phosphokinase) ( $p < .05$ ), higher Albumin ( $p < .01$ ) and lower  $\beta$ -Globulin ( $p < .05$ ) even if each parameter was within the physiological range considered for healthy animals. Improvement of metabolic parameters were especially detected in the first weeks of administration. These results suggested that SP could have been involved in a better adaptation to housing conditions due to experimental trial and different feeding behavior that could justify a better health metabolic status. In summary, *Spirulina* seems to positively affect metabolic status of calves and further investigations need to be carried out to study the effects of doses and supplementation conditions to deep understand interactions with metabolism and immune functions.



**P019****Effect of experimental covering texture on forage preservation and quality**

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Objectives of the present study were to assess stability and quality of alfalfa haylage sealed in round bales and covered by an experimental plastic layer (TRT), designed to avoid any oxygen entrance, versus a traditional coverage with plastic (CTRL). Alfalfa hay was cut and harvested from a single field on 26 June 2019. Bales were made within 24 h, at a moisture level of 50%. For the study, a final amount of 36 bales were sealed (18 treatment, 18 control). Multiple samples collection was carried out to evaluate haylage quality during the whole trial. For each time point, 3 bales per group were sampled. Time points were: 0, 30, 60, 9, 180, and 365 dd. For each bale, two samples were collected: one at the bale's core (C), and the second close to the surface (10 cm, S) to evaluate differences due to the distance from the layer. Once collected, samples were placed under vacuum and moved to the laboratory for analyses: moisture, pH, protein and fiber fractions, ash, in vitro NDF digestibility at 30–48–120–240 h, and in vitro crude protein digestibility (ruminal and intestinal). Each sample was analyzed in two replicates, with the exception of protein digestion, repeated in 3 replicates. Statistical analysis was performed using the software JMPpro v.15, and significance set at  $p < .05$ . Dry matter showed no differences among treatments, while it was higher in C compared to S (54.7% vs. 49.2% on average), and tend to increase during time. Ash and crude protein displayed no differences. Soluble protein was higher in TRT compared to CTRL (12.2% vs. 10.1% on average), while sampling site was not different. Fibrous fractions were similar among treatments and site of collection, with a tendency to increase during time. Protein digestibility was different among treatments, being higher in TRT compared to CTRL (85.3% vs. 81.1% on average). Similar situation was observed in fiber digestibility, which resulted higher in TRT compared to CTRL for almost all the collection time points (34.6% vs. 30.1% at 30h; 36.8% vs. 31.4 at 48h; 42.5% vs. 39.6% at 120h; 45.7% vs. 41.3%, on average, for TRT and CTRL respectively). No differences indeed resulted for sampling site. In conclusion, treatment group showed a higher stability compared to the control group, and moreover, a better product quality considering the nutritional fractions. This conclusion is supported by digestibility data of both fiber and protein, also underlined by the reduced amount of indigestible material.

**ROLE AND CHARACTERIZATION OF LOCAL BREEDS****P020****Optimal economic weight by restricted selection index for dual-purpose cattle breed**

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Economic selection indexes (SI) are commonly used to rank animals by combining EBVs of different traits by economic weights. Particularly, this approach is convenient for traits with intermediate optimum value. In dairy and beef cattle indeed, SI also include a wide number of morphological traits with intermediate optimum. The indexes then allow to maximize genetic progress for the most relevant traits and ensure marginal progress in the other. In dual-purpose cattle economic selection indexes are used to account for many traits, including productive (i.e. milk, fat, protein yield, and beef traits), functional (i.e. health, fertility, and longevity), and morphological traits. However, as most of these traits are negatively correlated between them, it is difficult to ensure a positive genetic progress or not worsening of all accounted traits.

In the last decades, several methods to restrict to zero the genetic gain of target traits have been proposed. Such restriction allows to prevent a detrimental effect on traits showing antagonistic genetic correlations with traits with greater economic value e.g. milk yield. Once the restriction has been applied, it is possible to re-calculate the economic weights of all the traits included in the selection index. This allows to obtain the same direction of genetic trends attainable before restriction but applying different economic weights. We used as case study genetic correlations from Grey Alpine cattle breed, which has a dual-purpose attitude for milk and beef. The dual-purpose represents a perfect chance to investigate how restricted selection indexes can be used to acquire optimal economic weight, because of selection index contains traits that are antagonistic to each other. In this study for simplicity purposes, we considered the milk yield (kg), fat yield (kg), protein yield (kg), SCS (score), and muscularity (score). The new economic weights were divided by the sum of their absolute values to obtain a sum of zero. Our results showed that applying the restriction for SCS and MUSC is equivalent to providing a negative economic weight of  $-0.008$  to SCS, an economic weight of  $0.010$  to MUSC, and weights of  $0.518$  and  $0.465$  to fat and protein. We showed that this approach could be convenient for selection indexes in which many antagonistic traits are included, and/or accounting for traits with an intermediate optimum value, such as a wide number of morphological traits.

## P021

### Genetic parameters for new phenotypes measured in alpine local breeds

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In recent years, the Italian dual-purpose cattle breeders' associations have focused their activity on assessing new breeding goals under the Dualbreeding project, a breeding program financed under the European Agricultural Fund for Rural Development by the Italian Ministry of Agriculture, Food and Forestry. Among the new breeding goals, new phenotypes have been identified and recorded aiming to exploit a possible use in selection. The new phenotypes were addressed to beef traits, pasture attitudes, temperament, milk-ability, etc. This study reports the first results of heritability estimates for some of the new phenotypes recorded in primiparous cows of local breeds from Alpine arc: Alpine Grey (AG), Rendena (RE), Aosta Red Pied (ARP) and Aosta Black Pied and Chestnut (ABP-CHE, unique herd-book). Data were recorded between 2017 and 2020 on a total of 4104 AG, 1457 RE, 3783 ARP, and 1788 ABP-CHE. Editing was carried out considering at least 2 cows within herd-year-classifier, and according to different ages at first parity (5 classes, 22–48 months of age) and days in milk (7 classes, 10–305 days). The final datasets contained 2394 records for AG, 1118 for RE, 2703 for ABP, and 1127 for ABP-CHE. Pedigree files accounted for a total of 17040 animals for GA, 7454 for RE, 21875 for ABP, and 9178 for ABP-CHE. Variance components were estimated under AIREML method after a preliminary analysis using REML. Body measures linked to beef attitude were taken at thigh (i.e. spiral girth from hook to pin bone crossing trough the internal part of the leg and the horizontal perimeter from stifle joint to the mid tail) and showed in general a moderate heritability ( $h^2 = 0.19$ ), ranging from 0.08 (ARP) to 0.29 (ABP-CHE). Direct or indirect pasture attitude showed a generally low  $h^2$ , i.e. 0.07, with a maximum  $h^2$  of 0.12 for muzzle width. Milk-ability obtained by interviewing farmers showed a further low  $h^2$ , ranging from 0.03 to 0.08, and similarly the temperament showed an  $h^2$  from 0.02 to 0.05 considering the different breeds. Coat typicality (based on the colour) highlighted moderate  $h^2$  (0.19 on average), but estimates highly varied among breeds, i.e. from 0.06 in RE to 0.47 in AG. In general, new phenotypes in local dual-purpose breeds revealed a low but effective additive genetic component, indicating a possible use in selection for some of them.

## Acknowledgements

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## P022

### Implementation of the first Italian semen cryobank of autochthonous turkey breeds: preliminary results

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In the last few decades in Italy, Farm Animal Genetic Resources of avian species have rapidly declined. In addition to in vivo management, in vitro conservation is also a strategic method used in order to secure genetic diversity within a wide range of lines and breeds. Semen cryopreservation is the most adapt technology within ex situ in vitro conservation programs, to maintain the avian genetic resources in gene banks. Recently, thanks the financed project 'TuBAvI' research groups from Molise and Milan have realized the first Italian semen cryobank of native chicken and turkey breeds. The aim of this work is to describe all of the activities fundamental for the creation of turkey semen cryobank: (1) the identification of a successful freezing protocols; (2) the write up of the standard operative procedures (SOP); and finally (3) the storage of turkey semen doses in the cryobank and hence its implementation. The identified reference cryopreservation protocol guarantees at least 30% of live and motile spermatozoa after the freezing process and ensures an adequate fertility rate. The freezing protocol reference involves the procedure which is reported here. The semen is pre-extended with Lake diluent, cooled at 4 °C for 25 min and diluted with freezing extender to reach the final concentration of 10% dimethylsulfoxide, 0.5 mM Ficoll and  $3 \times 10^9$  sperm/mL. Then, the semen is loaded into 0.25 mL plastic straws and equilibrated at 4 °C for 20 min and frozen by exposure at the height of 10 cm above liquid nitrogen vapor for 10 min. The draw up of the SOP results as important because there has been no regulation on the organization and management of an avian germplasm cryobank. However, the draw up of the SOP included the FAO indications and the following aspects: the choice of priority breeds, selection of semen donors, infrastructures and storage sites, cryopreservation process, doses traceability and management of cryobank. Currently, 85 turkey semen doses from Nero d'Italia, Romagnolo, Ermellinato di Rovigo and Bronzato breeds are stored in the first Italian cryobank.

The realization of a semen cryobank of native turkey breeds is a valid tool for the conservation and safeguard of the avian biodiversity in Italy. The doses stored in the cryobank could be useful in improving the genetic variability within farms, in correcting any selection errors and in reducing inbreeding problems. Second funding is foreseen to increase its consistency in term breeds and donators.

## P023

### The use of principal component and linear discriminant analyses to assess differences among four alpine goat breeds

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The evaluation of local breeds' unicity and distinctiveness might help to find their sustainable role in the current and future markets. Thus, the purpose of this study was to verify (i) whether, based on body conformation, some alpine goat breeds could represent homogeneous groups, and (ii) if it was, therefore, possible to discriminate the breeds based on body measures. To this aim, 327 adult female goats belonging to the Bionda dell'Adamello (BA, n.: 192), Pezzata Mochena (PM, n.: 53), Verzaschese (VE, n.: 43) and Camosciata delle Alpi (CA, n.: 39) breeds were submitted to sixteen body measures (withers and croup height; trunk length; chest length, width, height, and circumference; croup length; croup width; shin circumference; head and ears width and length). The data were processed by means of multivariate analyses, through principal component (PCA) and linear discriminant analysis (LDA), both on the complete database (model 1) and on a reduced one (model 2) represented by a balanced number of animals for each breed, randomly selected. In the PCA, both models identified 5 main components that explained, 78% and 81% of the variance, in mod.1 and mod.2, respectively. The first 2 components explained 55% and 59% of the variance, respectively, and the most involved body measures, based on loadings contributions, were represented by chest width (0.34) and circumference (0.32) (mod.1) and by chest circumference (-0.33) and head width (0.51) (mod.2). Based on the results of the PCA, the a posteriori identification of the breeds led to distinguish two groups, one represented by BA and PM, and one by CA and VE. This result agrees with the geographical distribution of the breeds in the Alps (BA and PM in the eastern part, VE and CA in the western part). For LDA, the complete database was splitted into a training and a testing one (80:20). The coefficients of linear discriminant functions explained a higher quote of

variance (99%, given by 59%, 36% and 4%, respectively for LD1, LD2 and LD3) than PCA. Based on LD1 and LD2 and regardless of the model adopted, the breeds were gathered into three groups (a: PM; b: BA; c: VE and CA). The LDA allowed to discriminate BA and PM better than PCA. The external validation on testing animals allowed to confirm the identification of the four Alpine breeds taken into consideration; however, while in the testing set BA and PM goats were all correctly attributed by LDA, the VE and CA breeds were correctly attributed in 92.8% of the cases.

## P024

### Semen cryopreservation for ex situ management of genetic diversity in chicken: creation of the Italian Avian Cryobank

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The project 'Conservation of Biodiversity in Italian Poultry Breeds' (TuBAvI), MIPAAFT PSRN 2017–2020, was dedicated to the safeguard, conservation and improvement of Italian poultry genetic resources. The planning and implementation of the Sperm Italian Cryobank of Local Poultry Breeds was an important task within TuBAvI project and semen doses of chicken and turkey breeds were stored in 2020. Adult Bionda Piemontese (BP;  $n = 25$ ) and Bianca di Saluzzo (BS;  $n = 18$ ) roosters were housed at the Poultry Unit, Animal Production Centre, University of Milan (Lodi, Italy). After a semen collection training period, semen donors were selected and semen doses frozen in liquid nitrogen. Each day of collection, quantitative (volume, concentration) and qualitative (viability, motility, progressive motility, kinetic parameters) sperm parameters were measured. Semen doses were frozen according to the procedure developed for the *Gallus gallus* species in the previous years of the project and reported in the SOP of the Cryobank. In brief, semen was diluted in two different steps to 1 billion sperm/mL with Lake pre-freezing medium containing 2% N-methylacetamide final concentration, equilibrated at 5 °C for 1 min, loaded into 0.25 mL French straws, frozen for 10 min over a nitrogen bath at 3 cm of height and stored in liquid nitrogen at -196 °C in cryogenic tank. The straws were thawed at 5 °C for 100 s and sperm quality was assessed. In total, 7 BP roosters and 6 BS roosters were selected as donors. The mean volume and sperm concentration recorded in fresh ejaculates of BP and BS were  $0.25 \pm 0.17$  mL and  $3.77 \pm 0.76$  billion sperm/mL, and  $0.33 \pm 0.11$  mL and  $2.89 \pm 0.62$  billion sperm/mL respectively. Semen quality of fresh samples was significantly different



between breeds. Higher values in sperm viability and motility were found in BP compared to BS semen, being viability 84.2% vs. 49% and motility 86.8% vs. 45.1%. As expected, a general significant decrease in sperm quality occurred after the freezing-thawing process and differences in sperm quality between breeds were no more present after thawing. The overall mean viability and motility values recorded after thawing were 16% and 17%, respectively. To date, the doses of frozen semen stored in the Italian Sperm Cryobank are 32 straws of BS and 67 straws of BP. A programme to store semen doses from further Italian poultry breeds was planned and will be implemented from 2021 to 2023.

## P025

### Parentage reconstruction using microsatellite markers in Valle del Belice sheep breed

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In the sheep breeding sector, the use of parentage test represents an important tool to avoid pedigree errors, due to the flock size and the difficulty to effectively recognize the parents of each offspring. Pedigree errors reduce genetic progress, due to incorrect estimation of breeding values, with 10–23% of detected errors. Therefore, the parentage analysis conducted using microsatellites markers could improve the animal breeding programs reducing pedigree errors. The traditional mating system in Valle del Belice sheep flocks was natural mating. Nowadays, in most farms, parentage records are based on the assumptions that the most probably father could be one of the selected rams used during mating seasons, and the most probably mother could be the one which suckle only their own lambs. All these reasons made pedigree errors notable increased. Microsatellite markers could define reliable parentage test to improve mating plans, reduce inbreeding, and maintaining genetic variability within Valle del Belice breed. A total of 23 microsatellites were chosen considering literatures and ISAG/FAO recommendations. Samples were collected from 4 flocks in Agrigento provinces and included 64 ‘offspring-mother’ pairs, included 9 twin pairs, and a total of 23 rams. Summary statistics of 151 individuals showed mean number of alleles per locus was 10.087, observed and expected heterozygosity values were 0.672 and 0.723 ( $p$ -value <0.05), respectively. The mean Polymorphic Information Content (PIC) value was ~0.70. Results of maternity test showed that 68.75% of the recorded/assigned mothers were correct but only for the

45.45% of those, was possible to assign a father and then to construct the most probably trios. Moreover, out of 9 twin pairs, only three pairs could be genetically correct while the other ones suggested us to hypothesize the rare adoption phenomena, and the heteropaternal superfecundation already observed in several poly-ovulatory species with an incidence of 35%. All our results demonstrated the difficult reliability of pedigrees drawn up on documentary basis like the ones of Valle del Belice breed. Application of DNA-based parentage test has the potential to assist the farmers in improving pedigree records, resulting in increase of genetic improvement, and consequent reduction of random genetic drift and inbreeding depression.

## P026

### Morphometric differences based on quantitative traits between different genetic lines in the Merino Español sheep breed

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The different traditional genetic lines of the Merina breed have been zoometrically characterized. These lines have been formed over time, through a combination of selection by farmers who have been looking for a type of animal with certain morphological, productive and behavioral characteristics, selection at certain environmental conditions, and in some case the genetic derivative due to reproductive isolation. Some lines have been forming in the last 100–200 years (generations of closed mating) such as those of Maesso, Egea, Granda and López-Montenegro, some external animal have been introduced in the last 50 years in Hidalgo and Perales lines, and Nuevo Serena has formed more recently (in the last 30–50 years).

Zoometric measurements have been made in 292 adult females chosen randomly in the herds: Granda (60), Egea (21), Hidalgo (50), López-Montenegro (40), Maesso (40), Perales (46) and Nuevo Serena (35). Thickness compass, zoometric rod, a measuring tape and an analog dynamometer had been used. The following zoometric indexes were calculated: Body index (BI), Compactness index (CoI), Ilio-ischial index (II), Thoracic index (TI), Cephalic index (CeI), Proportionality index (PI), Shinbone load index (SI).

High morphological variability between these lines ( $p < .001$ , and high homogeneity within the line) were shown. According to



these results, the Maesso sheep had the lowest live weight (36.53 kg) and registered a lower CoI and TI, but a higher SI, described as an elipometric type. The Hidalgo females had the highest live weight (62.10 kg), and had the highest BI, CoI, II, TI, but the lowest Cel and SI, as longilinear morphotype. The highest PI (between 100 and 102), and therefore the best heavier animals more related to meat production, were López-Montenegro, Perales and Nuevo Serena lines. These results show the evolution of different lines within the breed and determine a different capacity to adapt to different environments, as well as a different aptitude for meat production. It would be necessary to confirm if these morphological differences are reflected in a differentiated genetic profile that allow their official recognition within the Merino breed.

## P027

### Exploring pedigrees: an overall picture of biodiversity in Italian small ruminants

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To avoid the reduction of biodiversity and genetic erosion, local breed conservation continues to be a relevant topic of rural development policy. Optimal breed management requires careful control of the inbreeding level within a breed together with the availability of accurate demographic information. The calculation of the main demographic and genetic parameters allows us to better estimate the short- and long-term breed risk status, as well as to determine the best management practices for breeds with unreliable data and so which require particular attention. The Italian association of small ruminant breeders (ASSONAPA) monitored all goats and sheep breeds in a national project entitled 'Conservation, Health and Efficiency Empowerment of Small Ruminant' (CHEESR-PSRN 2014–2020). This study described the panorama of pedigree information for Italian sheep and goat breeds. In this study, 81 pedigrees (35 for goats and 46 for sheep) were analysed. Using optiSel R package we calculated the pedigree depth (full and maximum generation traced) and completeness, and the effective size ( $N_e$ ) applying Wright's formula. Furthermore, for the 10 breed case-study, we estimated the longevity and the percentage of animals eliminated from pedigree during their first 3 years of life (for the triennium 2007–2009). The analyses showed a median pedigree depth, for goats and sheep, equal to 2 and 3 full generations (interquartile range IQR

=2–4 and 2–5), and 5 and 7 maximum generations (IQR =3–10 and 5–12), respectively. Pedigree completeness was variable; breeds with a complete first generation (median, IQR) were higher in sheep (34%, 19–47) than in goats (14%, 5–28). Overall, the median  $N_e$  was 176 in goats and 234 in sheep (IQR =74–547 and 128–774). The mean  $\pm$  SD longevity was  $5.6 \pm 1.8$  (range =3.9–7.5) and  $5.5 \pm 1.1$  years (range =4.3–7.6), respectively, with a marked difference (equal to 3 years) between, more and less selected, goat breeds. On average,  $16 \pm 22\%$  goats and  $25 \pm 11\%$  sheep (range =1–41 and 5–40) were eliminated before 3 years of age. In conclusion, the Italian panorama is very variable and the pedigree analyses depict very different situations. Ovine populations are characterized by greater effective size, pedigree depth, and completeness than caprine populations. When information is lacking, pedigree analyses can be corroborated by genomics to determine good practice in breed management.

## P028

### Genetic diversity and runs of homozygosity in Rendena cattle

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The Rendena cattle is an autochthonous cattle breed from Val Rendena in the northern Italian region of Trentino Alto Adige. The breed is considered a dual-purpose breed (milk and meat) but the recent selection has emphasized milk production and quality. The breed is characterized by a small-medium size, good fertility and high longevity and is well adapted to the harsh Alpine environment. With a head count of ~4000 recorded cows, the Rendena cattle represent a source of income for many farmers in the Trentino Alto Adige region. In this study, 140 Rendena cows sampled in 31 different farms have been genotyped with the GGP Bovine 100K SNPchip (Neogen). Genotype data were used to estimate within-breed diversity and inbreeding and were compared with SNP data from 56 Eurasian cattle breeds to evaluate population structure and relationships. Principal Component Analysis (PCA) and Neighbour-net analyses performed on the Eurasian dataset indicated shared ancestry between Rendena and cattle breeds of the Original Brown Swiss group, but also revealed an original genetic makeup, thus confirming previous evidence from mitochondrial control-region data and lower density SNP profiles. Within-breed PCA highlighted the absence of

substructure and excluded the presence of crossbred individuals or showing a marked outlier behaviour. Some inbreeding was detected using a genome-wide analysis of runs of homozygosity (ROH) ( $F_{ROH} = 0.08 \pm 0.03$ ). The ROHs distribution across chromosomes appeared homogeneous and related to chromosome size. Most ROHs were private or common to very few animals. Exceptions were found in three genomic regions – on BTA6, BTA10 and BTA16 – where ROHs were shared by >25% of the animals tested. Noticeably, the ROH on BTA6 consisted in 33 consecutive homozygous SNPs shared by >50% of the animals. This region was found to harbour genes relevant for meat (NCAPG, LCORL) and milk production (LAP3).

## P029

### Role and characterization of dromedary breeding systems in northern Sahara: the PRIMA 'CAMEL-SHIELD' project

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Dromedary breeding has a great socio-economic potential in southern Mediterranean countries as it contributes significantly to ensure food security for smallholders, preserving their cultural and ethnic identity and protecting fragile arid ecosystems. It also contributes to reduce the economic vulnerability (and thus increase economic resilience) of rural communities living in border areas affected by modern human migration movements. Milk and meat production represent the two main segments of the dromedary value chain, and they could play a leading role in ensuring better and equitable integration of stakeholders and the dissemination of relevant technological innovations. Encouraging dromedary breeding in these areas is a strategic priority to promote endogenous and sustainable territorial development. A rapid, albeit fragmentary, intensification is underway in the dromedary sector, mainly in peri-urban areas, because of an increasing demand for milk and meat from mini-dairies and slaughterhouses. This ongoing intensification process in dromedary breeding poses potential risks to the sustainability of this production system. The CAMEL-SHIELD project, financed under the PRIMA 2019 call, will develop value chain analyses and techno-economic management tools to implement and design innovative strategies for resilience and efficiency, based on exploiting the adaptive capacity of the dromedary production systems. In particular, the characterization about the use of primary resources and about the genetic make-up of the dromedary populations in the study areas (Algeria: Ghardaïa, El Oued and Ouargla; Morocco: Guelmin, M'Hamid El Ghizlane and Errachidia), as well as an assessment of the genomic regions possibly underlying their environmental adaptation capacity, for orienting conservation

and improvement actions, will be performed. The project includes six partner institutions from four countries: the University of Kasdi Marbah – Ouargla (UKMO), in Algeria; the Agronomic and Veterinary Institute Hassan II (IAV), and the University Mohamed V, in Morocco; the Agricultural Research Centre for International Development (CIRAD), and the National Research Institute for Agriculture, Food and Environment (INRAE), in France; the University of Bari, in Italy. The project will provide a first opportunity to apply, and further validate, the dromedary HD SNP array, currently under development within the frame of the 11th Illumina® Agricultural Greater Good Initiative.

## P030

### Ancient mitotypes in the houndlike dog breeds native of the Mediterranean Basin

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The origin and evolution of extant dogs are still a puzzling question for geneticists. In order to assess the genetic diversity, phylogenetic relationships and the maternal origins among related modern dog populations, mitochondrial DNA (mtDNA), and in particular the D-loop hypervariable region 1 (HV1), has been widely exploited. The first investigations using maternally inherited mtDNA in modern dogs indicated that the southern part of East Asia is the place of origin. However, recent samples from archaeological sites have focused on Europe as much as possible, at least as a secondary center of domestication. In this study, eight dog breeds from the Mediterranean Basin that are

phenotypically similar, were investigated: Podenco Valenciano (PV), Podenco Ibicenco (PI), Podenco Canario (PC), Podenco Andalus (PA), Podenco Portugais (PP), Kelb tal-Fenek (KF), Egyptian Baladi dog (EG), and Cirneco dell'Etna (CI), with a total of 223 samples (ranged from 17 to 32 individuals per breed). The median-joining network defines four major haplogroups (Hg), with high prevalence of Hg A (55.2%) and B (40.8%). Four breeds (CI, KF, PI, and PC) showed only Hg A and B with almost homogeneous frequency. The other breeds (EG, PA, PP, and PV) showed the presence of Hg C with only one haplotype (Ht) per breed: EG and PP shared one Ht, while PA and PV both have unique Ht. EG was the only breed with Hg D, and only in one dog. Indeed, the principal component analysis showed the outlier position of EG breed. In order to verify possible genetic continuity since ancient times we compared our sequences to the ancient European dog mtDNAs and constructed the schematic phylogenetic tree encompassing modern (223 + one KF sequence from database) and ancient (56) mitogenomes. Even if ancient data showed an over-representation of Hg C and D, seven sequences fall in Hg A and one in Hg B and some Ht shared within ancient populations and extant dog breeds was also detected. Precisely, within Hg A one of EG sample shows identity with a sample from East France. Moreover, the Italian canid PIC-5 dated to the recent Bronze Age showed Ht identity with CI, PV and EG sequences. Finally, within Hg C, the shared clade was encompassed by EG, PV, PP, PA and eleven ancient sequences suggesting the conservation of ancient European mitotypes over several millennia in the Mediterranean native houndlike breeds. EG is the breed that shares more Ht and seems to be a stronger connection with the past mitogenomes.

## SUSTAINABLE INTENSIFICATION

### P031

#### Hemp cakes composition and ruminal degradability as influenced by the cold-pressing parameters

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The growing consumers interest in the oil has made available several hemp by-products for animal feeding. The main of them are the cakes resulting from mechanical extraction of oil which may contain remarkable levels of protein, but also varying amounts of fibre and fat in relation to both seeds' composition and extraction process. The aim of this study was to investigate

the influence of the pressure and the botanical variety on hemp cakes composition. Seeds from Futura75 and Uso31 cultivars with rather similar composition (respectively, in percent of dry matter, Crude protein, CP, 20.9 vs. 19.9, fat 23.6 vs. 23.3, NDF 48.8 vs. 49.6) were pressed in an experimental mechanical screw press powered by 2.2 kW electric motor and equipped with temperature sensors to control the oil extraction temperature. Each hemp variety was pressed at growing extraction pressures gained by fitting four different nozzle diameters on the screw press (14, 12, 8, 6 mm). For each pressure level, the seeds were pressed twice for a total of 16 cake samples which were analysed for chemical composition and in vitro digestibility of DM (IVDMD) and NDF (IVNDFD), determined based on a 48-h incubation in a Daisy II system. A two-way analysis of variance (proc GLM) was performed with variety ( $n=2$ ), pressure ( $n=4$ ), and their interaction as factors. Statistical significance was attained only between the extreme values of pressure (14 vs. 6 mm) and resulted in an increment of the concentration of NDF, ADF and lignin ( $p < .05$ ) as an effect of the reduction of the fat content ( $p < .05$ ). Despite the small differences between the whole seed compositions, the Uso31 and Futura75 cakes diverged for the CP, ADF ( $p < .05$ ), NDF and lignin ( $p < .001$ ), whereas no differences were observed for fat. It is self-evident that the pressure worked differently on the two varieties, though no significant interactions pressure\*variety were highlighted. The IVDMD and IVNDFD were affected by both pressure and variety ( $p < .001$ ) with the higher values observed for pressure at 14 mm and the variety Uso31. Overall, the nutritional characteristics of hemp cakes can be strongly influenced by the botanical variety even regardless of the chemical composition whereas they can be significantly modified only by more than doubling the pressure applied to seeds.

#### Acknowledgements

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### P032

#### Feed insects for aquaculture: use of *Hermetia illucens* L. meal for *Sparus aurata* L.: chemical and microbiological characterization of the diets

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The use of the *Hermetia illucens* meal (HIM), as an ingredient in feed, represents a way to achieve more sustainable production



of food. Under the current regulation in Europe (2017/893), the use of HIM is permitted mainly within aquaculture. The aim was to characterize the chemical and microbiological composition of diets for *Spaurus aurata* L. containing HIM in partial substitution of fishmeal (FM).

Four diets containing increasing levels of HIM (HIM0, HIM25, HIM35 and HIM50) with 0%, 25%, 35% and 50% of FM replacement, corresponding to an inclusion level of 0, 7.9%, 11% and 15.7% of HIM, respectively, were analyzed. Experimental diets, formulated to meet the *Spaurus aurata* requirements, were iso-energetic (22 MJ/kg gross energy) and isoproteic (42.7 g/100 g, as fed). Fatty acids composition was determined by gas chromatography. Mineral content, including the potentially toxic ones, was assessed via ICP-MS. Microbial flora was characterized following the ISO test methods. Data were analyzed using a one-factor ANOVA of XLSTAT statistical package.

No significant difference for fatty acid profile, Atherogenic, Thrombogenic and Peroxidation indices was observed among the diets. The hypocholesterolemic:hypercholesterolaemic fatty acid ratio (H/H) showed the best significant ( $p < .05$ ) values in the diets containing HIM. Twenty-six minerals were identified, nine of these showed significant differences among the diets (Hg, Na, Mg, Al, K, Ti, Mn, Zn, Sr). Hg content showed significant ( $p < .05$ ) lower values (0.04 ppm) in the HIM35 and HIM50 than those observed in HIM0 and HIM25 (0.1 ppm) diets; however, the Hg values were below the limit value provided by the EU regulation (2015/186). The microbiological analysis showed good microbiological quality and no significant difference of each microbiological parameter was observed among the diets. The counts of the aerobic colonies were lower than 70 CFU/g, Enterobacteriaceae and coliforms charges were  $<10$  CFU/g, while  $<100$  CFU/g for yeasts and moulds. No *Salmonella* spp., *L. monocytogenes* and *Clostridium* spp. were detected.

The HIM inclusion did not affect the fatty acid composition and the microbiological quality and positively influenced the H/H ratio and lowered the Hg content of the diets. Analyzes on the effect of dietary HIM inclusion on *S. aurata* performance and quality traits are carrying out.

#### Acknowledgements

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## NON-CONVENTIONAL LIVESTOCK SYSTEMS

### P033

#### Mealworm (*Tenebrio molitor*) as alternative feed ingredient for poultry production

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The use of insects in broilers farming may be a suitable alternative to conventional feed ingredients, such as soybean meal and oil, due to their high nutritional value as protein and fat sources. Insects rearing requires limited spaces and a small amount of water and can be fed with by-products and former foodstuff, decreasing the environmental impact. *Tenebrio molitor* (TM) is one of the most promising insect species for feed production. This review analyses all data from literature concerning the use of mealworm in broilers diet as partial or total substitution of conventional protein and fat sources. Broilers growth performance, in vivo parameters (blood parameters, digestibility and gut parameters), carcass traits and meat quality are evaluated to confirm the TM suitability as a feed ingredient. The body weight gain is positively affected by the TM inclusion, while the overcomes of feed intake and feed conversion ratio reported both positive and negative effects related to TM inclusion. Carcass and blood traits are slightly affected by the dietary treatments, with results comparable with the control groups and within the normal physiological ranges. Moreover, TM meal shows a high nutrients digestibility for broilers, such as dry matter and ether extract. Some authors report low digestibility value for crude protein, maybe due to the chitin content, but this does not influence broilers growth performance. Low amount of TM meal inclusion enhances broilers gut microbiota, improving the relative abundance of Firmicutes and Bacteroidetes, their ratio and the amount of short chain fatty acids such as butyrate and propionate produced by bacteria. Differently, the dietary treatments do not affect the mucin amount and the intestinal morphology.

The use of TM meal does not alter the physical parameters of meat quality, while it affects the chemical ones, as the fatty acids (FA) profile of broilers meat. Instead, the results highlight a correlation between broilers meat saturated (SFA), mono and polyunsaturated fatty acids (MUFA and PUFA) with the FA composition of the feeds. TM meal (rich in palmitic, stearic, oleic, linoleic and linolenic acids) inclusion leads to a decrease of SFA and an increase of MUFA and/or PUFA in broilers meat, especially in the liver, improving its nutritional value.

To sum up, the use of TM meal seems to positively influence broilers health status and productive performance, confirming its potential role as feed ingredient.



## NUTRITIONAL PROFILE

### P034

#### The role of heat stress and feed restriction on the physical traits and proximate composition of rabbit meat

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The present research aimed at differentiating the impact of the heat stress (with low feed intake as an indirect effect) from the low feed intake (feed restriction) alone, on physicochemical meat traits of growing rabbits. At weaning (5 weeks of age), a total of 180 rabbits were housed (3 animals/cage) in different conditions: a Control group was housed at 20 °C and fed ad libitum with a commercial pelleted diet for growing rabbits (20-AL), another group of rabbits (heat stress) was housed at 30 °C and fed ad libitum with the same diet of 20-AL (30-AL), and a third group of rabbits (feed restriction) was housed at 20 °C and pair-fed on the feed intake of the 30-AL (20-R). A total of 20 replicated cages/treatment group were thus designed. At 11 weeks of age, one rabbit/cage was slaughtered at a commercial abattoir and, after carcass dissection,  $n = 20$  *Longissimus dorsi* (LD) muscles/treatment were frozen at -40 °C and then used for the following meat physical evaluations: thawing (overnight at +4 °C), cooking (water bath until LD core temperature was 80 °C) and total (the sum of the previous ones) losses, pHu and  $L^*a^*b^*$  colour values (measured twice on the cranial and caudal side of each LD muscle). A total of  $n = 8$  LD muscles/treatment were then freeze-dried and analysed for their proximate composition. Data were analysed by a one-way ANOVA with experimental group as fixed effect. The LD of rabbits housed at 30 °C (heat stress) exhibited higher ( $p < .001$ ) cooking (29.1, 23.5, 28.4 % for 20-AL, 20-R and 30-AL) and total (33.0, 25.5, 31.4 % for 20-AL, 20-R and 30-AL) losses compared to the other groups, whereas LD of rabbits under feed-restriction had the highest pHu (5.82, 6.18, 5.90 for 20-AL, 20-R and 30-AL;  $p < .001$ ). Both heat-stressed and feed-restricted rabbits had leaner meat compared to that of the Control group (3.76, 3.10, 3.03 g/100 g meat for 20-AL, 20-R and 30-AL;  $p < .001$ ). Furthermore, the LD of feed-restricted rabbits had the lowest protein content (19.9, 19.3, 19.6 g/100 g meat for 20-AL, 20-R and 30-AL;  $p < .01$ ), while heat-stressed rabbits did not differ from the Control group for this trait. The LD meat of 20-R and 20-AL rabbits was also the richest in moisture ( $p < .001$ ). In conclusion, the present study indicated that feed restriction had a higher impact on the considered meat quality traits compared to heat stress, thus suggesting that rabbits may tolerate heat stress better than a forced reduction of feed intake.

### P035

#### Effect of cooking techniques on the protein digestibility and fatty acids profile of mealworms (*Tenebrio molitor*)

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Alternatives to the ongoing animal productions are required to sustain the increase of human population while lowering environmental impact. Insects could meet human nutrient requirements and increase production yields without negatively impacting the environment. Processing of insects is a crucial step to make them utilizable as food and reach consumers tables. As one of the last steps before consumption, cooking plays a key role in nutritional value of food affecting the bioavailability of nutrients in the digestive tract. In the present research *Tenebrio molitor* larvae were subjected to different cooking methods, such as: oven cooking at 70 °C for 30' (OC70-30) and at 150 °C for 10' (OC150-10), microwaving at 800 W power for 150' (MW), deep frying (DF) and pan frying (PF) in sunflower oil for 2', vacuum boiling (BO) for 30', steaming (ST) for 10'. All the cooking sessions were performed in triplicate on three different experimental units. Proximate compositions of cooked and uncooked larvae (UC, raw sample served as control) were carried out along with fatty acids composition. In vitro protein digestibility was also tested. Mealworms showed a high content of proteins (20% of fresh matter) and lipids (14% of fresh matter). These two parameters make mealworm nutritional value comparable to other conventional animals' products such as meat, eggs or milk. In vitro protein digestibility highlighted a decrease ( $p < .001$ ) after simulated gastric digestion in all the cooked samples (ranged between 24.10% and 40.90%) in relation to the UC (63%). In vitro protein digestibility increased in all the cooked samples after duodenal digestion, with significant changes in OC70-30, PF, DF and ST (respectively +37.48%, +19.39%, +16.18% and +16.83%;  $p < .05$ ). For all cooking methods a general decrease in SFA and MUFA was observed ( $p < .01$ ). This observation may be explained by the fact that both fatty acids classes are largely represented in neutral lipids and are more prone to migration. However, MUFA showed less variations than SFA. Furthermore, the PUFA contents were higher ( $p < .001$ ) in ST and DF groups (52.38% and 48.04%), followed by BO, OC150-10, PF and OC70-30 (30.00%, 29.58%, 28.83% and 22.73%, respectively) respect to UC (18.05%) and MW (14.55%). In conclusion, mealworm larvae represent a good source of macronutrients and may be part of human diet. Notably, the cooking section must be carefully checked in order to maintain the availability of nutrients in the final product.

## P036

### TBARs and ORAC: oxidative stability of lamb meat in three Italian sheep breeds

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Oxidation in lipid and protein fractions of meat is demonstrated as the main non-microbial cause of quality deterioration during its processing or storage. The meat oxidative stability is affected by several factors (species, breed, muscle type, feeding, pre- and post-slaughter treatments) resulting in a general degradation of meat quality, reduction of its nutritional value and palatability.

The aim of this study was to determine the meat oxidative stability in Bergamasca, Sopravissana, and Merinizzata Italiana breeds, assessing the ability of antioxidants to protect meat proteins from oxidative damage (ORAC assay) and detecting the malondialdehyde content, the main secondary end-product of lipid oxidation (TBARS assay).

For this experiment, 10 steaks for each breed between the 12–13th thoracic vertebrae were sampled from 30 right half carcass of male lambs. Longissimus thoracis samples were analysed, according to official methodologies, for antioxidant capacity using the oxygen radical absorbance capacity method (ORAC), and TBARS on fresh meat (1st storage day) and after 3rd and 6th day of refrigerated storage at 4 °C. Data were analysed with JMP software (ANOVA for ORAC and ANOVA-Repeated measurements for TBARS) to evaluate the effect of breed on oxidative stability parameters.

For ORAC assay, the analysis of variance highlighted a significant effect of breed ( $p < .001$ ) showing Bergamasca lamb meat with a lower antioxidant capacity compared to Merinizzata Italiana and Sopravissana lamb meat (712, 999, 1132  $\mu\text{mol TE g}^{-1}$ , respectively). Significant differences among breeds were also found for TBARS; Bergamasca lamb meat showed 0.156 mg MDA  $\text{kg}^{-1}$  muscle, higher compared to the levels detected for Merinizzata Italiana (0.146 mg MDA  $\text{kg}^{-1}$ ) and Sopravissana (0.127 mg MDA  $\text{kg}^{-1}$ ). An increase in TBARS level during aerobic storage for a six-day period at 4 °C, indicates a progressive loss of resistance to lipid oxidation of meat, and has been observed in the three breeds. Particularly, Sopravissana lamb meat compared to Bergamasca and Merinizzata Italiana ones showed a superior resistance to oxidative deterioration, which may be due to a slower oxidation of its unsaturated fatty acids.

The monitoring of antioxidant capacity (ORAC) and lipid peroxidation biomarker (TBARS) is essential to control the development of off-flavours and nutritional quality of lipid profile of lamb meat, which are the main quality characteristics determining the acceptability of meat by the consumer.

## P037

### Fatty acid profile of Massese lambs' muscles in different rearing systems

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Fat depot and fatty acid composition are strongly affected by feeding and rearing systems and this especially applies for autochthonous breeds, such as Massese dairy sheep.

The aim of this study was to evaluate the effect of different farming and feeding systems on tissues composition of main commercial joints and fatty acid profile of different muscles of Massese lambs slaughtered at 70 days of age. Massese lambs ( $n = 16$ ) were reared on two systems: stall (S), 6 males and 2 females, reared indoors and fed by concentrate and hay; pasture (P), 5 males and 3 females, reared exclusively outdoor and fed grass at pasture and hay. The lambs were kept constantly with their dams, having milk always available. At slaughter, final live weight and tissue composition of loin, shoulder and leg were recorded. On the main muscle of each region (Longissimus dorsi – LD, Triceps brachii – TB and Semimembranosus – SM) chemical and fatty acid composition of meat were determined. Data were subjected to ANOVA using the GLM procedure of SAS: for muscles tissue composition considering rearing system and sex as fixed effects; for fatty acid profile considering rearing system, sex and muscle type as discrete effects and the interaction between rearing system and muscle type as fixed effects.

The rearing system significantly affected final live weight ( $p < .05$ ) where S lambs were heavier than P (22.5 vs. 14.95 kg). The rearing system affected the weight of the commercial joints (expressed as proportion on carcass weight) and their tissue composition. Shoulder and hind leg were heavier in P than S group probably due to the major activity of locomotor apparatus, while no difference was observed in loin. Nevertheless, S lambs showed the highest proportion of fat in all cuts, while P animals had higher percentage of bone both in shoulder and leg. The results of chemical composition confirmed the lowest fat content of P lambs and in particular the difference emerged for TB and LD muscles, while within S lamb TB was fatter than SM.

The proportion of the main fatty acids in different muscles showed that C14:0 was higher in TB than SM in S lambs. Irrespective of muscle, S lambs presented the lowest values of n6 fatty acids except for C18:2 n6 where S and P lambs showed together the highest values for SM muscle. No difference among

muscles was found for C18:3 n3 whereas, for both S and P lambs, TB muscle had a content of C20:5 n3 (EPA) compared to SM. No difference among muscles was found for SFA, PUFA and MUFA. The rearing system seems to be the main source of variation.

## P038

### The dietary influence of partially defatted *Hermetia illucens* meal on the hepatic methionine metabolism in rainbow trout

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The interest in insect meal from flies, mealworms, and crickets to replace conventional protein sources in aquafeeds is continuously growing.

*Hermetia illucens* (HI), which belongs to the Diptera order, is one of the most promising insect species. Several studies have confirmed that replacing up to 50% of fishmeal (FM) with HI meal does not impair growth performance and feed digestibility of fish species, such as Atlantic salmon, European sea bass, and rainbow trout. Furthermore, research from our group has demonstrated a positive effect of dietary HI meal on the gut microbiota composition increasing the presence of *Lactobacillus* and butyrate – producing bacteria.

As a protein source, HI meal has a well-balanced amino acid profile that is closely comparable to that of soybean meal (SBM) and only slightly less optimal than wild-caught FM. With regard to the sulphur containing amino acids, such as methionine (Met) and cysteine, the levels are lower in HI meal than in SBM and FM. In particular, Met is often a limiting amino acid in many fish diets and its supplementation is necessary to meet the common cultivated fish species requirements, ranging from 1.8% to 4.0 % of dietary protein.

Accordingly, our study focused on Met metabolism of rainbow trout (*Oncorhynchus mykiss*) fed with diets based on partially defatted HI larvae meal without Met supplementation, to explore the effects on three genes and two metabolites involved in Met metabolism.

Three diets were formulated, an FM-based diet (HI0) and two diets in which 25% (HI25) and 50% (HI50) of FM was replaced with HI larvae meal.

Two target genes – betaine-homocysteine S-methyltransferase (BHMT), and S-adenosylhomocysteine hydrolase (SAHH) – are involved in Met resynthesis whereas the third one – cystathionine -synthase (CBS) – is involved in net Met loss (taurine synthesis). We also quantified the levels of two Met metabolites involved

in the maintenance of methyl groups and homocysteine homeostasis in the hepatic tissue: S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH).

Dietary replacement of up to 50% of FM with HI larvae meal without any Met supplementation, did not impaired rainbow trout growth and hepatic Met metabolism. In particular, Met availability from the insect-based diets directly modulated the transcript levels of two out of three target genes (CBS, SAHH) to maintain an optimal level of one-carbon metabolic substrates, i.e. the SAM: SAH ratio in the hepatic tissue.

## P039

### Histological evaluation of probiotic (lactic acid bacteria) effects on gilthead sea bream (*Sparus aurata*) gut

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The present investigation evaluated the morphological and physiological condition of the intestine of sea bream (*Sparus aurata*) fed with a diet containing *Lactococcus lactis* subsp. *lactis* (LAB) as probiotic. The study lasted 12 weeks and was performed using juveniles (70–80 g) reared in nine 500-L tanks with 40 fish/tank. Fish were fed in triplicate (3 tanks/diet) with 3 experimental diets: diet A (control group) and diet B and C, containing 2.0E + 09 CFU/kg and 5.0E + 09 CFU/kg dose of probiotic, respectively.

Intestines from six fish from each dietary group were collected, divided into proximal and distal portions, and then fixed and processed for standard histological techniques. The obtained tissue sections were stained with three different protocols: Hematoxylin and Eosin (H&E); Alcian Blue Periodic Acid-Schiff (PAS), and May Grünwald – Giemsa. Images acquired with digital cameras connected to a stereomicroscope and an optical microscope, were analyzed using ImageJ software (open-source Java-based imaging program). To detect the possible morphological differences between groups, due to the administration of different levels of probiotic, we assessed a combination of evaluation criteria as described in Baeza-Ariño et al. (2014); Gu et al. (2016); Purushothaman et al. (2016). The parameters taken into account were: gut mucosal folds height (Mh), mucosa folds width (Mw), the lamina propria thickness (LP), the sub-mucous layer (SML), and the goblet cells number (GC). Results showed that the general intestinal integrity, both for proximal and distal portions, was functionally maintained and no inflammatory phenomena were detected. In addition, significant differences were reported for the mucosal fold lengths that resulted higher in fish fed with diet C as compared to the other groups. In the same way, all the quantified parameters suggested, for diet C, a positive effect of



the probiotic in term of mucosal folds trophy and development. These results together with the growth performances data previously obtained suggest that LAB could play an important role in amelioration of the intestinal structure improving fish capacity to digest and absorb nutrients.

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## P040

### Sialyloligosaccharides content in milk of different cow breeds

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The MIQUALAT project aimed to improve the health image of bovine milk and to increase its use in nutrition. Milk oligosaccharides represent a class of bioactive molecules with potential beneficial effects on human health. Sialyloligosaccharides (SOS) play an important role not only in brain development and increasing immunity in infants, but also in adults for the prebiotic action on the bacterial flora with the improvement of immune defences and the intestinal microbiota. In the project, the presence and concentration of SOS was analysed in mature milk of different cow breeds. The study was carried out in four breeds: Holstein (HO), Simmental x Holstein (SMxHO), Simmental (SM) reared in the CREA farm in Monterotondo (Italy), and Podolica (POD) breed in a farm in Basilicata region. Individual milk samples of 25 animals for each breed were taken from milking morning at 60 and 120 days of lactation and stored frozen  $-20^{\circ}\text{C}$  until analysis. SOS (3'-sialyllactose = 3'-SL, 6'-sialyllactose = 6'-SL, disialyllactose = DSL, expressed in mg/L) contents were analysed with HPAEC-PAD, high performance anion-exchange chromatography with pulsed amperometric detection on a Dionex PA100 column with the use of external standards. Statistical analysis was performed by GLM procedure. The model included breed as fixed factor, comparisons between means were tested with the Tukey test. 3'-SL content, both at 60 and 120 days, was significantly higher ( $p < 0.001$ ) in the POD than all other breeds (at 60 days 115.97, 82.55, 76.56 and 71.04 mg/L in POD, SM, SMxHO, HO respectively; at 120 days 92.71, 63.72, 59.68 and 56.39 mg/L in

POD, SM, SMxHO, HO respectively). Furthermore, differences ( $p < .05$ ) were found at 60 days between SM and SMxHO, HO and SMxHO and higher significantly difference ( $p < .001$ ) between HO and SM. 6'-SL and DSL content, both at 60 and 120 days, was significantly higher ( $p < .001$ ) in POD than all other breeds. Results showed a significant effect of the breed on the SOS content which is more evident in autochthonous breeds.

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## P041

### Management, total mixed ration composition, and milk fatty profile in buffalo farming

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A survey was carried out on management practices, diet characteristics, milk yield, and fatty acid profile in buffalo farming. Thirty-nine farms (about 3% of the buffalo farms of Campania Region) were voluntarily enrolled in the study. To be eligible to be included, the farms need to use the same total mixed ration (TMR) for all lactating cows. Each farm was visited twice, at least 10 days apart, to record management practices, ration composition, and milk yield along with sampling TMR and milk. Chemical composition, in vitro digestibility of DM (IVDMD) and NDF (IVNDFD) based on a 48-h incubation in a Daisy II system, and particle size distribution by means of the Penn State Forage Particle Separator were determined for TMR. Milk samples were analyzed for fat and protein (Milkoscan 605), somatic cell count (SCC-Fossomatic250), and fatty acid (FA) composition by trans-esterification reaction using capillary gas chromatography. Descriptive statistics, Pearson's correlation and linear regression analyses were performed by using SAS statistical package. Herds averaged  $136 \pm 73$  lactating buffaloes producing about  $8.6 \pm 1.7$  kg at  $8.45 \pm 0.47\%$  fat,  $4.72 \pm 0.37\%$  protein,  $\text{SCC } 265.16 \pm 150.81 \times 10^3$ . Most of the farms used a combination of hays, silages, and concentrates while only 6 on them did not use ensiled forages. A larger variability was observed for ration characteristics. The TMR averaged  $51.9 \pm 8.5\%$  DM, crude protein  $13.9 \pm 1.9\%$  DM, and NDF  $57.3 \pm 5.4\%$  DM. Particle size analysis showed that 45.6% of the TMR was retained on the 19-mm sieve (range 12.3–78.7%), 20.4% on the 8-mm sieve (2.4–50.5%), 10.4% on the 4-mm sieve (3.2–20.7%), and 23.0% on the bottom pan (5.4–22.7%). Weak relationships were found between particle size and milk yield and



TMR composition, while IVNDFD was positively related to milk fat ( $R^2$  0.75,  $p < .001$ ). Saturated FA averaged  $70.3 \pm 3.8$ , mono-unsaturated FA  $26.1 \pm 1.3$ , and polyunsaturated FA  $3.6 \pm 0.5$ , data in line with previous reports. Weak relationships were found between TMR composition, TMR particle size and FA composition of milk fat. Overall, the survey highlighted that TMR particles larger than 19 mm were dramatically higher than the recommended values for lactating cows. Nevertheless, the low incidence of metabolic disorders and the satisfactory milk yield suggests the need of establishing particle size ranges of TMR specific for buffaloes.

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## P042

### Sea bream fillet quality as affected by alternative protein sources in diet

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Gilthead seabream (*Sparus aurata*) has been the object of intensive farming as early as the seventies. Nowadays it represents the most popular marine finfish in Italy and its farming is an important activity both for the productive volumes and economical value. As part of the aquaculture sector, seabream farm is suffering for feedstuff shortage, being fishmeal and oil highly unsustainable. Thus, the present study aimed to evaluate different protein sources in seabream diet: (i) a fish meal-based (54%) control diet (CF), (ii) a plant protein meal-based control diet (CV); the other four diets were based on the CV diet, with: (iii) 10% substitution with a mixture of microalgae (*Tisochrysis lutea* and *Tetraselmis suecica*) meal (M10), (iv) 10% substitution with the meal obtained by processing the invasive alien Louisiana red swamp crayfish (*Procambarus clarkii*) (R10), (v) 20% substitution with poultry by-product meal (PBM) (P20), (vi) 40% substitution with insect meal (*Hermetia illucens*) (H40). Ten fish per group were sampled at the end of the growing trial and the physico-chemical characterization of fish fillets was assessed. The results revealed that M10 diet slightly but significantly decreased fish total body weight (around 303 g against 335 g, as average of the other groups). Despite this, M10 fish had analogous fillet yield than the other groups (53%). Diets differently affected skin and fillet color values. Specifically, the results of skin color indexes (lightness,  $L^*$ ; redness,  $a^*$ ; yellowness,  $b^*$ ) displayed differences in the skin of the fish fed with M10, which was significantly lighter than the P20 group skin ( $L^*$ : 76.41 and 69.69,

respectively). Fish fed H40 showed a  $b^*$  index (1.28) significantly higher than the  $b^*$  value of CF group ( $-3.85$ ). Contrariwise, fillet color values were not modified by the dietary treatments. Looking at the nutritional values, the incidence of polyunsaturated fatty acid (FA) of the n-3 series was higher in CF fillets (23.86 g/100 g total FA) than in the other groups (19.51 g/100 g total FA, as average), while M10 and CV fillets had interesting amount of C18:3n-3 (8.37 and 8.46 g/100 g total FA, respectively). To sum up, microalgae seemed to induce the major changes in fish characteristics, while all the other ingredients could be considered to replace conventional protein sources in aquafeed for gilthead seabream.

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## P043

### Does honey bee pollen as dietary supplement effectively preserve sea bream fillets during frozen storage?

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Recently, the possibility to increase fish health, welfare, and fish storage stability through feed supplementation with natural additives or bioactive molecules has gained importance. Honey bee pollen (HBP) contains several interesting compounds such as polyphenols, which a new extractive technique called supercritical fluids extraction (SFE) seems to preserve. Indeed, SFE extract showed good antioxidant properties, high polyphenol content, radical scavenging activity, and reducing power. If these properties might act as antioxidants to preserve fish fillet quality during storage deserves to be investigated. Thus, our aim was to evaluate the effectiveness of honeybee extracts added to gilthead sea bream (*Sparus aurata*) feed formulation on the preservation of frozen fillet characteristics.

Two levels of HBP (5% or 10%, P5 or P10 treatments, respectively) and its supercritical fluid extract (0.5% or 1%, E0.5 or E1, respectively) were included in the sea bream diet. After 15 days of adaptation, fish were fed for 30 days, then 9 fish per experimental group were sacrificed and filleted. Fillets were stored at  $-10^\circ\text{C}$  for 110 days and their fatty acids (FAs) were detected at slaughtering (day 0) and at the end of the storage (110 days). Analogously, lipid oxidation was assessed by conjugated dienes

(CD, mmol hydroperoxides/100g meat) and thiobarbituric acid reactive substances (TBARS, mg MDA-eq/kg meat) quantification. The PROC GLM of SAS was used to analyse the obtained data with dietary treatment and storage time as fixed effects.

Frozen storage caused a loss of polyunsaturated fatty acids (PUFA) of the n-3 series, while a higher content of saturated fatty acid (SFA) was found at day 110th ( $p < .05$ ). Consistently, TBARS raised from 0.627 to 1.123 mg MDA-eq/kg fillet during storage ( $p < .05$ ). E1 group was the most effective in oxidative damage mitigation, by exerting a specific protection on C18:3n-3, C18:4n-3, C20:1n-9, and C22:1n-11 FAs. Noticeably, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid content did not decrease along storage.

In conclusion, administered HBP supercritical fluid extract at 1% seemed to slightly protect sea bream fillets from oxidative damages during long term frozen storage. Nevertheless, its action was limited to specific PUFA n-3 and few monounsaturated FAs. Due to the importance of finding natural bioactive products to improve fish metabolism, stress response, and fillet quality, further comprehensive studies are encouraged.

## P044

### Fatty acid profile of Sarda suckling lamb meat with different fat content

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Suckling lamb meat is one of the most relevant products in the traditional dairy sheep farming in many Mediterranean countries. The lambs are usually raised with their dams and fed almost exclusively maternal milk; they are slaughtered at 25–30 days of age and 9–11 kg of body weight. Body composition, carcass and meat characteristics are very heterogeneous among individuals, since they depend on farming management, breed, milk composition, etc. Meat from suckling lamb is one of the main sources of health-promoting natural fatty acids (FA), as the conjugated linoleic acid (CLA), the polyunsaturated fatty acids of n-3 series (PUFA n-3) and the long-chain fatty acids. Therefore, lamb meat with high concentration of PUFA n-3 and CLA could be of great nutritional interest. We conducted a study to investigate the variation of FA composition of meat from Sarda suckling lambs with different content of intramuscular fat. Sarda lambs, reared in Central of Italy, were slaughtered in autumn and spring season, and Longissimus thoracis muscle was taken at 24h after

slaughter to perform the analysis. Samples were analyzed for ash, crude protein, total fat content and fatty acids profile. The samples were classified according to the total fat content and the samples from 24 animals equally divided into 3 groups as follows: low-fattening  $2.3 \pm 0.2\%$ , medium-fattening  $4.5 \pm 1.5\%$ , and high-fattening  $6.3 \pm 1.8\%$ . The relative amounts of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, hypocholesterolemic/hypercholesterolemic index (h/H), atherogenic index (AI) and thrombogenic index (TI) were estimated. The results showed a significantly lower PUFA content in animals with high fat (21.41% vs. 25.32% as averages for medium and low-fat content respectively,  $p = .008$ ), but highest levels of MUFA ( $p = .049$ ) and SFA ( $p < .001$ ); this trend was probably due to a major intermuscular fat content. The low-fattening group showed the best values as far as all nutritional fat indexes such as the n-6/n-3 ratio ( $p = .038$ ), h/H index ( $p = .004$ ), in particular a high level of n3-PUFA (7.00% in low fat meat vs. 5.51% in high fat meat fatty acid,  $p = .007$ ). Percentage of total CLA did not differ significantly between groups ( $p = .208$ ). These results highlight the importance of fat accumulation in body lambs that makes substantial changes in fatty acids profile of meat. In fact, lean meat showed nutritionally higher values in particular as regards the n3 essential fatty acids.

## P045

### The incidence of fresh forage in sheep diet on Pecorino di Filiano DOP cheese quality

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Pecorino di Filiano DOP (PFC) is a ripened hard cheese made with ewe's raw milk of Gentile di Puglia breed, in the NW area of Basilicata, Southern Italy. The feeding system is based on pasture, with possible use of fresh forage, hay and grain exclusively produced in the cheese production area. The growing consumer interest in healthy quality cheeses demands tools for expressing it in a synthetic way. Our aim was to evaluate the effect of the fresh forage content in the diet on the PFC quality in terms of DAP (Antioxidant Protection Degree), HPI (Health Promotion Index) and GHIC (General Health Index of Cheese) indexes.

The experiment was carried out on the CREA farm in spring. Four groups of twenty sheep each, homogeneous by BCS and DIM,

were housed in pens and they were fed four different diets designed to have different levels of fresh forage (F), hay and mixed grains (HG): 100% F (F); 70% F and 30% HG (7F3HG); 30% F and 70% HG (3F7HG); 100% HG (HG). The experiment lasted 7d for adaptation and 8d for cheese making and sample collection. The raw bulk milk of groups was processed into PFC according to the product specification. Cheeses after 30 days of ripening were analyzed for fatty acid profile,  $\alpha$ -tocopherol, cholesterol, polyphenols and antioxidant capacity; cheese indexes DAP ( $\alpha$ -tocopherol/cholesterol), HPI ( $\omega 3 + \omega 6 + \text{MUFA}$ )/C12:0 + (4  $\times$  C14:0) + C16:0 and GHIC ( $\Sigma$ polyphenols, CLA isomers, PUFA,  $\omega 3$  and total antioxidant capacity scores) were calculated. Statistical analysis was performed by GLM procedure. The model included feeding treatment as fixed factor, comparisons between means were tested with the Tukey test.

The DAP index was able to discriminate PFC when the percentage of F changed. DAP index decreased from 9.7 (F), 8.6 (7F3HG), 7.8 (3F7HG), to 6.5 (HG) ( $p < .01$ ). No significant differences were found among groups for HPI, values were 0.44, 0.46, 0.47 and 0.45 in F, 7F3HG, 3F7HG and HG groups, respectively. A significant effect of the feeding treatment was detected for GHIC. The higher GHIC was observed in F group (31) compared to HG (14) group ( $p < .01$ ), intermediate values were found in 7F3HG and 3F7HG (20 and 18, respectively). This study has highlighted a relationship between level of F in the diet and DAP and GHIC in PFC. Both indexes are able to discriminate different feeding groups whereas HPI cannot do the same for feeding groups.

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## P046

### Bovine beta casein polymorphism and environmental sustainability of cheese production

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Beta casein constitutes up to 45% of bovine milk total casein and presents several genetic variants. Bovine beta casein variants may play an important role on milk production and composition; besides their possible influence on milk digestibility and human diseases, beta casein variants can influence cheese yield and quality and, in turn, environmental sustainability of cheese production.

The aim of the study is to investigate a possible effect on the environmental impact of cheese production, related to beta casein polymorphism. A Life Cycle Assessment was performed,

considering Grana Padano PDO (GP) and mozzarella cheese (MOZ) and three different genotypes of beta casein, A1A1, A2A2, A1A2.

Three groups of 10 lactating cows were reared under the same conditions; each group belonged to the three different beta casein genotypes. Separated cheesemaking procedures were performed for the three genotypes' milk. The evaluation of environmental impact of 1 kg of packaged cheese was performed using primary data as much as possible, together with secondary and tertiary data. Following classification, characterization was conducted through Recipe Midpoint (H) V1.10 method. With this approach, normalization was also performed, for identifying the impact categories which are important for this specific sector. Normalized results represent the fractional contribution of GP and MOZ production, to an average European Union citizen's cumulative annual environmental impact.

The results pointed out that cheese produced with A2A2 milk seems to be the most environmentally sustainable, both for GP and MOZ. This is probably due to the higher cheese yield (8.00%) of A2A2 milk, compared with the others, mainly related to the protein content. Carbon footprint (CF) of A2A2 was 16.6 kg CO<sub>2</sub> eq/kg packaged cheese and 10.7 kg CO<sub>2</sub> eq/kg packaged cheese for GP and MOZ, respectively. The main contribution to CF was given by raw milk production at the barn, for all the three genotypes and both for GP and MOZ. Normalization highlights that the first largest impact category was natural land transformation, followed by marine eutrophication and terrestrial acidification. In conclusion, cheese produced from A2A2 milk seems to favour environmental sustainability, mainly because of milk composition, which favours cheese yield.

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## P047

### Impact on goat milk composition and rumen microbiome of *Camelina sativa* cake and *Cynara cardunculus* supplements

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The study was aimed to evaluate changes in milk composition and rumen microbiome in response to *Camelina sativa* cake and *Cynara cardunculus* meal supplementation in dairy goats. Eighteen primiparous and multiparous Alpine lactating goats were divided into three balanced groups according to parity number, milk production and days in milk (1.83, 2.23 kg, 273 DIM), and assigned randomly to 3 treatment groups. The treatment groups were: 200 g/d of camelina cake (CAM;  $n = 6$ ), 200 g/d of cardoon meal (CAR;  $n = 6$ ), and control (CON,  $n = 6$ ) receiving the standard diet without supplementation. The supplementation lasted 21 days. Milk yield and composition were measured weekly. Proximate analysis was performed according to AOAC official methods while milk fatty acid profile was determined by means gas-chromatographic technic. Rumen content samples were collected on a subsample of 7 animals on days 0 and 21. The DNA extraction was performed using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA). The V3-V4 region of the 16S rRNA gene was amplified. Sequencing was performed by MiSeq Illumina (Illumina, San Diego, USA). Bioinformatic elaborations were performed in R 4.0.2 with the package DADA2. A non-metric multidimensional scaling (NMDS) and a permutational multivariate analysis of variance (PERMANOVA) based on Hellinger transformed genera abundance data were performed. The taxa with a different relative abundance between the conditions were identified by a Kruskal–Wallis test. Milk composition data were analyzed by PROC MIXED of SAS for repeated measures. Vaccenic acid was increased both in CAM and CAR (44%, 18%) compared to CON and also C18:1 trans 9, trans 10 and trans 12 ( $p < .01$ ). The alpha-diversity was similar between the tested groups and a tendency to a higher evenness was observed in the rumen of goats fed camelina. The classes Bacteroidia, Clostridia and Negativicutes (~53%, ~19% and ~12%, respectively) accounted for more than 80% of the community, regardless the diet. The relative abundance of the class Desulfovibrionia was higher ( $p < .1$ ) in CAM (~0.9%) compared to CON (~0.3%). Conversely the relative abundance of the class Saccharimonadia was lower ( $p < .1$ ) in CAM (~1.2%) compared to CON (~2.4%). Furthermore, a higher relative abundance ( $p < .1$ ) of the class Vampirivibrionia in the rumen content of CAR (~2.5%) compared to CAM (~0.7%) was observed. The most abundant genus in the rumen content was Prevotella, regardless the diet.

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Fatty acids (FA) contribute importantly to various aspects of meat quality and to its nutritional value. Unfortunately, the evaluation of the meat FA composition requires the sacrifice of the animal. Since drawing blood from cattle is a relatively simple and painless practice, to be able to predict the meat FA composition from that of blood it would be an important advancement. Partial Least Square Regression (PLSR) is a quite recent statistical tool able to handle multivariate regression models characterized by high collinearity among predictors. This study is a first evaluation of the use of PLSR to predict the meat FA composition of cattle from that of blood. Within a framework of a long-term research project (Kent'erbass), 15 calves ( $429 \pm 49$  kg live weight) were blood sampled from the tail and subsequently led to slaughter. Blood samples were centrifuged at  $1200 \times g$  for 15 min at  $10^\circ\text{C}$ , to separate and store plasma at  $-70^\circ\text{C}$  until FA analysis. At 24 h post mortem, samples of Longissimus thoracis et lumborum (LTL) between sixth and seventh thoracic vertebrae from each left half-carcass of animals blood-sampled were removed and submitted to FA analysis. PLSR was then applied to a dataset consisting of FA composition detected in both meat and plasma. The evaluation of the model in predicting FA was assessed by means of Pearson's correlation coefficient ( $r$ ), the coefficient of determination  $R^2$ , and root mean square error of prediction (RMSEP). Within the boundary of the ranges of FA values and despite the limited number of data used in the analysis, PLSR procedure was able to provide an estimate with a good degree of precision for many interesting FA, whose values are reported below as predicted and actual value, respectively: CLA,  $1.25 \pm 0.11$  and  $1.25 \pm 0.12$  % total FA, with a  $r = 0.91$ ,  $R^2 = 0.80$  ( $p < .001$ ) and RMSEP = 0.16. n-3 fatty acids,  $1.86 \pm 0.49$  and  $1.86 \pm 0.50$  % FA, with a  $r = 0.98$ ,  $R^2 = 0.95$  ( $p < .001$ ) and RMSEP = 0.36. n-3/n-6 ratio,  $0.22 \pm 0.02$  and  $0.22 \pm 0.03$ , with a  $r = 0.91$ ,  $R^2 = 0.82$  ( $p < .001$ ) and RMSEP = 0.04. C18:3 n-3 (linolenic acid),  $1.22 \pm 0.29$  and  $1.22 \pm 0.30$  % FA, with a  $r = 0.98$ ,  $R^2 = 0.96$  ( $p < .001$ ) and RMSEP = 0.18. C22:5 n-3 (docosapentaenoic acid, DPA),  $0.50 \pm 0.15$  and  $0.50 \pm 0.16$  % FA, with a  $r = 0.97$ ,  $R^2 = 0.94$  ( $p < .001$ ) and RMSEP = 0.13. This model represents an initial attempt to predict meat FA composition of cattle from blood. A more complete database is needed to increase the robustness of the model.

## P048

### Use of a partial least-squares regression model to estimate intramuscular fatty acid profile in beef from blood analysis

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## P049

### Variability of milk fatty acids profile between and within dairy farms producing milk for Grana Padano PDO cheese

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In recent years, a growing interest has been devoted to the nutritional value and health benefits of fatty acids profile of food of animal origin and there is a market tendency towards preferring milk with a higher level of unsaturation in the fatty acids profile for their benefit to human health. Milk fatty acids composition is influenced by many factors but those linked to the diet characteristics play a major role. The variability of this trait has been mainly studied in controlled experiments with marked differences in diet characteristics or in large surveys involving milk samples produced under very different conditions, but the variability that can be faced between farms with similar feeding management has been poorly investigated. As the cheesemaking process does not modify the fat composition, the fatty acids profile of milk fat is the main factor affecting cheese fat traits. As the milk for Grana Padano PDO cheese is produced under strict rules reported in the specification approved by the EU, a low level of variability in its fatty acid profile could be foreseen. A local investigation was carried out within a very short period on 20 dairy farms, associated with two small cooperative cheese factories but with different size and production level for the composition of the milk produced. Five milk samples from dairy cows in mid lactation ( $33.3 \pm 6.2$  kg/d milk yield;  $141 \pm 13$  days in milk) were collected from each farm and analysed for main components and for fatty acid profile of the fat. Samples of total mixed rations were also collected. Preliminary results show that a relevant variability exists within and between farms. The coefficient of variability of the singular fatty acids was exponentially related to their content in fat, ranging from less than 8 to 155%, that can be only partially attributed to analytical errors. Traits like parity, days in milk, milk yield and content of main components were not correlated to these differences. Further investigation on dietary factors is ongoing.

## P050

### Oxidative stability and antioxidant activity of Italian Holstein and Buffalo yogurt contaminated with Pb

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Pb is an environmental pollutant which induces oxidative damage in milk and dairy products. The objective of the research was to determine the effect of species on oxidative stability and antioxidant activity of yogurt obtained by Holstein (H) and Buffalo (B) milk contaminated with Pb. The milk samples (2 L for each species) were split into two equal batches: in the first lead acetate was added to obtain a final concentration of 1 ppm, whereas the second was milk control (without any addition). The milk samples were stored at 4 °C for 24 h before yogurt manufacture. Yogurt samples were obtained with a specific yogurt starter culture consisting of 1% (vol/vol) *Strep. thermophilus* and *Lb. delbrueckii* ssp. *Bulgaricus* and stored at 4 °C for 24 h. After, they were distributed to 250 mL in plastic containers and finally stored at -20 °C until analyzed. Thiobarbituric acid reactive substances method (mg malondialdehyde (MDA)/kg yogurt) and free thiol groups content (mol-SH/g yogurt) were used for evaluated in yogurt samples the lipid and protein oxidation, respectively; whereas, antioxidant activity was measured by 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays (expressed as  $\mu$ g trolox equivalent (TE)/g yogurt). On the yogurt samples, each determination was made five times. The statistical analysis was performed by means of ANOVA, using the GLM procedure of SAS. Overall, in the Pb-treated yogurt, the amount of MDA significantly increased as compared to the control yogurt (1.003 vs. 0.744 mg MDA/kg yogurt, respectively;  $p < .05$ ); whereas, the free thiol group content decreased (0.33 vs. 0.43 mol-SH/g yogurt, respectively;  $p < .05$ ). Considering the species, B yogurt contaminated with Pb showed the higher increase of MDA content (41.28%) compared to H yogurt (27.17%), which showed the higher decrease of free thiol groups (26.47%) than B yogurt (21.15%). The antioxidant activity of Pb-treated yogurt significantly decreased as compared to the control yogurt ( $p < .05$ ); in particular, ABTS value ranged from 353.60 to 231.92  $\mu$ g TE/g yogurt, whereas FRAP value between 204.06 and 162.92  $\mu$ g TE/g yogurt, respectively. Comparing two species, B yogurt contaminated with Pb showed the higher percentage decrease (expressed as  $\Delta\% = (\text{Control value} - \text{treated value}) / \text{control value} \times 100$ ) of ABTS value (39.14 vs. 25.53%) whereas H yogurt showed the higher percentage decrease of FRAP value (32.49 vs. 8.52%). The Pb contamination decreased the oxidative stability and antioxidant capacity of yogurt and the species influenced the extent of damage.

## P051

### Effect of breed on milk fat quality: comparison between Altamurana and Leccese

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The aim of this work was to investigate the effect of breed on morphometric characteristics of fat globules, fatty acid composition, and cholesterol content of milk from two autochthonous sheep breeds at risk of extinction: Altamurana (A) and Leccese (L) breed. The sheep used in this study (20 subjects for each breed), belonging to the same mounting groups within the considered breed, were reared under an extensive system on the same farm in the province of Potenza (southern Italy). The milk fat globules (MFGs) size were measured on milk individual by using a fluorescence microscope; fatty acid profile was determined by GC and cholesterol content by HPLC-UV. After determination of diameter, MFGs were divided into three classes: small globules (diameter <2 µm), medium-sized globules (2 ÷ 5 µm), and large globules (>5 µm). The percentage incidence of each globule class on total measured milk fat globules was calculated:  $\frac{\sum n_{\text{globules}}(\text{each class})}{\sum n_{\text{total globules}}} \times 100$ . The statistical analysis was performed by means of ANOVA. No significant difference was detected for fat content: 7.18 and 7.15 g/100 g milk, for A and L milk, respectively. However, a higher incidence of smaller and medium MFGs was associated with the A milk (29.21% and 65.37%, respectively) compared to L milk (24.17% and 58.93%, respectively); whereas, L milk showed a higher incidence of large MFGs (16.90%) than A milk (5.42%). GC analysis revealed differences in the lipid profile of each breed. In particular, a higher content of saturated fatty acids was detected in A milk (71.89%) than L milk (68.74%;  $p < .05$ ), which showed a higher content ( $p < .05$ ) of monounsaturated (25.09 vs. 22.69% for L and A milk, respectively) and polyunsaturated fatty acids (4.33 vs. 3.35% for L and A milk, respectively). Moreover, L milk showed a higher PUFA<sub>n-3</sub> content (1.72 vs. 0.93% for L and A milk, respectively); consequently, atherogenic and thrombogenic indices were significantly higher in A milk than L milk (3.14 and 2.92% vs. 2.38 and 2.22%,  $p < .05$ ). However, no difference were detected for PUFA<sub>n-6</sub> and CLA content (2.40 and 2.05% vs. 2.29 and 1.83% for L and A milk, respectively). The highest cholesterol content was detected in L milk (16.87 mg/100 g milk) compared to A milk (15.91 mg/100 g milk;  $p < .05$ ). The differences highlighted in this study on fat characteristics could be ascribed to the genetic peculiarities of the genomes of the two considered breeds, which are the result of a selective process linked to adaptation.

## P052

### Fatty acid profile, liposoluble vitamins and antioxidant capacity in three historic cheeses of southern Italy: Piacentinu ennese DOP, Maiorchino e Canestrato di Moliterno IGP

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Piacentinu Ennese PDO (PE), Maiorchino (MR) and Canestrato di Moliterno IGP (CM) are historical cheeses belonging to the dairy tradition of southern Italy. PE, produced in Sicily, is a pressed pasta cheese with a unique yellow colour, due to the use of saffron in the milk; it is produced with raw, whole ewe's milk using traditional tools predominantly on wood. MR is a Sicilian hard cheese, still produced with traditional techniques, from raw, whole ewe's milk sometimes mixed with goat's milk (ewe's milk 30–50% and goat milk 70–50%). CM cheese is a PGI hard cheese produced in Basilicata with a combination of raw or heat-treated goat and sheep milk (70–90% sheep's milk and 30–10% goat's milk).

PE (5 samples), MR (3 samples) and CM (5 samples) cheeses were chosen at ripening phases mostly appreciated by consumers (4, 10 and 6 months, respectively). Cheeses were analysed for fatty acid composition (% of Fatty Acid Methyl Esters, %FAME), retinol,  $\alpha$ -tocopherol (mg/kg of cheese), total phenol content (TPC) and antioxidant capacity with FRAP (ferric reducing ability) and TEAC (trolox equivalent antioxidant capacity) tests. MR cheese was characterised by a lowest content of SFA (Saturated Fatty Acids) 65% vs. 70% and a highest content of monounsaturated fatty acids 27% vs. 24% vs. 22% respect to CM and PE, respectively. As concern beneficial fatty acids, MR cheese showed a highest content of CLA c9 t11 (1.8% vs. 1.4% vs. 1.1%) and of Polyunsaturated Fatty Acids- $\omega$ 6 (PUFA- $\omega$ 6, 2.2% vs. 1.9% vs. 1.8%) compared to PE and CM, respectively. PE cheese was featured by a highest content of PUFA- $\omega$ 3 (2.1% vs. 1.6% vs. 1.4%) and consequently the lowest  $\omega$ 6/ $\omega$ 3 ratio value compared to MR and CM cheeses. Retinol content (mg/kg of cheese) was significantly higher in PE (4.1) than MR (3.2) ( $p < .05$ ) and it was intermediate

in CM (3.5) cheeses;  $\alpha$ -tocopherol values were 9.7, 8.2 and 7.9 (mg/kg of cheese) for PE, MR and CM, respectively. TCP values were 4.66, 3.75 and 3.93 (g GAE/kg cheese) in CM, PE and MR, respectively. FRAP assay detected a wide range of values: 1.77, 3.10 and 2.56 (mmol FeSO<sub>4</sub>/kg cheese) in CM, PE and MR, respectively. TEAC values (mmol Trolox/kg cheese) were significantly higher ( $p < .001$ ) in CM (63.1) and MR (44.6) respect to PE (16.2) cheeses. In conclusion, values of fatty acids, vitamins, TCP, FRAP and TEAC characterize the single cheese because they are linked to the breed, feeding system, production season and ripening phase. This gives them the characteristic of uniqueness.

#### Acknowledgements

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## P053

### Poultry meal in feed for Gilthead seabream (*Sparus aurata*): results from an ‘on farm’ feeding trial

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An ‘on farm’ feeding trial was conducted on Seabream (*Sparus aurata*), with inclusion of poultry meals (PM) in the diet to replace fishmeal (FM) as protein source. The aim of the study was to evaluate the possibility of a complete substitution of FM; therefore, the inclusion of other alternative protein sources, such as plant-derived proteins and an addition of AA, including taurine, was adopted. Three floating cages of 5600 m<sup>3</sup> each, moored in the North Adriatic Sea, were loaded with 173,589–185,213 fish/cage of 267–285 g mean initial weight, total biomasses ranging from 46,348 to 52,786 kg. Fish were fed for 83 days with either a Ctrl (a commercial diet) or two experimental diets: A (20% PM +10% FM), and B (20% PM +10% corn gluten meal). At the end of on-growing, total biomass and fish average size were measured. Gut samples were collected for histological and microbiota analysis.

The fish cage that received Ctrl feed (49,700 kg in total), reached a production of 24,742 kg, SGR 0.514, FCR 2.001, with a mortality of 620 fish. The cage that received diet A (52,900 kg feed), reached a production of 27,032 kg, SGR 0.495, FCR 1.957, with mortality of 143 fish. The third cage, receiving diet B (41,975 kg feed), reached a biomass of 20.816 kg, SGR 0.474, FCR 2.016, with a mortality of 595 fish.

Histological examination revealed alterations attributable to enteritis in all fish, in particular in those receiving zero FM and

unexpectedly, in those fed the Ctrl diet, whereas fish receiving 10% FM the enteritis was significantly less severe. Gut microbiota analysis is still in progress and results will be shortly available. The apparently higher biomass reached with diet A may be explained by an higher feed consumption and a lower FCR. The Ctrl diet led to a better SGR, nevertheless FCR was the same as FCR of diet B (FM free). The results showed that the performance of fish fed with diet B with a complete substitution of FM, did not differ markedly from fish fed with the Ctrl diet, nevertheless the intestinal morphology indicated a nutritional imbalance in diet B. Therefore, in spite of similar performances observed among the cages, before the marketing of FM-free commercial diets, further studies need to be conducted at a laboratory scale, to identify how to further balance the feed formulations.

## P054

### Quails meat quality as affected by *Tenebrio molitor* larva meals in feeds

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Insects have been rapidly emerging as an alternative high quality, efficient and sustainable protein source to the conventional livestock feed protein. *Tenebrio molitor* (TM) is considered one of the most promising species in this sense. This study evaluated the effect of a partial replacement of soybean and corn meal with TM larval meal in the diet of common quails (*Coturnix coturnix*) on physical and chemical characteristics of raw and cooked meat. To this purpose, 96 quails, divided into four groups (24 quails per group), were fed with four different diets for a period of 42 days. A group with soybean and maize meal (C), the other three groups with a partial replacement of soybean meal at 5, 10, 20% with TM meal (T5, T10, T20, respectively). All the diets were isonitrogenous, isolipidic, and isoenergetic. At 42 days of age, quails were slaughtered, and the peeled carcasses were used for meat quality evaluations. Specifically, each carcass was divided into two symmetrical halves to analyze the left side as raw, allotting the right half to the cooking trial (baking in oven at 200 °C for 35 min). Data related to the raw and cooked samples were analyzed separately by means of one-way ANOVA using PROC GLM of SAS statistical software. Marketable and physical parameters showed that the inclusion of TM meal did not compromise the carcass traits and cooking losses of the product. The T20 raw breasts were



more tender ( $2.16 \text{ N/g}$ ;  $p < .01$ ) than breasts of T5 and T10 groups ( $2.48$  and  $2.62 \text{ N/g}$ , respectively), but did not differ from the C one ( $2.36 \text{ N/g}$ ). Regarding the fatty acid profile, slight differences were found in raw and cooked meat in TM groups, with a higher content of C18:3n3 in the cooked meat of TM10 group ( $p < .01$ ). The TM inclusion in feed significantly affected the oxidative status of breast raw meat. Specifically, the T20 group was scarcely subjected to lipid degradation, having  $0.033 \text{ mg MDA-eq./100 g}$  meat that was significantly lower than TBARS content of T5 and C ( $0.040$  and  $0.045 \text{ mg MDA-eq./100 g}$  meat, respectively). In contrast, the T10 meat was the most oxidized ( $0.053 \text{ mg MDA-eq./100 g}$  meat) group. No significant differences were observed for cooked meat. In conclusion, results highlighted that TM meal represents a valuable protein source in the diet of common quails when included by up to 20% in their diet, without any detrimental effect on meat quality parameters and fatty acid profile.

## FOOD PRODUCTION AND PROCESSING

### P055

#### The effects of donkey meat cooking processes on vitamins and minerals content

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Meat is an important source of minerals and vitamins, and for this reason largely contributes to the daily intakes of these micronutrients in human diet. Meat cooking techniques showed significant effects on vitamin and trace element contents, with important losses of B-vitamins. Losses in minerals after cooking occur too, so the amounts of these nutrients really ingested with meat intake could vary greatly. The aim of the present study was to detect minerals and vitamins B-complex content in raw donkey meat and to analyze the influence of the cooking process on the level of these micronutrients. Twelve male entire crossbred donkeys born and reared in the same farm were slaughtered at 20 months of age, with an average final body weight of  $246 \pm 20 \text{ kg}$ . After slaughtering four samples of  $600 \text{ g}$  were taken from the muscle *Longissimus thoracis* (LT). Two samples of LT were used for raw meat chemical analysis, the other two LT samples were cooked in an oven at  $170^\circ \text{C}$  for 45 min. B-vitamins were quantified by HPLC, whereas macro (Ca, K, Mg, Na, P) and microelements (Cu, Mn, Fe, Zn) were determined by means of atomic absorption spectroscopy. Data were analysed by the method of least squares using the GLM procedure. Niacin content was the most abundant vitamin determined in raw meat,  $6.9 \pm 0.27 \text{ mg/100 g}$ , followed by pantothenic acid,  $1.13 \text{ mg/100 g}$ ,

vitamin B<sub>6</sub>,  $0.61 \pm 0.12 \text{ mg/100 g}$ , then riboflavin,  $0.22 \pm 0.07 \text{ mg/100 g}$ . Thiamine content was  $0.09 \pm 0.01 \text{ mg/100 g}$ , vitamin B<sub>12</sub> content was  $1.8 \pm 0.15 \mu\text{g/100 g}$ . Cooking procedure decreased B-vitamins complex content, mainly thiamine, that resulted significantly ( $p < .01$ ) reduced (trace) by thermal degradation. Niacin content showed a significant ( $p < .01$ ) decrease after cooking ( $5.22 \pm 0.16 \text{ mg/100 g}$ ); riboflavin resulted more stable to heat. Vitamin B<sub>12</sub> showed a significant ( $p < .05$ ) decrease in cooked donkey meat ( $1.10 \pm 0.04 \text{ mg/100 g}$ ). Potassium is the most abundant mineral in raw donkey meat ( $375 \pm 23.4 \text{ mg/100 g}$ ), followed by phosphorus ( $261 \pm 14.4 \text{ mg/100 g}$ ) and sodium ( $44.7 \pm 2.11 \text{ mg/100 g}$ ). Considering the microelements, raw meat iron content was  $2.87 \pm 0.28 \text{ mg/100 g}$ , whereas zinc was  $5.60 \pm 1.01 \text{ mg/100 g}$ . Cooked donkey meat did not show a significant decrease in minerals content compared to raw meat, both in macro and in microelements. Donkey meat can represent a valuable niche food in human diet; use of indigenous donkey breeds can help in preserving local animal biodiversity.

### P056

#### Use of L-ascorbic acid diesters to preserve rabbit meatballs

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Consumers appreciate rabbit meat because of its lean meat rich in polyunsaturated fatty acids (PUFA). However, PUFA are highly prone to be oxidised, thus they need to be preserved during handling and storage. Among the others, antioxidant addition to meat products is considered an effective strategy; however, consumers' demand for natural products is rising. L-ascorbic acid (Vit. C) is one of the most potent water-soluble antioxidants whose functionalisation of the hydroxyl groups at the C-5 and/or C-6 may represent a solution to obtain effective L-ascorbic acid derivatives with improved lipophilicity. On this basis, we aimed to evaluate whether L-ascorbyl-5,6-O-dialkanoates prepared from myristic (MA) and stearic (SA) acids could be employed as novel semi-synthetic antioxidants to protect rabbit meatballs from oxidation. The carcass meat (without hind legs, and *Longissimus thoracis et lumborum* muscles) were obtained from nine male rabbits (New Zealand White  $\times$  California) for a total of  $1400 \text{ g}$ . After mincing, meat was divided into three groups: no additive (control, C), L-ascorbyl-5,6-O-dialkanoates from myristic acid (MA group), and L-ascorbyl-5,6-O-dialkanoates from stearic acid (SA group). Antioxidant powder was *ad hoc* synthesized and added at  $0.1\%$  (w/w). Overall, 48 meatballs were prepared and 4 meatballs for



each group were analyzed immediately (T0) and after 20 (T20), 40 (T40), and 80 (T80) days of frozen storage ( $-10^{\circ}\text{C}$ ). Weight loss (WL, %), primary (conjugated dienes CD, mmol hydroperoxides/100g meat) and secondary (thiobarbituric acid reactive substances, TBARS; mg MDA-eq/kg meat) lipid oxidation products were quantified. Fatty acid (FA) profile was determined by gas chromatography. The PROC GLM of SAS was used to analyse the data with antioxidant and storage as fixed effects. Antioxidants showed a marginal protection against meat oxidative damages. The WL of SA was higher ( $p = .076$ ) than C and MA, being 2.59, 2.15, and 2.49%, respectively. No effect on CD emerged, while TBARS was lower ( $p = .061$ ) in MA and C group than SA (0.512, 0.556, 0.815 mg MDA-eq/kg meat). Between T0 and T20, CD increased from 0.030 to 0.079 mmol Hp/100g meat and saturated FA from 35.37% to 36.21% of total FAME, contrariwise PUFAn-6 passed from 35.54% to 33.03% total FAME. Between the two tested antioxidants, only L-ascorbyl-5,6-O-dialkanoate from myristic acid seemed to slightly preserve meat quality during storage. Further trials are necessary to identify if higher concentrations would show more specific protection.

## P057

### Effect of heat treatment on bovine milk in three genetic groups

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Milk contains several antioxidant compounds that have positive effects on health. However, lipid oxidation occurs during milk pasteurization and processing due to the effect of heat treatments. Thiols actively participate in redox regulation processes by protecting against oxidative damage and their amount could be related to oxidation status in milk. In this study, we assessed the oxidation level in bovine milk after heat treatment by determining malondialdehyde (MDA) content as index of lipid oxidation. Moreover, thiols, glutathione (GSH), and lipoic acid were determined on raw milk as antioxidant compounds. The research was carried out on milk samples from Holstein Friesians (HO), Italian Simmental (SM) and crossbreed (SMxHO) cattle, collected from 30 animals for each genetic group at 60 and 120 days of lactation. Animals were reared, in the same feed conditions, in Research Centre for Animal Production and Aquaculture (CREA-ZA) in Central Italy. The main milk characteristics such as somatic cells, lactose, urea, protein, and fat, were determined on raw milk. MDA was quantified by HPLC method on raw milk and milk aliquots subjected to heat treatments: at  $63^{\circ}\text{C}$  for 30 min (simulating a pasteurization) and at  $90^{\circ}\text{C}$  for 15 min (high

temperature treatment). The content of thiols and GSH were not statistically significantly different among the milk of the different breeds (3.19 nmol/mL,  $p = .181$ ; 0.04 mg/100 mL,  $p = .728$  respectively on the average) whilst in lipoic acid HO showed the lowest value (158.03  $\mu\text{g/L}$  vs. 274.90  $\mu\text{g/L}$  in mean for SMxHO and SM  $p < .012$ ). MDA content increased in heated milk compared to raw milk (from 1.30 to 1.49 nmol/mL on average for the two heat treatments,  $p < .001$ ) whilst no significant difference was reported between  $63^{\circ}\text{C}$  and  $90^{\circ}\text{C}$  treatment (1.43 nmol/mL and 1.51 nmol/mL,  $p = .307$ ) confirming the oxidant effect of heat treatments, without statistically significant differences among genetic groups ( $p = .992$ ,  $p = .528$ ,  $p = .831$  respectively for MDA in raw,  $63^{\circ}\text{C}$  and  $90^{\circ}\text{C}$  treated milk). However, there was a tendency to a less MDA increase (at  $63^{\circ}\text{C}$ ) in SM milk than in HO milk ( $p = .096$ ). No significant differences were found for oxidation level between the milk at 60 and 120 days of lactation, although there was a decrease in yield and an increase in protein content. No relationship was found between the main milk characteristics and oxidation parameters. There was an inverse correlation between thiols and MDA content in both raw milk ( $r = -0.87$ ) and milk heated to  $63^{\circ}\text{C}$  ( $r = -0.84$ ) and  $90^{\circ}\text{C}$  ( $r = -0.85$ ) showing the protective effect of thiols from lipid oxidation. In fact, when thiols were higher, the increase in MDA was lower. These results could explain the higher resistance to lipid oxidation due to heat treatment in milk containing higher levels of thiols.

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## P058

### Breeding techniques and post maturation systems on biomolecules content in buffalo meat

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The aim of this study was to understand if a production system could affect some biomolecules content in buffalo meat such as Glycine betaine (GlyBet),  $\gamma$ -Butyrobetaine ( $\gamma\text{BB}$ ),  $\delta$ -Valerobetaine ( $\delta\text{VB}$ ), L-Carnitine (Cnt), Acetylcarnitine (C2Cnt), Propionylcarnitine (C3Cnt), Butyrylcarnitine (n-C4Cnt). Moreover, another aim was to understand whether a prolonged post dry aging (PDA) maturation process in a defined system (Maturmeat<sup>®</sup>) could influence biomolecules level in raw buffalo

meat. We firstly evaluated if animal welfare had an influence on functional molecules level in buffalo meat and then we evaluated if the diet could modify functional molecules content in meat. For the first experiment, we measure biomolecules content in two groups of animals ( $n = 8/\text{group}$ ) reared in different space allocation (15 vs. 10  $\text{m}^2/\text{animal}$ ). The space availability (15  $\text{m}^2/\text{animal}$ ) of the group with a higher production of biomolecules was chosen and the other two groups of animals for experiment 2 were reared with that specific animal density. Two different diets were created, and the animals involved in the trial were divided into two groups ( $n = 8/\text{group}$ ) with or without the use of green forage. The meat of the animals bred with higher space availability and fed green forage was chosen to see if a PDA maturation process could influence the amount of biomolecules in buffalo meat. To evaluate differences among means, Test- $t$  or Tukey's test were performed for each significant effect ( $p < .05$ ) while data on PDA parameters were analyzed by ANOVA for repeated measures. No differences were recorded for the daily average weight gain for the fattening period in both experiments. Biomolecules content in the meat of the group reared at lower density was statistically higher for most of the molecules taken into account ( $54.5 \pm 2.5$  vs.  $66.6 \pm 1.1$  GlyBet;  $44.4 \pm 1.2$  vs.  $55.8 \pm 1.8$   $\delta\text{VB}$ ;  $26.3 \pm 2.3$  vs.  $37.2 \pm 1.7$  C3Cnt,  $p < .01$ ; and  $271.7 \pm 5.4$  vs.  $285.5 \pm 3.2$  Cnt;  $236.4 \pm 11.2$  vs.  $294.1 \pm 13.7$  C2Cnt;  $p < .05$  respectively for 10  $\text{m}^2$  and 15  $\text{m}^2$  animals) compared to the higher density group. Similarly, the group fed the inclusion of green forage showed higher content of biomolecules ( $68.9 \pm 0.6$  vs.  $79.7 \pm 1.5$  GlyBet;  $56.8 \pm 1.8$  vs.  $71.1 \pm 1.4$   $\delta\text{VB}$ ;  $162.6 \pm 4.5$  vs.  $174.6 \pm 1.6$  n-C4Cnt  $p < .01$ ; and  $293.1 \pm 3.4$  vs.  $317.4 \pm 5.1$  Cnt,  $295.2 \pm 4.0$  vs.  $330.2 \pm 5.7$  C2Cnt;  $p < .05$  respectively for without and with green forage) compared to the group fed without forage. Most of the biomolecules, except for the  $\gamma$ -butyrobetaine, increased during maturation time in the Maturmeat<sup>®</sup>, up to 60 days ( $p < .01$ ). In conclusion, the present study showed how breeding techniques and a PDA maturation system could enhance meat production in terms of functional quality.

## P059

### Comparison of two lab scale techniques for ensiling whole plant corn

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In the Po Valley, dairy cow nutrition is largely based on ensiled forages, in particular maize-silage. Although ensiling can reduce the preservation losses and furnish succulent forages of high quality though the year at a relatively low cost, the silage preservation quality can affect animal production and health. Various additives have been proposed and some are commonly used to

improve or guarantee the fermentative processes, but their effects need to be verified under controlled conditions. To this purpose, laboratory-scale silos allow higher-throughput approach and are cheaper compared to farm-scale studies. Different laboratory systems have been developed but they are rarely compared regarding the results obtained. Vacuum-bags are less labour intensive and time-consuming compared to the wider used glass jars system, and they are increasingly used. We aimed to compare the vacuum-bags technique compared to the wider used glass jars system on maize ensiling. Whole plant maize forage at the two-thirds milkline stage, treated (TRT) or not (CTR) with a LAB additive was ensiled, in triplicate, either in vacuum bags either in 0.75 l glass jars at a density of 0.75 (fresh basis). Mini silos were opened 3, 5, 7, 15, 30 and 60 days after ensiling and controlled for pH, lactic acid, volatile fatty acids, and ammonia. At the 60 day- end point, most of the main descriptive parameters of the silage quality appeared affected by the kind of experimental silo, as pH was higher (3.70 vs. 3.65), total lactic acid (3.12% vs. 3.29% ss) and D/L lactate ratio (1.02 vs. 1.20) was lower in vacuum-bags ( $p < .05$ ), although the differences were quantitatively limited. LAB did not affect ( $p > .05$ ) final pH and lactate concentration, but lowered D/L lactate ratio (1.01 vs. 1.21,  $p < .05$ ).  $\text{NH}_3\text{-N}$  was not affected by treatment or kind of silo. Considering the whole preservation period times, a significant ( $p < .05$ ) interaction kind of silo by treatment by time was evident for all the parameters mentioned above, suggesting that patterns of fermentation were different within glass jars and vacuum bags. These highlight the opportunity to standardize the lab scale ensiling technique to achieve more comparable results between research laboratories.

## OTHER

### P060

#### The influence of access time to pasture grazing on dairy sheep performance in an organic feeding system

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We compared two feeding systems for organic dairy sheep farming based on different access time to pasture grazing, with the aim to evaluate the implications in terms of ewe performances. Both systems had the same pasture type based on grass-legume mixtures including *Lolium multiflorum* Lam. with *Sulla coronaria* (L.) Medik. or *Medicago polymorpha* (L.) or *Trifolium resupinatum* (L.): P4 (4 grazing hours/day, 8:00–12:00.) and P6 (6 grazing hours/day, 8:00–14:00.). The pasture was rotationally

grazed from 12 March to 5 June 2019, with a grazing period (GPn) of 15 days (from GP1 to GP7) by two groups of 24 Sarda ewes, homogeneous for milk yield ( $1426 \pm 213$  g milk ewe<sup>-1</sup> day<sup>-1</sup>) body weight (BW,  $49.9 \pm 4.5$  kg) and BCS ( $2.30 \pm 0.2$ ). After grazing, the ewes were stall-fed with ryegrass-based hay (0.6 in P6 and 0.8 kg head<sup>-1</sup> day<sup>-1</sup> in P4), corn grain and faba beans organic supplementation (0.3 in P6 and 0.6 kg head<sup>-1</sup> day<sup>-1</sup> in P4). Herbage mass, ewe BW, BCS were measured at the beginning of each GP. Individual milk production and quality were recorded fortnightly. Supplementation intake was measured daily. Data were analysed by a mixed model using system, GP and their interaction as fixed effects and ewe within GP as random effect. Herbage mass availability was 1.9 (P4) and 2.8 t DM ha<sup>-1</sup> (P6) and it increased throughout the experiment (GP effect  $p < .05$ ). BCS and BW were not affected by the system whereas they increased during the observation period. BCS was higher in P6 than P4 in GP3, BW was higher in P6 than P4 in GP1. No differences were observed between groups for milk yield,  $1383 \pm 53$  (P6) and  $1275 \pm 53$  g day<sup>-1</sup> (P4), which decreased during the period reaching the lowest value in GP7. The milk production pattern was different between the two groups: P4 had a sharp decrease from GP6 to GP7 ( $p < .05$ ) whereas P6 showed a linear decrease ( $p < .05$ ). Milk fat ( $5.89\% \pm 0.09$  vs.  $5.64\% \pm 0.09$ ;  $p = .05$ ) and protein ( $5.55\% \pm 0.07$  vs.  $5.32\% \pm 0.07$ ;  $p < .05$ ) content were higher in P4 than in P6, respectively, and increased during the GP ( $p < .05$ ). Lactose was not significantly different between groups, but it was influenced by the GP. Increasing the access time to pasture allowed P6 to cover 79% of total energetic requirement with grazing pasture compared to P4, where pasture covered only 51%. The results showed the positive effect of a higher access time to pasture in the self-sufficiency of the organic feeding system, but a slightly negative effect on milk quality.

## P061

### Effect of salty or sweet food leftover based diets on serum metabolites in piglets

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Nowadays, agriculture and livestock production face several challenges for what concerns sustainability. In particular, animal feed production competes with human food production for the use of lands and natural resources. The use of alternative feed ingredients could thus increase the sustainability of livestock production. In this study, conventional cereal grains have been partially

replaced by confectionery or bakery former food products (FFPs) in pigs' diets to evaluate their effect on pig serum metabolites. FFPs are safe and healthy ingredients produced for human consumption, but no longer intended for this purpose. Specifically, 36 post-weaning female piglets were fed with three different diets: (1) control diet (CTR), 0% FFPs; (2) FFP-C diet, (30% cereals replaced by 30% confectionery FFPs); (3) FFP-B diet, (30% cereals replaced by 30% bakery FFPs). The diets were formulated to meet the NRC requirements and to be iso-energetic (15.3 MJ/kg DM) and iso-nitrogenous (19% CP DM). During the 42 days of trial, blood samples were collected on days 0 (t0), 21 (t1) and 42 (t2). The serum metabolites, measured using a standard enzymatic colorimetric analysis, were: total proteins, albumin, globulin, albumin/globulin (A/G), urea, alanine aminotransferase (ALT-GPT), aspartate aminotransferase (AST-GOT), alkaline phosphatase (ALP), total bilirubin, glucose, triglycerides (TG), non-esterified fatty acids (NEFA),  $\alpha$ -amylase, total cholesterol, calcium, phosphorus, magnesium. Data were analyzed using one-way analysis of variance (one-way ANOVA) and further explored by principal component analysis (PCA). The results showed that the levels of serum metabolites were similar across the three experimental groups in the three different time points. The only exceptions were observed for  $\alpha$ -amylase and TG. In particular,  $\alpha$ -amylase tended to be lower in both experimental groups (FFP-C and FFP-B) in comparison with CRT group throughout the experiment, whereas TG were lower in the FFP-C experimental group only at t1. In conclusion, the inclusion of FFPs (up to a level of 30%) in post-weaning piglets' diet does not lead to a significant perturbation of the level of several serum metabolites, thereby enhancing the hypothesis of their reuse in animal nutrition. However, further studies are needed to strengthen these results and increase the knowledge.

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## P062

### Farming wild-caught glass eels for habitat restocking in Sardinia

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Conservation programs of some endangered species involve the artificial restocking of habitats that sometimes rely on wild-captured populations. As in the case of the European eel *Anguilla anguilla* (L.), which cannot be artificially reproduced, stocking



material is always caught in nature. The aim of the work was to evaluate the effectiveness of restocking using wild-caught farmed glass eels. We explored the growth and survival rate of glass eels raised in captivity and their adaptation when released into the wild, and the effectiveness of restocking. The captivity phase of the experiment was carried out from February to November 2018. A total of 1450 g (7398 specimens estimated, with average length  $6.34 \pm 0.66$  cm and weight  $0.20 \pm 0.24$  g) of glass eels were captured using a fike-nets during February 2018 in Pramaera river (Sardinia, Italy). Glass eels were immediately transported in an intensive eel farm and fed with a diet based on mullet eggs and fish feed. Every 2 weeks, a sample of 50 specimens was analyzed. Total length (TL) and total weight (TW) were measured, and their sanitary status was evaluated. Environmental parameters were recorded every sampling day using a multiparameter probe: temperature, salinity, pH, dissolved oxygen. Environmental conditions in breeding tanks were stabilized by controlled systems. Initially, glass eels showed mean TL of  $5.97 \pm 0.41$  cm and mean TW of  $0.18 \pm 0.04$  g. After nine months of growth the specimens were 2073, for a total survival of 28.02%. and the mean TL was  $17.29 \pm 4.24$  cm, and mean TW was  $8.06 \pm 7.75$  g, showing increases of 190% and 4378 %, respectively. The total biomass of eels was 22.3 kg (increase in 1438%). A biomass of 10.8 kg of eels (1367 specimens) was returned to the original capture site, and released after brief acclimatization. A biomass of 11.5 kg (706 specimens) was released into the wild in the Ulassai stream (Sardinia, Italy), where 136 eels have been marked with PIT tags. Monitoring activities are currently underway to verify the growth of specimens in nature and some of them have now been found, suggesting that they have been readjusted to wildlife. In conclusion, we suggest as a management practice, as well as for breeding for production purposes, for eels population the restocking with farmed elvers or juvenile yellow eels starting from glass eels. This action may represent a solution to ensure a greater percentage of success, rather than the direct release of glass eels in natural environment.

## P063

### Awassi sheep and Baladi goat milk composition in extensive production systems in Lebanon

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Small ruminant production represents an integral part of the Lebanese economy, where extensive systems for sheep and goats are the most widespread type of production. In these systems, Awassi sheep and Baladi goat represent the majority of small

ruminants (100% of sheep and 95% of goats). The aim of this work is to study the socio-economic status of extensive systems of sheep and goats in Lebanon and to analyze the milk samples collected from Awassi sheep and Baladi goats. A structured survey was assessed and 43 shepherds from all over Lebanon were interviewed, to have an overview on the flock structure, milk and dairy production, the different types of feed used and the health problems and constraints facing the extensive production systems. Sheep and goats milk samples were collected from 10 traditional farms (semi nomadic, transhumant and sedentary systems ) from different regions and at different altitudes, at the final stage of lactation in August. Results showed a dominance of farms with both species represented by Awassi sheep and Baladi goats. The yearly management of the flock in these farms were similar for both species and animals were dry during August-September and January-February. Grazing is practiced all over the year and is supplemented during winter by hay, concentrate and green leaves (100%, 41% and 27% of the farms respectively). The average milk yield for Awassi is  $1.44 \pm 0.45$  L/head/day, while for Baladi goats is  $1.66 \pm 0.61$  L/head/day. Milk is sold directly to the market, industry or cooperatives (55%, 28% and 17% respectively) to produce yoghurt, cheese and labneh. The sanitary and health services are supported by a veterinary only in 32% of the farms. The most small ruminant diseases encountered were: Pasteurella, Enterotoxemia, and foot and mouth diseases. The analysis of the milk samples has shown that the average fat content in Awassi sheep milk is  $9.17 \pm 1.65\%$  while the average protein content is  $6.76 \pm 0.81\%$ . For Baladi goats, the average fat is  $4.08 \pm 1.43\%$  while the protein content is  $3.52 \pm 0.62\%$ . The high values of fat and protein may be due to the sampling carried out in August at the advanced stage of lactation. In conclusion, in Lebanon, efforts should focus on ameliorating the productivity of local races and feed quality thus increasing the quality of the dairy products.

## P064

### Effect of queen cell size on acceptance rate and morphometric characteristics of queen honey bees (*Apis mellifera*)

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Rearing techniques are important to determine a successful honey bee production. For example, queen cell cup size may affect the acceptance rate of grafted larvae and queen's size, which in



turn may have an effect on the quality of the colonies (e.g. larger queens give rise to larger colonies). The present study was carried out to compare the effect of different queen cell cup sizes (0.8 vs. 0.9 mm diameter) on the acceptance rate of grafted larvae and on morphometric characteristics of queen honey bees. Sixty-five larvae were allocated to each treatment. Grafting took place once a week in June and once at the end of August. As soon as they emerged from the pupal case, virgin queen bees were collected and immediately frozen at  $-20^{\circ}\text{C}$  in order to prevent weight losses and dehydration. Head, thorax and abdomen width (mm) were measured using an electronic caliper; the weight of each of the three segments was recorded using a precision scale. One-hundred and ten grafted larvae out of 130 were accepted. The acceptance rate was higher in larger cell cups, but differences were not statistically significant (larger cells: 89.2%; smaller cells: 80.0%;  $\chi^2 = 2.1273$ ;  $p = .1474$ ), confirming the results of previous research on the effect of cell cup size on acceptance rate. All morphometric traits measured on the accepted larvae (large cups:  $n = 58$ ; small cups:  $n = 52$ ) were significantly higher in queen bees raised in larger cell cups ( $p < .001$ ; GLM with cell cup size and grafting period as fixed factors), except for head width. Principal Component Analysis on morphometric traits confirmed a trend of queens raised in larger cells to show higher values on PC1 (58.4% of explained variance), characterized by particularly high loadings of variables related to the weight of the three segments. In conclusion, increasing cell cup size seems to have a positive effect on queens' quality, especially in terms of a higher weight, which is expected to positively affect the dimension of the colonies, with no detrimental effect on the acceptance rate of grafted larvae.

## P065

### Animal Bio Arkivi: establishment of a phenotype and tissue repository for farm animal and pet at the University of Milan

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Thanks to the new omic technologies, research on veterinary, and animal sciences will take advantage of large repositories of biological materials.

At the University of Milan, Dipartimento di Medicina Veterinaria, a biorepository (Animal Bio Arkivi) has been established. Animal Bio Arkivi consolidates, organizes, and promotes the collection, cataloging, and conservation of biological samples of different veterinary interest species. Samples and data are all available for research on biomedical aspects, risk assessment, sustainable breeding and conservation, leading to improvements for animal and human health.

The collection is registered in a database organized by species, but with uniform criteria for recording and storage. Each animal record includes: signaling (studbook ID, microchip), pedigree data, phenotype data (clinical information/ productive features/ other traits under selection), and genotype data (coding loci, microsatellite markers, SNVs profiles) when available. Samples are conferred with owners' permission and non-disclosure agreement. Procedures of periodic backups are set up.

The collection preserves samples from different animal tissues, mainly blood, but also frozen muscles and other organs, semen, hair and feathers. Specimens are stored (at  $-20^{\circ}\text{C}$ ,  $-80^{\circ}\text{C}$  or liquid nitrogen) to ensure proper organization and quality of the conservation. The repository also hosts DNA extracted from relevant samples. The first collected specimens date back 35 years. Overall the repository includes specimens of approximately 45,000 animals of several species: 34,000 Equidae, 5000 Bovidae, 5000 Felidae, and 1000 Phasianidae. Cosmopolitan breeds from historic routine typing service are mainly represented. Many local and rare populations or cohorts/families segregating relevant traits and samples from wild species are also included. The repository benefits from close long-lasting strategic partnerships of the University scientists with breeding associations, service laboratories, Academic institutions, and scientific initiatives of practitioners, such as the Osservatorio Veterinario Italiano Cardiopatie, which provide valuable additional information on phenotypes and genotypes, bringing together the public and scientific communities.

Animal Bio Arkivi benefits from the newly established location in Lodi and its potential additional synergy with Veterinary Clinical and Husbandry Center of the University of Milan.

## P066

### Non-invasive acoustic detection of wolf's attack to livestock. Preliminary results of a prototype in two sheep farms

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The ongoing expansion of wolf population in Italy raises conflicts with farmers due to livestock predations, which are added predations from free-ranging dogs and wolf-dog hybrids. Livestock predations represent one of the main issues for wolf conservation in Italy, since most of the known illegal killings occur in areas with high farm densities and high levels of damages and, at the same time, they induce considerable economic losses to farmers. A series of prevention measures could be applied, such as guardian dogs, electric fences, and animal enclosure; compensation

strategies are put in place to alleviate economic losses. However, each solution has disadvantages, either in the lack of functionality or in the high cost of application. Aim of this study was to implement a prototype based on non-invasive acoustic detection of wolf or other canids' attack to livestock. The hardware consists of three main components: (i) a signal and a peak level detection/amplification circuit; (ii) an Arduino UNO© microcontroller for managing the firmware and the entire system and, (iii) an Arduino GSM Shield 2 for alarm transmission to farmer's mobile telephone via GSM. In this case, sounds are picked up by a directional microphone (Sennheiser brand mod. ME67 powered by K6 modules), that are acquired by Arduino UNO© system and transformed into MIDI signals (on/off) by a sound switch. Sounds above a certain volume, attributable to an increase in environmental noise caused by the attack on animals, are recorded as alarm signals and a warning message is sent to the farmer. The first results of tests conducted at two sheep farms showed that the system is working and regularly sends signals via text message when the preset noise threshold is exceeded. However, further implementations are needed to reduce false positives: selection of specific bands with which to filter sounds not connected to the farm environment (e.g. wind, rain, etc.), apply video recording systems, so that the farmer can verify the truth of the attack signals and implement defensive measures; install a power supply system that does not require connection to the electricity network (for example a photovoltaic panel). Further implementations of hardware and software systems will be developed through on farm tests.

## P067

### Circular Economy: Live Larvae recycling Organic Waste for rural Poultry (CELLOW-FeeP)

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The CELLOW-FeeP project, funded by the Cariplo Foundation (Circular Economy call), is coordinated by the Department of Veterinary Medicine of the University of Milan. Researchers from the Department of Agricultural, Forest and Food Sciences and the

Department of Veterinary Sciences of the University of Turin are also involved.

Various experimental protocols are planned within the project, aiming at identifying the optimal inclusion levels of live black soldier fly (*Hermetia illucens* L.) larvae into diets destined for slow-growing chickens, based on the growth and slaughtering performances, health, welfare and meat quality of the birds.

FAO has identified insects as an innovative, alternative and sustainable source of nutrients for animal feed. From a Circular Economy perspective, the insect rearing residues (insect frass) may find use as an organic fertilizer. Preliminary data have also indicated that an energetic valorisation (aerobic and anaerobic processes) is feasible but all these aspects need to be deeper investigated is needed on this topic.

Insects are biotransformers of organic waste (which most of time represents a production cost for the agro-industry) into high quality nutrients to be reused. Live insect larvae are suitable to be directly administered as feed ingredient to poultry and are also suggested to play a positive general effect on bird's health and welfare. Black soldier fly is the best insect candidate to be used for animal feed purposes.

Live larvae fed to poultry provide good nutrients (proteins, amino acids, lipids, minerals, vitamins) and bioactive compounds with positive effects on health, also enabling birds to express their normal behavior (i.e. foraging activity), with expected positive impacts on animal welfare and product quality. Nevertheless, related data are still missing and further research is needed.

Following a multidisciplinary and sustainable approach, the CELLOW-FeeP project intends to fill the above-mentioned gap of knowledge directly involving the potential stakeholders and trying to align both the project processes and outcomes with the values, needs and expectations of the European society.

## P068

### Effect of dietary supplementation of olive oil wastewater on gut health and growth performance

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The study was carried out in a commercial pig farm, in which suckling piglets destined for the production of typical regional dishes are raised. The economic losses in the weaning transition period are well known. The main problems are premature removal from the mother, feeding changes when the gastrointestinal

tracts are not completely formed and other social and environmental shifts. This situation leads to an alteration of piglets' welfare, which induces temporary anorexia, unbalanced gut microbiota and diarrhea. To optimize growth performances and to prevent weaning problems, antibiotics and minerals are often used, e.g. zinc oxide (ZnO), even if the latter represents a risk for the environment. Thus, alternative solutions, such as the use of by-products, are needed to avoid environmental damages. Bioactive compounds of olive oil wastewater (OOW) have antimicrobial, anti-inflammatory and antioxidative effects. The aim of this study was to investigate the effect of OOW on the growth performance and gut health of piglets. A total of 27 piglets (Large White  $\times$  Landrace) was selected one week before the weaning and fed a pre-starter diet without ZnO. After the weaning ( $28 \pm 1$  days of age), piglets were randomly assigned to three dietary treatments balanced for litters and sex: a control group (CON, control diet + ZnO), L-dose (control diet + 7.44 g/kg of OOW) and H-dose (control diet + 14.88 g/kg of OOW). Molasses was added on L-dose and H-dose to avoid problems in palatability. Body weight (BW) and weight gain (WG) were recorded every day and analyzed using the following model  $BW(WG) = \text{diet} + \text{time} + \text{diet} \times \text{time} + e$ . Diet was not significant, and time was significant ( $p < .0001$ ) for both BW and WG; interaction diet  $\times$  time was significant only for BW ( $p < .05$ ). However, WG on the fifth day was greater (even if not significant) in CON group (531 g) compared to H-dose (215 g) and L-dose (156 g). The non-significant effect of the diet could be due to the large variability and the small number of animals. Animals receiving OOW were not significantly lighter than those receiving ZnO and, therefore, our results support the hypothesis of beneficial effects of the bioactive compounds on the negative aspects of weaning transition in piglets.

## P069

### ATP12A expression in ruminant spermatozoa

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The  $H^+/K^+ - ATPase$  Type 2, also known as ATP12A, is an heteromeric integral membrane protein, member of the P-type ATPases, also including  $Na^+/K^+ - ATPase$  and  $H^+/K^+ - ATPase$  type 1. ATP12A is responsible for  $H^+$  efflux in exchange with  $K^+$ . The functional ATP12A consists of two subunits: a specific  $\alpha$  subunit, known as  $\alpha 2$  and encoded by the gene ATP12A, and a nonspecific glycosylated  $\beta$  subunit. The aim of the present study was to demonstrate the presence and characterize the protein encoded by the ATP12A gene in sperm cells from *Bubalus bubalis*, *Bos taurus* and *Ovis aries*, as a preliminary step before its functional

analysis. Immunoblotting revealed in all the three species a major band with a molecular mass of about 100 kDa, corresponding to the expected  $H^+/K^+ - ATPase$  type 2  $\alpha 2$  subunit molecular mass. The immunolocalization pattern observed using an antibody against the ATP12A  $\alpha 2$  subunit, showed, consistently in all the considered species, a strong fluorescence signal at the acrosome region of the sperm head and a weaker signal at the middle piece of the flagellum. These results, described here for the first time in sperm cells, are consistent with the immunolocalization pattern, observed here, as well as in previous work, for the  $\beta 1$  subunit of  $Na^+/K^+ - ATPase$ , thus suggesting that this subunit may assemble with the  $\alpha 2$  subunit to originate a functional ATP12A protein also in sperm cells. A markedly differentiated localization pattern was observed in this study for the  $\alpha 1$  and  $\alpha 4$  subunits of  $Na^+/K^+ - ATPase$ , with the fluorescence signal being observed only at the middle piece of the flagellum, indirectly supporting the specificity of the antibody against the ATP12A  $\alpha 2$  subunit, also highlighted through *in silico* analysis. When studying the expression of the ATP12A  $\alpha 2$  subunit during different stages of sperm maturation, a progressively increasing fluorescence intensity was observed moving from the testis lobules to the deferent duct. Considering both its cellular localization, and the intensity of the fluorescence signal in mature spermatozoa within the deferent duct, we hypothesize that ATP12A may contribute to the acidification of the luminal environment in the epididymis, thus helping to keep spermatozoa in a dormant state, while it may play a role in the acrosome reaction once approaching the ovum in the female reproductive tract. Further studies will be needed to address the functional role of this target protein in sperm physiology.

## P070

### Farm silage facilities and their management for spore former prevention

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Corn silage represents a big share of the dry matter intake for lactating dairy cows in the left Po Valley area, in the milk production for long-ripened cheese manufacturing. Silage quality can be affected, during storage or at feed-out, by the aerobic deterioration which allows the growth of anaerobic spore-forming bacteria (ANSB), causing an increase in their spore numbers. Therefore, corn silage can become the main source of ANSB that, through the contamination of the total mixed ration (TMR), drives their spread in the farm environment, also affecting the



milk contamination and increasing the risk of late blowing defect in cheese. A survey on silage facilities and their management practices was carried out during the summer months in 108 farms of the left Po Valley, to study the relationships with ANSB spore content of silages and TMR samples. Descriptive statistics, simple correlations, and linear regressions were calculated among structural, management, and microbiological items at silo as well as at farm level. The high variability in ANSB in the different silages as well as in the other feeds, within the adopted sampling procedure, does not explain the ANSB content in TMR, confirming that the microbiology of this mixture is strongly affected by critical contamination from spoiled peripheral areas of the silos. The distribution of ANSB values has a wider range for bunkers than for bags stored silages, suggesting a higher variability in the factors affecting their microbiological quality. Bunker silos older than 16 years were more prone than recently built silos toward higher ANSB spore values. The unloading equipment affected the ANSB count; the front-end loader with cutter was associated with a lower ANSB, probably as a result of a reduced and compact surface left after the daily silage removal. Silo length and silo surface area daily removed were the main factors affecting spore contamination of silage during summer months: longer silo and wider surface removal were related to a reduced ANSB contamination, probably as a consequence of a reduced aerobic deterioration at silage surface. It has been found that the features of silage facilities and their management are an important first step to prevent the ANSB contamination chain at the farm level, namely by the reduction of silage face exposition to the aerobic spoilage effects.

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## P071

### Flow cytometry study of blood and milk parameters in dairy ewes supplemented by cocoa husks

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Cocoa husks (CH), the principal by-product derived from *Theobroma cacao* (L.), could be used as a supplemental feed for animals. However, this use is constrained by the presence in the cocoa by-products of a natural alkaloid, theobromine which has been found to be toxic to animals when ingested in large amounts. The aim of this trial was to study the effects of CH on some

immune indicators through flow cytometry on blood and milk of ewes. Twenty-four Sarda dairy ewes were randomly allotted to three experimental groups fed basic diet (CON) and a supplement of 50 g (low dose group; LD), and 100 g (high dose group; HD) of CH/d per head for 5 weeks. Individual blood and milk samples were collected at the beginning, in the middle and at the end of the experimental period. Whole-blood samples were processed to purify peripheral blood mononuclear cells (PBMCs) by gradient concentration, using the density separation medium Histopaque-1077. PBMCs pellet was suspended in PBS for flow cytometry analysis. Individual post-dipping milk samples were collected weekly at morning milking by cleaning and sterility practices. Milk samples were prepared to flow cytometry by centrifugation and defatting. The cell pellet was re-suspended in PBS + EDTA buffer and stored at  $-80^{\circ}\text{C}$  until flow cytometry analysis. A mix of antibodies was prepared to label PBMCs samples to determine Lymphocytes T-helper (CD4), T-Cytotoxic (CD8), T-activator (CD25), Macrophages (CD14), Natural Killer (CD56/16) and milk cell samples to determine Total T-Lymphocytes (CD3), Lymphocytes T-activator (CD25), and viability of cells/apoptosis by Hoechst 33258 (H58)/Caspase-3. CD4+ percentage tended to increase in LD than HD ewes ( $p = .06$ ). No significant differences were found for other blood parameters. Diet affected milk parameters, for Caspase3+ percentage that was higher in HD group ( $p < .05$ ) and H58+/Caspase-3+ percentage that was higher in LD ewes ( $p < .01$ ). It seems that high dose of cocoa increased cell apoptosis, but low dose increased the early apoptosis of cells, so thanks, probably, to its antioxidant effect, to delay apoptosis. In conclusion, results suggest that the addition of CH as a supplement to the diet of sheep could exert positive effect at low dose, whereas high dose seems to affect negatively immune response.

## ANIMAL EFFICIENCY

## P072

### Long-term administration of calcium laurate to sows and piglets: effects on growth performance, fecal calprotectin and intestinal status of weaning piglets

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Newly weaned piglets are commonly exposed to nutrition, environmental and physiological stress that can negatively affect growth and health. Maternal nutrition may represent a valuable



strategy to benefit postnatal offspring development and decrease the susceptibility to diseases in adulthood. The aim of the study was to evaluate the effects of dietary lauric acid saponified with calcium (C12-Ca) given during late gestation to sows and piglets on offspring growth and intestinal health. At 3 weeks before parturition, 16 sows were randomly assigned to two dietary treatment: basal diet (CTR) or basal diet with 1 kg/ton C12-Ca (T). At weaning, 96 piglets were chosen from the litters and distributed in 4 post-weaning dietary treatments belonging to the same descent group: CTR-CTR and T-CTR fed basal diet, CTR-T and T-T fed basal diet plus C12-Ca (1 kg/ton). Body weight (BW), average daily gain (ADG), and average daily feed intake (ADFI) were measured and feed efficiency (FE) was calculated. Fecal samples were taken at 14 and 26 d to evaluate calprotectin (Calp) concentration as marker of gut inflammation. Piglets were slaughtered at 26 d and mucosa intestinal samples were collected and used to investigate the impact of C12-Ca on the total antioxidant capacity (TAOC) and secretory IgA concentration (SigA). Growth performances were analyzed applying a MIXED procedure for repeated measurements and accounting for the effects of treatment, time and their interaction. One-way ANOVA was used to analyze Calp, TAOC and SigA. The differences between means were compared using Tukey's test and considered statistically significant at  $p < .05$ . BW and FE were not affected by C12-Ca while ADG and ADFI resulted higher in CTR-T piglets ( $p < .05$ ), although there was no significant effect of treatment x time interaction. Calp concentration was lower in T-CTR and T-T compared with the other groups ( $p < .05$ ) after 14 d and no differences were observed at 26 d. SigA concentration in duodenum was lower in T-CTR and T-T compared to CTR-CTR and CTR-T group ( $p < .05$ ) whereas no differences were observed for SigA in ileum and TAOC in both duodenal and ileum mucosa. Although the administration of C12-Ca soaps in the diet of the mothers did not show clear effects on the growth of the offspring, our findings suggest that the administration of C12-Ca soaps modulates intestinal inflammatory status during the first two weeks after weaning, improving gut health.

## P073

### Interaction between stearoyl-coenzyme A desaturase polymorphism and feeding system in affecting the fatty acid profile of sheep cheese

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Stearoyl-coenzyme A desaturase (SCD) catalyses the synthesis of monounsaturated fatty acids, by introducing a cis double bond at the delta 9–10 position. The study investigated the effects of a polymorphism at the ovine SCD gene and its interaction with the inclusion of fresh forage in the diet on cheese fatty acid (FA) composition in Valle del Belice ewes. A region of 527 bp of the ovine SCD gene including the partial sequence of the promoter, the complete sequence of the 5' UTR and exon 1 and the partial sequence of intron 1 were analyzed in order to identify the g.87C > A SNP located in the promoter region. A feeding trial was carried out using 16 Valle del Belice ewes selected on the basis of their genotype (6 CC and 10 AC). Each genetic group was fed, according to a latin square experimental design, with diets based on conserved or fresh forage, consisted of sulla hay (SH) or sulla green forage (SGF), integrated with 1 kg/d of concentrate feed. Cheese made from individual milk was analyzed for FA composition. The SCD genotype had no effect on cheese monounsaturated and polyunsaturated FA, whereas significantly influenced the desaturation activity, as demonstrated by the higher desaturation indices in the CC ewes compared to AC ewes (C14 index: 0.021 vs. 0.017; C18 index: 0.71 vs. 0.68; trans-vaccenic index: 0.38 vs. 0.32;  $p < .05$ ). This result seems consistent with the possible role of this polymorphism on the enzyme desaturase synthesis and its activity in the mammary gland. However, this does not appear to be supported by the levels of monounsaturated FA or conjugated linoleic acid (CLA, C18:2c9t11) which were not affected by the g.87C > A polymorphism. The SGF diet significantly increased trans vaccenic, linoleic and CLA acids of cheese, compared to the SH diet (respectively: 2.30 vs. 1.31; 2.53 vs. 2.25; 1.00 vs. 0.80;  $p < .05$ ). No significant interaction resulted, thus the effect of SCD genotype on desaturation indices was evident regardless of the diet. The results obtained, while showing an effect of SCD polymorphism on the level of desaturation, do not allow to highlight a role of the more favourable CC genotype in further improving the acidic composition of the cheese in health terms due to the presence of green forage in the diet. However, the genetic polymorphism of sheep SCD and its interaction with diet should be further investigated, given the lack of information on this species.

## P074

### Comparison of growth performance and whole body composition of two strains of Rainbow trout (*Oncorhynchus mykiss*) juveniles fed different diets

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The aim of this study was to evaluate the growth performance and the whole body composition (WBC) of two strains (Italian and American) of rainbow trout (*Oncorhynchus mykiss*) juveniles fed Italian and American commercial diets. Two trials were conducted contemporarily.

All fish were individually weighed at the beginning of the trials. In the first trial, 125 American rainbow trout (USA trout; Initial Body Weight – IBW: 10.64 g), and 125 Italian rainbow trout (ITA trout; IBW: 10.63 g) were allocated into 10 tanks (5 tanks/strain) and fed an Italian commercial diet (ITA diet) containing both plant (PP) and processed animal proteins (PAP). In the second trial, 100 ITA trout (IBW: 13.47 g) and 100 USA trout (IBW: 13.46 g) were divided into 10 tanks (5 tanks/strain) and fed a commercial USA diet containing only PP sources. The fish were fed by hand, twice a day, six days per week.

After 105 days, the fish were anesthetized, individually weighed (Final Body Weight [FBW]), and the following performance parameters were calculated: Survival Rate, Specific Growth Rate, Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER). Five fish per tank were sacrificed, grounded, freeze-dried and analysed for dry matter (DM), crude protein (CP), ether extract (EE), and ash contents. Data were statistically analysed by one-way ANOVA, using IBM SPSS Statistics v. 25.0 ( $p \leq 0.05$ ). In the first trial, USA trout reached higher FBW (64.64 g) and showed improved FCR (0.93) and PER (2.16) when compared to ITA trout (FBW: 57.62g; FCR: 0.99; PER: 2.04;  $p \leq 0.05$ ). In the second trial, no differences were observed between strains in terms of growth performance. The WBC of the strains did not vary when the fish were fed the ITA diet, while ITA trout showed the lowest CP content and higher DM and EE content than USA trout when fed the USA diet.

In conclusion, the USA strain showed good efficiency when using PAP and PP sources. On the other hand, the USA diet did not lead to differences in growth performance between the two strains, while led to significant differences in the content of DM, CP and EE of the whole fish.

## P075

### Growth and fillet composition of gilthead sea bream (*S. aurata*) after long term feeding fish meal-free diets including poultry by-product meal singly or combined with insect meal

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In formulating sustainable aquafeeds that conform to the principles of the circular economy, processed animal proteins such as poultry by product meal (PBM) and insect meals, deserve increasing attention to complement vegetable protein-rich ingredients as alternatives to fish meal. On this basis, the aim of the present study was to evaluate growth performance, dressing out yield and fillet composition of gilthead sea bream (*S. aurata*) fed diets deprived of fish meal, where vegetable protein-rich ingredients were replaced by PBM, singly or combined with *Hermetia illucens* larvae meal (HM). Four isoproteic and isolipidic dietary treatments denoted as FISH, VEG, PBM, and PBM + HM were fed in triplicate over 25 weeks to groups of sea breams each consisting of 20 fish (initial weight  $106 \pm 10$  g) kept in 500-L squared tanks in a marine recirculating aquaculture system (T,  $23.6 \pm 1$  °C, Salinity  $30 \pm 2$  psu). The treatment FISH consisted of 90:10 and 70:30 fish to veg. protein and lipid ratios respectively. An opposite ratio between protein sources was applied in treatment VEG with a 50:50 fish to non-fish lipid sources. Dietary treatments PBM and PBM + HM were obtained by replacing 40% protein from veg. sources of the VEG treatment with protein from PBM alone or PBM (30) plus HM (10), respectively. At the end of the trial, the growth performance was recorded per group. Nine fish per dietary treatment were analysed for dressing out yield and fillet composition. After 25 weeks on trial fish nearly quadruplicate the initial weight ( $400 \pm 15$  g). Fish subjected to treatment PBM + HM resulted in the best growth performance and feed conversion ratio which did not differ from the outcome of treatment PBM ( $p > .05$ ) but were significantly improved when compared to those attained with treatments FISH and VEG ( $p < .05$ ). Carcass and fillet yields as well as fillet proximate composition were not affected by the diet ( $p > .05$ ). Similarly, dietary treatments did not alter the MUFA, overall PUFA as well as (LC)n-3 PUFA profiles of the fillet lipid fraction, whereas treatment FISH as expected resulted in significantly diminished n-6PUFA and higher n-3/n-6 ratio when compared to the other treatments which did not differ

from each other. Besides improved farming performance, treatments PBM and PBM + HM also resulted in improved indicators of sustainability (FIFO; ECR) which make PAPs from rendered poultry by product and insect larvae meals very attractive ingredients for a next generation of aquafeeds.

## P076

### Utilization of solvent extracted cardoon meal in the diet of lactating Sarda dairy ewes

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The aim of the study was to compare the effects of two different doses of solvent extracted cardoon meal (21.9% CP, 72.3% NDF, fat 1.02%, DM basis) included in the diet of Sarda ewes during their mid-lactation.

After 21 d of adaptation, 36 ewes were divided into 3 groups of 12 ewes each, balanced for body weight (kg  $44.06 \pm 5.15$ ), body condition score (BCS  $2.68 \pm 0.10$ ) and milk production (kg  $1.83 \pm 0.33$ ). For 28 days, during the experimental phase, a group (control group; Con) received a diet consisting of a total mixed ration (TMR), dehydrated ryegrass and soybean meal. For the other two groups, the experimental diets the TMR was given in the same proportion of the control group but part of the soybean meal and the dehydrated ryegrass were replaced by cardoon meal in two different doses: low (250 g/d per head as fed; group LC) and high (550 g/d per head as fed; group HC). The substitution aimed to keep the diets iso-proteic and iso-fibrous (17.3% CP, and 37.3% NDF, on a DM basis). The ration was supplied 4 times a day. The results were analyzed by a linear mixed model, with diet, time and their interaction as fixed effects and ewes a random effect.

Body weight and BCS were not significantly affected by treatments. The group feed intake was similar among treatments (1.909, 1.983, 1.963 kg/d of DM for Con, LC and HC, respectively). Dry matter intake increased during the experimental period in all groups. The feeding treatments did not affect milk yield (1.62, 1.62, 1.65 kg/d for Con, LC and HC, respectively; SEM 0.069 kg), milk fat content (5.84%, 5.80%, 5.61% for Con, LC and HC, respectively; SEM 0.48%), milk protein content (5.14%, 5.52%, 5.22% for Con, LC and HC, respectively; SEM 0.14%), milk lactose, and milk fatty acids. Time had a significant effect on all these variables ( $p < .001$ ).

In conclusion, cardoon meal was introduced in the diet of ewes without negative effects on intake, milk production and milk quality and body reserve variations.

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## P077

### Effects of calcium laurate on broiler growth performance and antioxidant intestinal activity

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Reducing the use of antibiotics is one of the largest challenges for animal production. The scientific community has investigated numerous alternative substances to antibiotics, including medium chain fatty acids, due to their antimicrobial and protective effects on gut health. The way of administration represents a critical point since free fatty acids dissociates in the stomach and are partially adsorbed and metabolized in the proximal gastro-intestinal tract, reducing their effects along the distal intestinal part. The present study investigates the effect of lauric acid saponified with calcium on the growth performance and intestinal antioxidant status in broiler chicks. A total of 720 female Ross308 chicks were assigned to one of three dietary treatments: CTR (basal diet alone), T1 (basal diet and lauric acid, 1 g/kg) and T2 (basal diet and lauric acid saponified with calcium (C12-Ca), 1 g/kg) considering Starter (0–11 d), Grower (12–21 d) and Finisher (22–42 d) basal diet. Body weight (BW), average daily gain (ADG), and average daily feed intake (ADFI) were measured and feed conversion ratio (FCR) was calculated. Chicks were slaughtered at 42d and intestinal samples were collected by scraping off the duodenal mucosa using a sterile glass microscope slide and used to investigate the impact of C12-Ca soaps on intestinal antioxidant status (superoxide dismutase SOD, catalase CAT and total antioxidant capacity TAOC). C12-Ca significantly ameliorated ( $p < .05$ ) FCR during the starter period (0–11d) compared to lauric acid while no differences were found on BW, ADG and ADFI. SOD and CAT activities at intestinal level were significantly ( $p < .05$ ) higher in chicks treated with C12-Ca. Our findings suggest that C12-Ca may not completely dissociates in the stomach and modulates the gut antioxidant status.



## P078

### A snapshot of functional and reproductive traits of Italian Mediterranean Buffaloes (*Bubalus bubalis*)

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The Italian Mediterranean Buffalo (IMB) is located in the Mediterranean area and distributed mainly in south-central Italy, an area characterized by offering optimal conditions for its development and production. The total census in Italy has increased considerably over the last decade, making it one of the most important dairy species in the country. Like any other species, IMB has also evolved and undergone changes due to the selective pressure to which it is subjected, for this reason the objective of this study was to analyze the current situation of various functional and reproductive parameters of IMB. A descriptive analysis was carried out using 153,256 buffaloes born between 1990 and 2018 and distributed in four regions, namely Campania (CAM), Lazio (LAZ), North (NOR), and South (SUR). The analyzed parameters were age at first calving, longevity (date of death – date of first calving), age of culling, herd size, number of lactations and milk yields. Results show a clear increase in the IMB population, especially from year 2000 onwards. The age at first calving showed differences among regions, being earlier in the NOR and later in CAM and LAZ. Regarding the number of lactations, no differences were observed between regions (~3). Longevity, one of the most important parameters in the IMB, showed that there are differences between regions, with LAZ being the region with the longest-lived animals (~1804 d), while CAM region where the highest number of buffalo is located and with a higher selective pressure, the mean was 1495 d. The herd size shows that LAZ and NOR have a smaller size, with a mean between 892 and 906, respectively. The trend of milk, protein and fat production by regions showed that in CAM the main objective is to obtain more kilos of milk (2268 kg), followed by the SUR region. On the contrary, in LAZ the protein content (4.71%) was more important, while fat had more relevance in the NOR (8.25%). The results of this descriptive analysis in the IMB show a clear diversification between regions but following common selection objectives. This analysis is a starting point to be able to determine the differences with respect to the past and to identify which of the parameters could be improved.

## P079

### Tributylin as feed supplement for young animals

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In the last decades, antimicrobial resistance increased exponentially, so valuable alternatives are urgently needed. Functional feed additives contain bioactive compounds that can improve the animals' health status and immune defence. In this scenario, tributyrin possesses antibacterial activity, promotes both gut health maintenance and nutrient absorption. The aim of this study was to evaluate the effect of tributyrin supplementation in young animals' diet on animal growth and health status. Firstly, 120 piglets were weaned at  $28 \pm 2$  d and divided into two experimental groups fed for 40 days an isoenergetic and isoproteic diet differentiable only for the inclusion of 0.2% of tributyrin (CTRL and TRIB group). Individual body weight and feed intake were recorded weekly. Faecal and blood samples were collected for the evaluation of principal metabolic parameters and the gut microbiota analysis. Secondly, at birth, 12 calves were randomly allotted in two experimental groups differentiable for the inclusion of 0.3% of liquid tributyrin in milk replacer (CTRL and TRIB group). The trial lasted 42 days. Individual body weight, feed intake, faecal score (0–3; considering diarrhoea  $\geq 2$ ) were recorded to assess performance and diarrhoea incidence. Faecal samples were collected weekly for the evaluation of principal microbial families. Piglets in the TRIB group revealed improved zootechnical performance ( $p < .05$ ) and a higher level of serum insulin and high-density lipoprotein was observed ( $p < .05$ ). Gut microbiota revealed a decrease in *Lactobacillus* spp. and *Bifidobacterium* spp. simultaneously with an increased  $\beta$  diversity ( $p < .05$ ). In calves, TRIB group highlighted no differences in zootechnical parameters, while a statistically significant reduction in diarrhoea incidence was observed ( $p < .05$ ) during the whole experimental period in TRIB compared to CTRL group. Calves from TRIB group revealed a decreased incidence of moderate diarrhoea (faecal score = 2;  $p < .01$ ) without observing a significant difference in the principal bacterial families.

In conclusion, the supplementation of tributyrin in young animals could promote zootechnical performance and health status suggesting tributyrin as promising feed additive as alternative to antimicrobial drugs in food-producing animals.



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## P080

### Characterization of feed efficiency in young Holstein bulls

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Agronomic and livestock sectors are recognized among the major contributors of greenhouse gases (GHG) emissions. Methane is strongly associated with global warming and is the major GHG produced by ruminants. The characterisation of feed efficiency with the selection of the most efficient animals is an important step towards the reduction of GHG emission in the dairy sector. Therefore, the aim of this study was to characterise the feed efficiency in young Holstein bulls, candidates to artificial insemination. Data were collected between May 2018 and July 2020 in the genetic centre of the Associazione Nazionale Allevatori della Razza Frisona e Jersey Italiana (ANAFIJ, Cremona, Italy) on 111 young Holstein bulls. The pens of the genetic centre were equipped with Roughage Intake Control system (RIC; Hokofarm Group, Voorsterweg, The Netherlands) to record dry matter intake (DMI, kg/d) and one pen was equipped with the Automated Head-Chamber System (AHCS; GreenFeed C-Lock Inc., Rapid City, SD, USA) to record CH<sub>4</sub> emissions (g/d). Average daily gain (ADG) was estimated as the regression of body weight (BW) on days of test. Feed efficiency was expressed as feed conversion ratio (FCR), i.e. the ratio between DMI and ADG. Means of age, BW, ADG, DMI and CH<sub>4</sub> emissions were 276 ± 42 d, 298 ± 63 kg, 1.12 ± 0.29 kg/d, 8.24 ± 2.20 kg/d and 220 ± 41 g/d, respectively. Average FCR was 7.55 ± 2.48, with the lowest values indicating more efficient animals. Moderate to low positive Pearson correlations were assessed between BW and DMI (0.49;  $p < .001$ ) and between BW and CH<sub>4</sub> emissions (0.24;  $p < .01$ ). Methane production was weakly positively correlated with DMI ( $r = 0.23$ ;  $p < .01$ ). Regarding FCR, a low positive favourable correlation with CH<sub>4</sub> (0.20;  $p < .01$ ) was estimated, meaning that improving (i.e. reducing) FCR would lead to less CH<sub>4</sub> emitted in the atmosphere. Also, a moderate positive correlation between FCR and BW (0.46;

$p < .001$ ) was observed. Large variability for FCR among individuals has been observed, suggesting that there is potential to improve (and therefore to reduce in terms of absolute values) FCR in Italian Holstein population.

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## P081

### Effects of boar taint compound concentrations on sexual behavior of young boars

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Boar taint (BT) is a sensory off-odour found in meat from entire male pigs, mainly caused by the accumulation in adipose tissue of two compounds: androstenone (5 $\alpha$ -androst-16-en-3-one; AND) and skatole (3-methylindole; SKA). A strategy to reduce the incidence of BT in meat is to select pigs for reduced AND and SKA concentration, but this might have an unfavourable correlated response on male reproduction, as the defect is associated with sexual maturity achievement and testosterone secretion. The aim of this preliminary study was to investigate the association between the concentration of BT compounds and sexual behaviour in 206 young commercial breeding boars.

Concentrations of AND and SKA were measured by HPLC with fluorescence detection in backfat samples (0.5 g) collected when animals reached a minimum body weight of 140 kg, before the start of the training of the boars in mounting the dummy sow. The training consisted in three mounting sessions performed over one week. Six sexual behaviour traits (SBT; excitement, salivation, mounting aptitude, interest in the dummy sow, penis unsheathing, overall libido score) were scored at each of the three sessions in a scale from 1 to 5, where five represented the best performance. The time from the start to the end of the mounting session (TTS) was also recorded. Sexual behaviour was assessed in all the animals by the same trainer. A total of 520 records of SBT were available for analysis.

Variation in SBT was analysed by ordinal logistic regression, whereas TTS was analysed by a linear mixed model. For all traits, the model included the fixed effect of the session number (from 1 to 3), farm (4 levels), AND concentration (5 levels), SKA concentration (5 levels) and the linear effect of the age of the boar (ranging from 170 to 250 d), and the random permanent environmental effect of the animal.

More favourable SBT scores and shorter TTS were observed with increasing session number. Concentration of SKA did not affect SBT, but SBT tended to exhibit less favourable scores at high AND levels, even though effects were generally not statistically significant. Increasing AND concentrations were significantly associated ( $p < .05$ ) with a greater interest/curiosity in the dummy sow and shorter TTS; we observed that the TTS of animals classified in level 5 of AND was 7 min shorter compared to the animals classified in level 1. Results indicate that associations between SBT and AND should be investigated more in-depth.

## P082

### The effect of an extender inducing sperm capacitation on fertility of Italian dairy cattle: a field experience

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The use of Heifer Plus, an additive for bovine semen extender that induces sperm capacitation, was associated with an increase in female births (about 70–75% if used 18 h after heat starts) and a higher conception rate than with conventional semen. The aim of this work was to evaluate the efficacy of Heifer Plus additive in combination with artificial insemination (AI) on the fertility of Italian Holstein Cattle (with a field trial). We analyzed the results of 3800 AI, carried out from September 2019 to July 2020, in 24 farms located in Northern Italy, using both Heifer Plus and conventional semen obtained from 9 bulls. We recorded the starting times of heat and AI; the data of the animals used; the type of semen, conventional and Heifer Plus; the number of progressively motile spermatozoa (NSPM) per batch of semen, and the result of the pregnancy diagnosis. With JMP®13 software we performed descriptive statistics and chi-square tests to evaluate the effect of the type of semen and other relevant variables collected on fertility evaluated through the positive or negative result of the diagnosis of pregnancy. 85.5% of the inseminations led to a diagnosis of pregnancy. The conception rate was 4% higher in cows inseminated with Heifer Plus semen than those who received conventional semen: 40.34% vs. 36.33%, respectively ( $p = 0.019$ ). Interestingly, a significant effect of the calving order ( $p < .001$ ) was also observed with a higher conception rate in heifers and a difference between conventional semen and Heifer Plus equal to 57.75% and 50%, respectively. The highest value of NSPM of the Heifer Plus semen (12.7 million/dose versus 8.2 million/dose of the conventional semen) did not affect the results significantly. Finally, there was also a significant variability between bulls and farms. However, we noticed that breeders did not always respect the ideal incubation time for Heifer Plus

semen and this could have affected the results. In conclusion, the use of this technology seems interesting, but it should be adopted with better management of the reproductive programs including stricter compliance with the ideal incubation time of AI, which entails higher costs but yields better results. Due to the high degree of variability among bulls, it would also be better to use the semen of different bulls in each farm.

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## P083

### Effect of sow's diet enriched with Arg and/or BCAA on health and performance of sows and newborn

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This study tested the hypothesis that dietary supplementation of branched-chain amino acids (BCAA; L-leucine, L-isoleucine and L-valine) and/or L-Arginine (Arg) to lactating sows enhance their immune status and productive performance. Sixty-eight sows were divided into 4 groups according to parity and body weight (BW): (i) control (CO) group (fed a corn lactating based diet); (ii) CO +22.5 g/d/sow of BCAA; (iii) CO +22.5 g/d/sow of Arg; (iv) CO +45 g/d/sow of BCAA and Arg. Amino acids were supplemented on top. Diets were fed to the sows from 4 days before farrowing (d-4) until weaning (d27). After weaning, piglets received a standard post-weaning diet. Piglets were individually weighed at d0 (farrowing), d7, d14, d27 and post-weaning, at d34 and d41; health status of piglets was followed over the whole period. Blood samples were collected on half of the sows at d-4, d10 and d27 for haematological analyses, urea, glucose, insulin, prolactin and immunoglobulin contents. Colostrum and milk were sampled at farrowing and at d10 and d20 and analysed for polyamines and immunoglobulin concentration. Data were analysed in a 2 × 2 factorial design by GLM or GENMOD procedure including batch, parity order, BCAA and Arg supplementation and their interaction as factors. A trend for higher lymphocytes percentage was observed in sow serum at d10 and d28 for BCAA ( $p < .1$ ). Both BCAA and Arg supplementation increased glucose and prolactin content ( $p < .05$ ) in serum at d27. Arg increased IgM content in serum at d10 ( $p = .05$ ) and tended to increase it in colostrum ( $p = .08$ ). BCAA increased IgA content ( $p = .004$ ) in milk at d20. Two weeks post-weaning, piglets born from sows supplemented

with amino acids were heavier than piglets born from CO sows ( $p = .03$ ). Arg supplementation reduced piglets' mortality pre- (d7 and d14 post-birth) and post-weaning (d41) ( $p < .05$ ). The administration of BCAA affected the sows' blood and milk immunological profile, while Arg increased the serum and colostrum IgM improving the piglets' survival rate.

## P084

### Evaluation of sow colostrum composition and somatic cell count as indicators of litter characteristics

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Sow colostrum is essential during early piglet life providing nutrients and immunity to piglets, therefore, its quality and quantity can influence the growth and survival of piglets and contribute to the differences in productive traits of gilts and multiparous sows. The aim of the present study was to characterize the colostrum composition, the somatic cell count (SCC) and Immunoglobulins (Igs) contents and to evaluate the relationship between them and the litter performance.

A total of 133 sows (parity  $4.24 \pm 2.56$ ; litter size =  $14.63 \pm 2.14$ ) were included in the study. Colostrum samples were collected at farrowing after the birth of the first piglet and before the parturition of the last, by manual milking. Colostrum was analysed in triplicate for fat, lactose and somatic cell count (SCC) contents with infrared spectroscopy using a Milkoscan FT2 (FOSS A/S, Padova, Italia). Immunoglobulins were quantified using an Elisa technique. Litters size and body weight (BW) were recorded at farrowing and at weaning (day 27). Pearson correlation was conducted between colostrum components and an Anova model was carried out to associate the colostrum component and sow' parity class ( $1 = 1^\circ$ ,  $2 = 2^\circ$ ,  $3 = 3^\circ$ ,  $4 = 4^\circ$  and  $5^\circ$  and  $5 = > 5^\circ$ ). A step-wise and Anova model were performed to associate the colostrum composition and parity class to the percentage of weaned piglets. Fat was positively correlated with the number of SCC ( $r = 0.49$ ,  $p < .0001$ ), IgM ( $0.403$ ,  $p < .0001$ ) and negatively correlated with lactose ( $-0.49$ ;  $p < .0001$ ).

Parity class significantly influenced the fat, lactose, SCC, IgM and IgA concentration ( $p < .005$ ). Primiparous sows had a higher level of fat and SCC and IgM compared with the other parities ( $p < .05$ ). The percentage of weaned piglets was associated with an increase of SCC ( $p = 0.02$ ; coef =  $0.009$ ), litter size ( $p = .001$ ; coef =  $-2.28$ ) and piglet BW at birth ( $p = .01$ ; coef =  $1.18$ ) while parity was not associated to it.

In conclusion, colostrum composition of primiparous sows can differ for fat, IgM and SCC which consists of leukocytes and epithelial cells, confirming a different status of the mammary gland

of these younger animals, however, parity does not appear to affect the litter performance.

## P085

### Genome-wide analysis of twinning rate using canonical discriminant analysis

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Twinning in cattle is an undesirable trait that, in dairy cows, entails reduced calf survival, increased incidence of abortion and dystocia, increased calving interval.

Nevertheless, several studies have shown that the increased production of twin calves, in beef cows, may represent an opportunity to enhance profitability in extensive livestock system.

Recently many Genome-wide association studies (GWAS), carried out using the single marker regression approach, highlighted the complexity of twinning trait, which seems to involve a large number of genes involved in several complex pathways. Anyway, analyses based on a multivariate approach could be performed to capture the covariance among markers.

In this study, a GWAS was performed to identify candidate genes associated with twinning rate in a suckler herd, using Canonical Discriminant Analysis (CDA). This technique allows investigating the relationship between experimental groups, by the extraction of new maximum variance components (canonical variables) from within-between (co)variance ratio matrix. The biological meaning of canonical variables can be inferred by the canonical coefficients (loadings) that represent the correlation with the original variables.

128 Maremmana cows, reared at CREA were genotyped using the BovineSNP50k BeadChip. After quality control and data editing, 39,085 markers and 124 samples were retained for the analysis.

CDA was performed by dividing the animals into two groups, single birth (SB) or twin birth (TB), then the canonical variables were extracted at genome-wide level using non-overlapping window of 100 markers according to sample dimension (Dimauro et al. 2011). Only markers that exceeded the 99th percentile were retained for subsequent whole-genome analysis.

We found several SNPs highly linked to genes that could be considered involved in genetic determinism of this trait. The largest loading values were observed on BTA 2, 5, 11, 14 and 24. Moreover, the strong signals were in correspondence with QTL previously reported for twinning in dairy and beef populations, and many signals not yet reported in the literatures.



## P086

### Modelling pig growth curves using daily weight recorded in automatic weighing systems

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Automatic feeders for livestock are becoming increasingly common. Besides their use in animal feeding and management, data coming from these machines can be used for animal breeding purposes. Aim of this work was to investigate individual pig growth curves using information from automatic feeders in a pig private farm. A total of 322 boars, belonging to four different genetic lines (P1, P2, Landrace (L), P3; where P is a private line), were raised in fattening cycles with different lengths, starting from  $84 \pm 21$  ( $35.8 \pm 8.91$  kg) to  $232 \pm 22$  ( $145.3 \pm 6.9$  kg) days of life. Aim of the trial was to test sires of sows candidates. During the trial, the amount of feed consumed, and the animals' weights were registered each time that animals accessed to the manger. Daily average weights and sum of feed consumption were used as data to construct individual growth and feeding curves. Data were fitted with a linear regression. In the linear model ( $y = b_0 + b_1 \cdot x$ )  $b_1$  was considered as the average daily gain (ADG) since it represents the increase in weight for each day. Principal component (PC) analysis was also applied to daily consecutive weights (from 105 to 200), considered as different variables, for 128 animals. The first two extracted PCs accounted for 80% and 8% of the total variance, respectively. Average  $b_1$  was  $0.85 \pm 0.13$  kg, ranging from 0.50 to 1.23 kg. Average adjusted  $R^2$  was  $0.98 \pm 0.02$ . The first PC was related to the general growth, whereas the second one to the beginning (positively) and to the end (negatively) of the trial. Animals were then divided in six classes according to their PC scores to highlight differences in their growth. The average ADG highlighted from the linear regression is in agreement with the values registered in the considered genetic lines. The analysis of PC1 scores allowed to model the pig growth curves, showing that the weights at the end of the trial ranged from 100 to 141 kg. Though PC2 scores, different slopes (i.e. different growth rate) were identified. Both techniques showed that data coming from daily weight registration could be used to highlight individual variability. Thus, this new information can be incorporated within animal breeding schemes: PC2 scores could be used as new phenotypes to select animals that grow earlier than others.

## P087

### Impact of the age at first calving on milk-related performances in Italian Mediterranean buffaloes

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The aim of the present study was to assess the influence of age at first calving (AFC, years) on milk-related parameters of Italian Mediterranean buffaloes. A total of 1,414,449 milk test day records (TD) collected between January 2013 and December 2017 in 106,388 buffaloes were provided by the Italian Breeders Association with information on birth date, milk yield (MY, kg/d), parity, and days in milk (DIM). Fat (FP, %), protein (PP, %) and somatic cell count (SCC, cells/mL) were available and SCC was converted into SCS by using a logarithmic transformation. For the purpose of the study, only buffaloes with both first and second lactations were kept. The editing included restrictions on the number of TD present per each lactation, i.e. at least 3 in both first and second parity. Moreover, AFC had to be within the range  $3.3 \pm 1.3$  years. Considering MY, FP, and PP, values were retained only if within the range of mean  $\pm 3$  SD, whereas for SCC the minimum and maximum were fixed at 1000 and 10,000,000 cells/mL. Restrictions have led to 396,674 final TD, where AFC showed quartile 1 at 2.73 years, median at 3.00 years, and quartile 3 at 3.34 years. By using such statistics, the TD were divided in 4 classes as: Class 1 (25.04 % TD) if AFC was  $\leq$  quartile 1, Class 2 (24.74% TD) if AFC was between quartile 1 and median, Class 3 (25.11% TD) if AFC was between median and quartile 3, and Class 4 (25.11% TD) if AFC was  $>$  quartile 3. A mixed model was adopted for the analysis of variance of MY, FP, PP, and SCS. The model included the fixed effect of parity (2 levels), DIM (10 stages), AFC (4 classes), and season of calving (4 seasons) and the interaction between AFC and parity and the interaction between AFC and DIM. Buffalo and herd were the random effects. No differences were observed in terms of FP and PP among the AFC classes, likely due to a dilution effect; however, AFC affected both MY and SCS ( $p < .01$ ). Indeed, there was a linear increase in both MY and SCS with greater AFC. Milk SCS in Class 1 was equal to  $2.73 \pm 0.04$  and significantly differed from SCS in Class 4 ( $2.78 \pm 0.04$ ). Similarly, MY estimated for the 4 classes were significantly different to each other ( $p < .05$ ), with least squares



means of  $78.04 \pm 0.75$ ,  $79.82 \pm 0.75$ ,  $81.24 \pm 0.75$ , and  $82.40 \pm 0.75$  kg/d in the four classes, respectively. Results show that AFC has a deep impact on daily MY and SCS in the first two lactations of the buffaloes, without affecting FP and PP.

## P088

### Relationship between temperature-humidity index and growth in pigs

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The Italian pig breeder association (ANAS) keeps the herd books of pig breeds and manages several breeding programmes. The genetic evaluation of Italian Large White (ILW), Landrace (IL) and Duroc (ID) boars is based on the sib-testing, in which three piglets of the same litter (four females and one castrated male) are individually performance tested at the ANAS control station. The aim of this contribution was to study the effect of temperature and relative humidity on the growth of the pigs of the three breeds. From August 2016 to August 2019 a total of 1.786 pigs (799 ID, 313 IL e 674 ILW) was raised in multiple groups (max 48 animals). On average, animals spend  $135.23 \pm 6.84$  days at the control station starting and ending the trial at  $103.80 \pm 4.22$  and  $239.03 \pm 7.94$  days of age, respectively. During this period, individual's weights are registered every two months, whereas feed intake is recorded daily. Average daily gain (ADG, in g) and feed gain ratio (FGR) were then calculated. The temperature (T, divided into seven classes from lowest to highest) and humidity data came from the nearest regional meteorological station. Temperature Humidity Index (THI) was calculated and divided in nine classes (from lowest to highest). During the considered period, the maximum T ranged from  $-1.5^{\circ}\text{C}$  (January) to  $39.3^{\circ}\text{C}$  (August), while the THI values from 36 to 75.6. ANOVA analysis was carried out to evaluate the effects of group, breed, class of THI on ADG using the following linear model ( $\text{ADG} = \text{group} + \text{breed} + \text{THI} + \text{breed} \times \text{THI}$ ). All considered factors were highly significant ( $p < .0001$ ), except breed ( $p = .24$ ). Predicted ADG values increased up to THI class 4 (48–53) and then remained constant. Even if breed was not significant, interesting variations of ADG according to THI classes within breeds could be observed. Results of this preliminary study showed that ADG values are affected mostly from low then from high temperature or THI values. However, THI values almost never reached the alert zone during the experimental period. Further studies are needed to better

understand the relationship between pigs growth and THI, for example also considering feed intake daily variations.

## P089

### Effects of corn grain of different particle size on sheep feeding behavior and fecal pH

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The effect of corn grain particle size and distribution on the intake, feeding behaviour and digestibility of the starch source itself was studied in 24 dry Sarda sheep divided into four groups of six ewes/dietary treatment. Each sheep was fed a basal diet of 900 g of alfalfa hay and a concentrate supplement consisting of 40 g of soybean and 400 g of corn grains; the latter were processed to have different particle sizes: whole ( $>4.7$  mm, WH), cracked (average 3.0 mm, CR), medium flour (average 1 mm, ME) and fine flour ( $<0.6$  mm, FI), respectively. Concentrate supplement was individually fed at 8.00 AM, whereas hay was available all day. Time spent for concentrate intake and feeding behaviour were recorded for 12 h after concentrate supplementation. Faecal pH was measured at 7:30, 11:30, 15:30 and 23:00 h. The results showed that in the first 90 min after supply the intake of concentrate (g/d) was complete in the groups receiving corn grains of larger particle size, i.e. WH and CR, compared to the FI group, in which the lowest intake was recorded (262.4 g/d). Concentrate intake rate (g/min) was significantly higher ( $p < .01$ ) in the WH (86.53 g/min), vs. the CR, ME and FI (60.1, 21.1 g/min and 12.6 g/min, respectively). Alfalfa hay intake in the FI group (707 g/d) was significantly lower than in the WH, CR and ME groups (755, 770 and 785.0 g/d, respectively). Indeed, total intake was highest in the groups with largest grain sizes, i.e. WH and CR. The percentage of time spent eating alfalfa hay in the 12 h following maize intake was highest in the FI and lowest in the CR group (19.81 vs. 15.21%; respectively;  $p < .001$ ). The percentage of time devoted to concentrate intake increased as corn grain size decreased (0.65% vs. 2.87% for WH vs. FI, respectively). The rumination time was greater ( $p < .001$ ) in the WH and FI (20.05% vs. 18.12%, respectively) and lower in CR and ME groups (13.96% vs. 12.99%, respectively). Hourly variation of faeces pH at different times of the day showed the highest values at h.15.30 and the lowest values at h.7.30 (8.45 vs. 8.09, respectively;  $p < .05$ ). The lowest average pH was recorded in WH and CR (8.18 vs. 8.39,

respectively), the highest values in the medium and fine flour groups (8.53 vs. 8.48; respectively) ( $p < .05$ ). In conclusion, the intake rate and the rumination time can be markedly reduced by reducing the particle size of the corn grain below 1 mm.

## GENETIC VARIABILITY, INBREEDING AND CROSSBREEDING

### P090

#### Signature of selection in specialized and rustic Italian native beef cattle breeds

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Ever since domestication, livestock species have been submitted to a selection process developed by humans. Cattle has been one of the most selected species, as the breeding programs account for the improvement of well-defined traits based on the specialization of the animals, and this is enhanced by the common usage of reproductive technologies such as artificial insemination. The availability of SNPs arrays allows identifying genomic regions that have undergone the selection process and thus improve the knowledge about the genetic basis of the selected traits. In this study, we aim to identify genome-wide selection signatures in 5 Italian beef cattle breeds. A total of 3581 animals of the specialized beef cattle breeds Marchigiana, Chianina, and Romagnola and from the rustic breeds Maremmana and Podolica were genotyped with the GeneSeek® Genomic Profiler™ Bovine LDv4 33K chip (Illumina Inc., San Diego, CA, USA). Quality control was performed using plink v1.9, considering only individuals with a genotype call rate of 0.9 and autosomal SNPs with a MAF >0.05. Animals were analyzed as two separate populations, specialized and rustic breeds, and two selection scan analyses were carried out, one based on the FST-outlier approach with BayeScan software, and one based on the haplotype frequencies in different populations using the software hapFLK. The results from both methodologies were contrasted and common regions were selected to analyze their gene content. The BayeScan analysis yielded 14 significant genomic regions in bovine chromosomes (BTA) 1, 5, 6, 8, 9, 10, 13, 14, 15, 18, 19, 21, 24, and 26, while the

analysis performed with hapFLK showed 3 significant regions in BTA 6, 13, and 20. Common regions were found in BTA 6 and 13. The genes mapping to these regions are involved in immune response (e.g. WFDC, CD40, STK4), reproduction and embryo development (e.g. TKDP1, SPATA2, SPATA25), coat color (e.g. KIT), and beef quality (e.g. CEBPB). This study provided an insight into the genomic patterns of selection specific for Italian beef cattle breeds. Many of the genes found mapping to the putative selection regions are related to traits of economic interest in this species, allowing us to know more about the genetic basis of these traits.

#### Acknowledgements

The authors wish to thank the ANABIC for selecting and providing SNPs data and phenotypic information of Italian beef cattle breeds. Thanks to CRAG for providing the tools to perform the analyses.

### P091

#### A genome wide association analysis for male fertility traits in three Italian beef cattle breeds

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In beef cattle, male fertility is involved in two distinct fields: clinical medicine, which aims to identify subfertility, and livestock breeding, which relies on optimal fertility rates for efficient reproduction. Male fertility indicators are used in the evaluation of the breeding capacity of livestock species, and they present a low heritability based on complex phenotypes, controlled by multiple genes. The objective of the present study was to identify genetic markers and genes associated with eight fertility traits, namely scrotal circumference, right testicle length and diameter, sperm volume and concentration, percentage of abnormal spermatozoa, and total and progressive sperm motility in 3 autochthonous Italian beef cattle breeds using GWAS approach. The traits were recorded in 2692 young bulls representing three of the main Italian beef cattle breeds: Chianina (CHI, 909), Marchigiana (MAR, 879), and Romagnola (ROM, 904). Samples were collected at the ANABIC genetic station during the performance tests

carried out between 1985 and 2019. We genotyped the animals in 2019 using the GeneSeek® Genomic Profiler™ Bovine LDv4 33K chip (Illumina Inc., San Diego, CA, USA) and performed GWAS using the GEMMA software. We found significant SNPs in all the studied breeds by the GWAS analysis. At the chromosome-wide level, a total of 79, 91, and 66 SNPs were identified considering all analyzed traits for MAR, CHI, and ROM, respectively. Pathways and gene ontology analyses suggest promising candidate genes located in overlapping or in proximity with the significant SNPs and involved in reproductive traits. Results show genetic relationships between the breeds, as well as relevant genes for the breeds' improvement, which will allow performing better selection programs. In this study for the first time, three of the main Italian beef cattle breeds were investigated by a genomic approach for fertility traits. The new genomic tools allow us to identify different markers of interest for the selection of the breeds, preserving the history and the traits that distinguish them. The selection programs used for the improvement of the Italian beef cattle breeds will benefit from the significant SNPs found in this study, especially those which are in common between breeds.

#### Acknowledgements

The authors wish to thank the ANABIC for providing SNPs data and phenotypic information of Italian beef cattle breeds. Thanks to CRAG for providing the tools to perform the analyses.

## P092

### Cryobanking of native Mediterranean brown trout semen as a tool for the safeguard of genetic resources

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The Native Mediterranean brown trout (*Salmo cettii*) populations inhabiting Biferno and Volturno rivers in Molise are decreasing due to poorly regulated fishing activities, river pollution and the introduction of allochthonous strains. This last factor has triggered an overall genetic impoverishment, therefore reducing the original genetic variability. Because of these factors, the conservation status of *S. cettii* in the Mediterranean area is now considered as 'critically endangered' by IUCN Italian Red list. In this regard, the project 'LIFE' Nat.Sal.Mo, recently funded by the EU, aims to ensure the recovery and the conservation of native trout in Molise river basins. In particular, the creation of a sperm cryobank is a milestone in the Nat.Sal.Mo project and is an effective strategy for the protection of the biodiversity of the local

Mediterranean brown trout populations. The purpose of this work was to depict the results achieved during the first 2 years of the project when it comes to the creation of the first European cryobank and its practical use in the artificial fertilization schemes to safeguard the genetic resources and improve the genetic variability of native trout. Autochthonous specimens were captured using fixed traps allocated in the main attractive spawning sites. Semen was collected by stripping and was extended to reach a final cryoprotectant concentration of 0.15 M glucose and 7.5% methanol and loaded into 0.25 mL plastic straws, obtaining a final sperm concentration of  $3 \times 10^9$  sperm/mL. The straws were equilibrated for 15 min above ice at a height of 3 cm, then frozen by exposure to liquid nitrogen (LN2) at 3 cm above LN2 level for 5 min and finally placed in LN2. During the first 2 years of activities 1287 semen doses from 93 native individuals were stored. About 25% of these doses were used for artificial fertilization. In total around 90,000 eggs were fertilized with frozen semen, obtaining a yield to eyed eggs of about 70–75%. The creation of the first sperm cryobank of pure Mediterranean trout populations with a high genetic variability will be useful even after the end of the project for Molise river basins as well as for other Italian basins where *S. cettii* is at risk of extinction. Besides being a supportive tool in ensuring the sustainability of the project in the future, it represents a powerful multiplying effect which will affect other neighbouring areas at a national level and other European river basins.

## P093

### Interspersed repeated elements analysis in *Camelus dromedarius* CSN1S2 gene

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The  $\alpha$ 2-casein (CN) is a milk protein fraction encoded by the CSN1S2 gene. Previous studies on dromedary milk proteins mainly focused on the other three fractions ( $\alpha$ 1-,  $\beta$ - and k-CN), and, although there is globally growing interest in camel milk, studies focusing on  $\alpha$ 2-CN are very limited. In this respect, no information has been reported on retrotransposons (RTs). These repeats, also known as Interspersed elements (INEs), are informative genetic markers useful as phylogenetic tools for clustering, evolutionary and population structure studies, rather than for the setup of food traceability methods. Aim of this study was to identify and characterize INEs in the dromedary CSN1S2 gene. Analysis has been carried out using RepeatMasker as bioinformatic tool. Three long interspersed elements (LINEs) were found



in introns 1, 10 and 11. The first and the last elements are truncated LINEs 2, respectively 50 bp and 97 bp long. Conversely, the repeat in intron 10 is 149 bp long and it is a truncated LINE 1. In addition, three short interspersed elements (SINEs) have been found in introns 8, 9 and 16, with a size of 190 bp, 149 bp and 207 bp, respectively. The observed six repetitive elements cover 5.6% of the whole dromedary CSN1S2 gene sequence. Camel CSN1S2 gene has more INEs compared with CSN2 gene (five RTs) and they belong to the same types except for the MER5B, which was not found in CSN1S2 gene. Conversely, the CSN1S2 has less INEs compared with CSN3 (7 RTs) and is distinguished by the presence of LINE 2 type, which was not found in camels CSN3. Camel CSN1S2 also shows less INEs than the homologous bovine gene (8 RTs, 5 of them are SINEs and 3 are LINEs). These results agree with previous studies on CSN2 and CSN3 genes and confirm that camel casein genes have lower repetitive elements than cattle. This characteristic is an indication that Tylopoda diverged from Ruminantia before additional retrotransposition events took place at different times during the divergence.

## P094

### Artidactyla retroposons detection at the Mediterranean river buffalo CSN1S2 gene

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The  $\alpha$ s2-casein is an important phosphoprotein secreted in ruminants' milk but, nowadays, the encoding gene (CSN1S2) has not been characterized in buffaloes. In this study, we report the first characterization of buffalo CSN1S2 gene and its artiodactyla retroposons (RTs) distribution. For this purpose, we amplified and sequenced the whole gene of 2 Mediterranean river subjects reared in Campania (Italy). The gene includes 1025 bp of coding (18 exons) and 17578 bp of intronic regions, 937 bp at the 5'-end and 707 bp at the 3'-end and it is characterized by 13 RTs. The first (named A) is located in the promoter region and appears to be a Bov-tA2. Further 2 elements are located in intron 1 (B and C) and show a strong similarity with L1\_Art sequence. Two Bov-tA2 are located in the intron 2 (D and E). The intron eight includes a Bov-B (F), whereas the intron 12 contains a Bov-A2 (G). Five additional retroposons (H, I, L, M, N) are located in the intron 13 (Bov-tA1), 15 (Bov-A2) and 17 (Bov-tA1, Bov-B, Bov-A2). Finally, a Bov-tA2 (O) is located in the 3' UT, near to the last exon. The observed RTs represent 19.45% of the whole gene. This figure decreases considerably in the bovine and caprine/ovine counterpart because of presence/absence of RTs observed in these species. In particular, the bovine CSN1S2 does not show the elements

B and C and, at the same time, includes expanded Bov-A monomers (Bov-A3 in cattle and Bov-A2, G in buffalo). Sheep and goat have a similar gene structure and, when compared to buffalo, the absence of elements C, G, I and N was noted together with the presence of an extra Bov-tA3 and of Bov-A4 in the promoter region and the intron 1, respectively. Ruminant retroposons are highly informative genetic markers that can be considered a powerful phylogenetic tool for clustering studies, animal evolutionary history, population structure and demography, rather than the setup of methods for species discrimination in meat and dairy products.

## P095

### Characterization of the Pura Raza Español Carthusian horse strain after 500 years of close matings using genomic data

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The Carthusian strain is recognized within the Pura Raza Española (PRE) horse originally formed by the Carthusian monks in the 15th century in Andalusia. Since then, the strain was bred as a close population, being selected mostly by morphological traits and tameness. However, this breeding system has produced different extents of close-relative mating for nearly 500 years, thus increasing the inbreeding levels in the population (mean  $F=0.11$ ). In this study, we analyzed the genomic landmarks of such matings using runs of homozygosity (ROH). To this, we determined the ROH patterns in 50 pure Carthusian horses (CART), which were compared with 64 PRE horses with 0% of Carthusian blood according to pedigree records. We determined the average ROH count, length, and FROH (molecular inbreeding value determined as the proportion of the genome covered by ROH) per individual and chromosome using the DETECTRONS package of R software. Carthusian horses showed an increased number of ROHs (91.3) and FROH (0.182) per animal in comparison with PRE, which showed 64.27 and 0.128, respectively ( $p < .001$ ). Whereas the average ROH length per animal was similar in both populations (4.96 Mb and 4.92 Mb), suggesting that differences between CART and PRE were produced a long time ago. However, the number of ROH was highly variable between chromosomes. For example, ROH was increased in ECA30 and ECA31 in CART and PRE, respectively, in comparison with the rest of chromosomes per group. Furthermore, ECA30 showed the



greatest difference in FROH between CART and non-CART (0.386 vs. 0.16, respectively;  $p < .0001$ ). Considering the different ROH lengths, FROH has been decreasing considerably in CART strain over the generations (FROH<sub>9g</sub> = 0.103 to FROH<sub>3g</sub> = 0.035), indicating that the large inbreeding events occurred in the past. Finally, we determined the existence of two regions in which ROH incidence was significantly increased (ROH islands) only observed in CART (30:10,120,984–10,354,052 and 30:11,756,033–15,171,027). Within them, we detected 13 candidate genes related to immune response and morphogenesis, according to gene ontology analysis. Overall, our study suggests that the Carthusian strain had a differential selection process over last 500 years which caused a marked increase in inbreeding, as well as the existence of a selected pattern in terms of homozygosity in comparison with the PRE horse, mostly related to genomic regions associated with immune response and morphogenesis.

## P096

### A conservation strategy for Italian honey bees

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The presence of different breeds of *Apis mellifera* in the same area can cause progressive hybridization between genetic types and issues of conservation of genetic diversity. This is due to the fact that a virgin queen freely mates only once in her life, in flight and with many drones (10–15) of potentially different varieties/genetic types. For this reason, the introduction of mating control in beekeeping can be seen as a proper conservation approach to preserve biodiversity. In this study we propose a strategy to conserve diversity in honey bees by the use of isolated mating areas (IMA). The scheme involves the participation of numerous beekeepers who submit their best mother colonies to a test of conformity to the genetic type of interest. Secondly, batteries of sister virgins queens are obtained by each of the mother colonies that have passed the test and are then committed to the production of drones, the drone-producing queens (DPQ). It is very important that DPQs conserve the greatest possible genetic variability. Therefore, we recommend to increase the number of mother colonies instead of the number of queens, given the same number of DPQ. Subsequently, the DPQs are located in a well-defined IMA protected from the presence of other colonies of unknown variety, where interested beekeepers can bring their virgin queens for mating. From the synergy between the BEENOMIX Project (Regionally Funded project) and the AISSA association, three IMA

have been created in Italy where beekeepers can bring their own mating nucleus. The three areas are located in San Pietro Island (South West Sardinia), Ponza Island (LT) and Val Bodengo (SO), to be activated in the next few years and are guaranteed by specific local ordinances.

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## P097

### Frequency assessment of casein genes variants in Tunisian dromedary (*Camelus dromedarius*) population

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Recent years have witnessed an increasing interest in camel milk and in its processing into various products due to its great nutritional and therapeutic value. This growing focus has led researchers to investigate the genetic variation of some candidate genes associated with camel milk yield and quality such as caseins, major proteins in this milk. The molecular characterization of those genes has been then studied in different camel populations and multispecies comparison of the casein gene cluster has been conducted. The aim of this study was to investigate the distribution of genetic variants c.150G > T, g.2126A > G and g.1029T > C at CSN1S1 ( $\alpha$ S1-casein), CSN2 ( $\beta$ -casein) and CSN3 ( $\kappa$ -casein), respectively, in Tunisian camel population. Blood samples were collected from unrelated Maghrebi camels ( $n = 159$ ) reared in different localities. Genomic DNA was extracted from 5 mL of whole blood and specific DNA fragments were amplified according to already published protocols to generate amplicons of the 903, 659 and 488 bp. Restriction Fragment Length Polymorphism (RFLP) methods, using Sml I, Hph I and Alu I endonucleases, were used for genotype identification. Allele frequencies and Hardy-Weinberg (HW) equilibrium were calculated. Distribution of genotypes for the CSN1S1 gene highlighted that the most common genotype was GG (0.87), whereas no homozygous TT was found in the investigated population. Despite that, the frequency of T allele (0.066) is very similar to that reported in previous published papers carried out on Sudanese and Nigerian

populations, confirming that T allele is rare. Regarding the CSN2 and CSN3, all possible genotypes were found and the minor allele frequencies (MAF) were G (0.462) for the CSN2 and C (0.384) for the CSN3. Population resulted in HW equilibrium at each locus. The comparison of the obtained frequencies with those of Sudanese and Nigerian populations did not show any significant difference between them. Nevertheless, as far as we know, this is the first reported study on casein genes in Tunisian camels, which may contribute to a better understanding of the genetic variability of this population and be useful for future association studies with milk traits, besides the characterization of different camel ecotypes.

## P098

### Study of candidate genes for the biosynthesis of prebiotic molecules

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The MIQUALAT project is focused on the identification of cows that produce milk naturally enriched of bioactive prebiotic and protective molecules with nutraceutical properties and beneficial effects on human health. One of the aims of the project consists in the analysis of some free oligosaccharides, sialic acid, alpha lipoic acid, thiols and glutathione in milk of four cattle breeds (Holstein, Simmental, Simmental×Holstein (Monterotondo experimental farm), Podolica (Bella herd) at 60 and 120 days after calving. The genetic characterization of the studied cattle breeds is being carried out using genotyping by bead chip and RNA-seq. Moreover, we have focused on the study of some candidate genes associated with the bioactive molecule's biosynthesis and specifically: beta-1,4-galactosyltransferase 1 (*B4GALT1*) and lactalbumin alpha (*LALBA*) for lactose, cytidine monophosphate-N-acetylneuraminic acid hydroxylase (*CMAH*) for sialic acids, beta-galactoside alpha-2,3-sialyltransferase 4 (*ST3GAL4*), beta-galactoside alpha-2,3-sialyltransferase 5 (*ST3GAL5*), and beta-galactoside alpha-2,3-sialyltransferase 6 (*ST3GAL6*) for 3'-sialyllactose (3'-SL), beta-galactoside alpha-2,6-sialyltransferase 1 (*ST6GAL1*) for 6'-sialyllactose (6'-SL), alpha-N-acetylneuraminide alpha-2,8-sialyltransferase 2 (*ST8SIA2*), alpha-N-acetylneuraminide alpha-2,8-sialyltransferase 3 (*ST8SIA3*), and alpha-N-acetylneuraminide alpha-2,8-sialyltransferase 5 (*ST8SIA5*) for disialyllactose (DSL), and lipoic acid synthetase (*LIAS*) for lipoic acid. RNA was extracted from milk somatic cells of 10 animals for each breed and was reverse transcribed to produce long cDNAs. cDNAs were amplified with specific primers and the obtained amplicon were sequenced.

Preliminary results have identified new splicing variants. In *ST3GAL4* and *ST6GAL1* we have found a transcript with an exon skipping and in *LALBA* a transcript with intron retention. In the amplified regions of *B4GALT1*, *CMAH*, *ST6GAL1* and *ST3GAL4* genes some already annotated SNPs (rs110112208, rs210697833, rs440366763, rs42184639, rs208302929, rs209522477) have been confirmed. The allele frequencies showed differences between breeds even if a small number of animals has been included. Analyses are ongoing to better characterize the expressed specific transcripts and to amplify the other candidate genes.

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## P099

### Genome-wide association study for residual concentrate intake Italian Brown Swiss calves

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Residual feed intake (RFI) is the most used measure of feed efficiency. However, considering the importance of concentrates in the ration composition, a new index, the residual concentrate intake (RCI), was defined. RCI, as RFI, aims to identify efficient and inefficient individuals in converting, in this case, the concentrate into animal products. 736 Brown Swiss young bulls, housed in boxes (no more than six bulls/box) equipped with an automatic feeding system able to recognize the animal and to record the concentrate intake, were involved in the study. The diet consisted of concentrate and hay, being hay administered ad libitum. Animals were genotyped with the Illumina BovineSNP50 BeadChip and data were analyzed combining two multivariate methods. The first is based on the idea that calves belonging to the tails of the phenotypic distribution of RCI share different allelic combinations for genes involved in its determinism. Consequently, genes related to associated markers would act differently in the two groups. With this aim, after RCI was corrected for the fixed effects of birth month and birth year, and for the random effect of the animal, individuals belonging to the first and last quartile of the phenotypic distribution were flagged as low (LRCI) and high (HRCI) RCI. The stepwise discriminant analysis selected 698 most discriminant SNPs and the canonical

discriminant analysis significantly separated LRCI from HRCI. Then the Bayesian GWAS (B-GWAS) was developed by using the BayesR software and, at the end of the procedure, markers were ranked according to their posterior inclusion probability and the first 698 SNPs were selected. Only markers simultaneously selected in the two procedures (11) were considered associated with RCI. The subsequent gene discovery analysis highlighted 50 genes in the neighborhood (250 kb downstream and upstream) of the 11 SNPs with 17 genes directly associated with RFI and average daily gain.

## P100

### Application of single-step GBLUP in Italian Comisana sheep

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Comisana is a dairy sheep breed reared in central and southern Italian regions used to produce PDO cheeses. Since 2000 a nucleus flock has been established at the breeders association experimental station aiming at improving both milk yield and composition using pedigree-based EBVs and fitting a lactation model. Recently, within the national CHEESR project, ewes of the experimental station were genotyped (Illumina OvineSNP50 Beadchip) and a repeated test-day model was implemented using both pedigree-based (TD-PBLUP) or single-step genomic best linear unbiased prediction (TD-ssGBLUP). The main objective of this study was to compare results from TD-PBLUP and TD-ssGBLUP. Data consisted of daily milk production and weekly fat and protein contents for 1138 Comisana sheep, 50K SNP genotypes for 309 animals and 2062 animals in the pedigree. Overall, a total of 169 individual – dam pairs were available. After a quality check 45,343 SNPs were retained. A multiple-trait repeatability test-day model was fitted including year of birth, month of calving, class of days in milk as fixed effects, and flock-test-date, permanent environmental and additive genetic as random effects. Phenotypes of 100 ewes (50 with genotypes) were masked to create a validation group. Heritability ranged from 0.09 (fat content) to 0.33 (daily milk yield). The average accuracy of TD-PBLUP and TD-ssGBLUP EBVs for the validation group was 0.43 and 0.45 for milk yield, 0.48 for fat content and 0.62 for protein content. When considering only ewes with genotypes the average accuracy

of TD-ssGBLUP was higher than that of TD-PBLUP only for milk yield (0.40 vs. 0.45) while for protein and fat content did not change being 0.60 and 0.48, respectively. Results from the current research confirm the usefulness of a single-step approach but they depend on the trait and its heritability, the percentage of genotyped individuals and the population structure. The upcoming inclusion of ram genotypes is expected to increase the accuracy of the TD-ssGBLUP EBVs.

## P101

### Genetic structure at CSN1S1, CSN2 and CSN1S2 loci of four autochthonous goat breed reared in Lazio and Campania regions

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One main aspect of safeguard plans for livestock Autochthonous Genetic Types (AGTs) is to ensure economic sustainability of their farming. This encourages farmers to contribute to the preservation of AGTs. The aim of this study was to characterize the genetic structure of four AGTs of endangered goats at the  $\alpha$ S1,  $\beta$  and  $\alpha$ S2 calcium sensitive caseins encoding genes CSN1S1, CSN2 and CSN1S2, respectively. The peculiar polymorphisms detected at these loci are associated with milk qualitative characteristics and have been largely investigated in widespread dairy goat breeds. On the contrary, there are very few studies carried out in AGTs goats. This study was performed on 50 Neapolitan goats, typical of the Campania region, and on 61 goats which included the three breeds Monticellana, Capestrina and Grigia Ciociara, reared in Lazio region (Lazio AGTs goat). Genotyping of quantitative alleles at CSN1S1, CSN2 and CSN1S2 loci was performed according to previous studies. At the CSN1S1 locus a high variability was observed in all four AGTs. In particular, a higher frequency of the null allele N was observed in Neapolitan goats than Lazio AGTs goats (0.420 vs. 0.008). On the contrary a higher frequency of F allele, associated with a low  $\alpha$ S1 casein content in milk, was observed in Lazio AGTs goat (0.442) than Neapolitan goat (0.120). All four AGTs do not carry the null alleles CSN1S1 01, CSN2 01 and CSN1S2 0 and the alleles associated with an intermediate content of  $\alpha$ S1 (CSN1S1 E) and  $\alpha$ S2



(CSN1S2 D) caseins in milk. The genotyping data obtained provide useful information regarding the productive aptitudes of the four AGTs studied. In fact, the high frequency of CSN1S1 N allele in Neapolitan goat, predisposes this breed to produce low-protein milk for fresh consumption while the high frequency of the allele CSN1S1 F in the Lazio AGTs goat addresses their milk to processing for the production of typical cheese with unique organoleptic and flavor characteristics. Developing safeguard plans that take these results into account will have a higher chance of success, with a consequent increase in the number of animals raised.

## P102

### Breeders' management in a heritage breeds' rabbitry

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Population size reduction and genetic erosion are two critical events in domestic animals' productions. The aim of the present research is to analyze breeders' management data recorded in a heritage breeds' rabbitry to determine breeders' husbandry strategies and their effects on some reproductive parameters. Litters' registrations including buck id, doe id, day of birth, litter size (born alive) and weaned kitten were analyzed. Three breeds' litters were studied in a 11 years time lapse: Belgian Hare (BEHA;  $N = 151$ ), Burgundy Fawn (BUFU;  $N = 105$ ), Californian (CALI;  $N = 212$ ). Inclusion of external breeders was considered too (buck origin 0, 1; doe origin 0, 1; litter origin 0, 1, 2). Data analysis was performed by ANOVA using the General Linear Model procedure of SPSS. In our model dependent variables were litter size (born alive) and percentage of weaned kitten, and sources of variation were breed, year of birth and litters' origin (three levels: both parents internal, one parent internal and one parent external, both parents external). The post hoc Bonferroni test was used to investigate the significant differences ( $p \leq .05$ ). The dependent variables number of litters per buck and number of litters per doe were analysed with breed as source of variation. Frequencies distribution of buck origin, doe origin and litter origin were analysed using SAS PROC FREQ procedure and the Chi Square test was used to investigate significant differences. Breed had no effects on litter size (means  $\pm$  SE; BUFU  $5.75 \pm 0.25$ , CALI  $5.77 \pm 0.18$ , BEHA  $6.06 \pm 0.20$ ) and percentage of weaned kitten on born alive ones (mean  $\pm$  SE; BUFU  $87.18 \pm 2.45$ , CALI  $88.89 \pm 1.74$ , BEHA  $89.86 \pm 1.97$ ). Year of birth affected significantly the litter size and percentage of weaned kittens in all the considered breeds: in the last years, the percentage of weaned kittens was higher than in the first years of recording. The inclusion of external breeders did not significantly influence recorded

reproductive data. The number of litters per buck (mean  $\pm$  SE; BUFU  $1.91 \pm 0.21$ , CALI  $2.34 \pm 0.16$ , BEHA  $1.94 \pm 0.12$ ) and per doe (mean  $\pm$  SE; BUFU  $1.61 \pm 0.17$ , CALI  $1.77 \pm 0.13$ , BEHA  $2.10 \pm 0.16$ ) were not influenced by breed. The frequency of use of external breeders is breed specific. Data recording is a basic step in conservation plans; our results supply some information about breeder's management strategies in a heritage breed rabbitry aimed to produce morphologically typical rabbits limiting genetic variability reduction.

## P103

### A machine learning approach for the identification of population-informative markers from trout genotyping data

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Cost-effective commercial SNP arrays are now available for several species and this has had a substantial impact on livestock as well as on fields of natural ecology, evolution and conservation biology. Nowadays, genome-wide SNP analysis is the method of choice for the characterization of natural populations. In this context, the identification of a minimum number of SNP with the maximum information to differentiate populations is becoming important but challenging. This may have interesting implications for several downstream applications such as allocation of individuals and comparative analyses of selection signatures. Recently, the use of machine learning approaches and notably of random forest classifier (RF) has been proposed for the identification of the most discriminating genetic markers among thousands of SNP. Here we used the RF algorithm to analyse genotyping data obtained with 57K Trout BeadChip array (Affymetrix) from autochthonous and allochthonous trout populations of Molise rivers and their tributaries. The 48 highest ranked SNP were obtained and compared with the list of the most informative SNP estimated using traditional statistical approaches: Delta,  $F_{ST}$  and principal component analyses. In total, 103 specimens were enrolled in the study, from a larger cohort of ~300 fishes caught in 30 different sites of Volturino and Biferno basins. The samples were chosen based on results obtained by PCR-RFLP and preliminary fine-scale population structure outcomes. Trout considered in this study were representative of four different native trout subpopulations and one Atlantic species. Four reduced informative panels were obtained and their performances estimated using correct prediction proportions from RF classification. The correct assignment of the



specimens to their subpopulations had an average of ~92% for all tested approaches. RF shared the highest number of SNP with FST method (19 SNP). Chromosome 3 harboured the largest number of selected SNP across all panels. Six SNP resulted in common among the tested approaches resulting in a correct assignment performance of ~69%. For the first time SNP-array technology and machine learning were combined to identify population informative markers in trout species. Further studies with larger populations and samples size are required to evaluate the validity of the approach.

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## P104

### Genetic biodiversity study in Mediterranean trout population using SNP array: a case study in Molise region

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Mediterranean trout (*Salmo cettii*) is a freshwater fish with economic significance for fisheries management, aquaculture, and conservation biology. Unfortunately, native trout populations' abundance is significantly threatened by anthropogenic disturbance and the introduction of commercial hatchery strains of *Salmo trutta* for recreation activities has compromised the genetic integrity of native trout. This work assesses the genetic structure of Mediterranean trout of the main streams of Molise region (Biferno and Volturno rivers), considering that no previous genetic population structure data are available. Ultimately, this research aims to provide useful information to be considered in conservation management. In total, 288 specimens were caught in 30 different sites (15 per basins). Adipose fin tissues were collected and DNA isolated. Samples were genotyped using the Affymetrix 57K SNP-array. Sample and SNP quality control were applied considering no-call threshold of 0.05 and missing data cut-off of 0.90. SNP with a minor allele frequency below 0.05 were discarded. Since no animals were discarded the final dataset consisted of 288 specimens and 920 polymorphic SNP. Population differentiation was analyzed using global weighted average F-statistic over loci and pairwise FST among sampling sites. All tests were implemented in Arlequin software. The SNP data set was also processed through principal component analysis and Bayesian clustering analysis using Plink and ADMIXTURE

software, respectively. Our data provided a good representation of the population structure of Molise Mediterranean trout at fine-scale and results indicate a high degree of introgression and genetic admixture among introduced Atlantic lineage and native trout, particularly on Biferno river, where the population seems to be exposed to a high risk of genetic integrity loss. In Volturno basin, a high proportion of genetically low-introgressed trout was found strictly connected with the role of artificial and natural barriers. Subpopulations within each river and their related habitats were identified. These outcomes provide useful information to preserve native species in conservation and management programs. In conclusion, this work provided an insight into the genetic relationships and spatial distribution of populations in Molise rivers and the first use of SNP-array technology in research of Italian trout population.

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## P105

### Relationship between somatic cell score and milk production in Spanish Florida goats

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Genetic improvement of Spanish dairy goats is basically based on milk production traits. Selection for high milk production and ignoring its relationship with other traits in selection programs could lead to undesirable outcomes in the correlated response of certain traits such as fertility and health. During the last years, there has been a growing interest in estimating genetic parameters of other important traits for farm profitability, such as female fertility and mastitis resistance. Mastitis is one of the major diseases in dairy species causing substantial economic losses for dairy farms. Selection for improved resistance to mastitis is commonly done by selecting against somatic cell count (SCC), a trait strongly correlated with mastitis. Herein, the aim of this study was to estimate the genetic parameters of somatic cell score (SCS) and to determine its genetic relationship with milk yield (MY) in Spanish Florida dairy goats. Pedigree and

production records were provided by the National Association of Florida Goat Breeders. Animals from this breed are raised under semi-intensive production systems. Test-day SCC was transformed to SCS by logarithmic transformation to achieve normality of distribution and a mean lactation somatic cell score (LSCS) was computed as the geometric mean of SCS. In total, 162,127 lactation records from 56,769 Florida females were analyzed and the number of animals in the pedigree was 109,583. Covariance components and genetic parameters were estimated by fitting a repeatability animal model using the restricted maximum likelihood (REML) approach. Non-genetic effects affecting LSCS and MY were herd-year-season of lactation, parity number, litter size and days in lactation, and random effects were the additive genetic animal and permanent environment. Heritability estimates were 0.17 and 0.19 for LSCS and MY, respectively. This result shows that SCS is a heritable trait in the Florida goat breed and can be used as a selection criterion for the genetic improvement of resistance to mastitis in Spanish goat breeds. The phenotypic correlation between LSCS and MY was -0.05 and the genetic correlation was -0.01 (SE =0.09) indicating that selection for milk production that is taking place in this breed is not causing an increase in SCS.

this mutation in canine breeds and populations in Italy, supporting the usefulness of this genetic analysis to optimize medical care in dogs at risk of CEA and to create an objective basis in breeding programmes definition in breeds at risk. Genotype data for 7.8 kb deletion in intron 4 of the NHEJ1 gene from 504 dogs belonging to Border Collie (BC), Shetland Sheepdog (SS), Australian Shepherd (AS), Nova Scotia Duck Tolling Retriever (NSDTR) and Rough Collie (RC) breeds were collected. The healthy carriers were 50%, 45%, 29.6%, 17.3% and 12.5% in SS, NCDTR, BC, AS and RC, respectively. The affected recessive homozygotes were 81.3%, 10.8% and 1.5% in RC, SS and BC, respectively. These results show that in RC there is a relevant presence of healthy carriers to be strictly monitored. On the other hand, in BC, a breed that has been undergoing a screening programme for at least 10 years, we found very few affected recessive homozygotes. The quite high percentage of BC healthy carriers is due to their use as breeders in mating with clear partners in order to limit the excessive genetic variability reduction. The results support the effectiveness of this genetic analysis to optimize medical care in dogs at risk of CEA and to adopt adequate breeding programs to monitor and prevent the diffusion of this hereditary oculopathy.

## P106

### Genotypic and allelic frequency of CEA (Collie Eye Anomaly) gene in dogs in Italy

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Collie eye anomaly (CEA) is a hereditary oculopathy affecting the development of the choroid and the sclera. British sheep herding breeds and their descendants can frequently present the analysed mutation, anyway, CEA mutation is recorded in breeds from different groups. The clinical phenotype can considerably vary: in most cases dogs exhibit no obvious clinical signs and conserve normal vision throughout life, whereas other phenotypes are defined as 'severely affected' because they can develop secondary intraocular hemorrhage, retinal detachment and blindness. CEA mode of inheritance is autosomal recessive with incomplete penetrance. Thanks to the CEA mutation genetic test, breeders can genetically identify affected recessive homozygotes, clinically healthy carriers of the mutation and clear mutation subjects. The present study is a retrospective investigation on the presence of the CEA mutated gene in Italian dog population from 2012 to 2020. At present, this is the first available survey about breed-specific distribution of the mutation in Italy. The secondary aim of the present work is to offer a deep knowledge of the presence of

## P107

### Genomic characterization of the Spanish Merino Sheep and situation of the mains historical genetics lines

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The Spanish Merino Sheep breed is formed by two lineages, the White Spanish Merino (animals with the classical white coat and commonly known in Spain as White Merino) and the Black Spanish Merino (animals with black-brown wool color, the Black Merino). The aim of this study was to characterize genetically the population of the Spanish Merino Sheep breed and the differentiation degree of the main historical lines after more than 200 years of closed mating. The analysis included 39 individuals belonging to the Black Spanish Merino lineage, and 284 belonging to the 5 historical lines of White Spanish Merino (Maesso, Egea, Granda, Lopez-Montenegro and Hidalgo). In addition, 60 White Spanish Merino animals from a commercial line focused on meat traits were included (Serena type) as divergent control. All the individuals ( $n=383$ ) were genotyped with the Axiom™Ovine Genotyping Array 50K chip. Expected (He) and

observed ( $H_o$ ) heterozygosity analysis did not revealed differences among populations with an overall value of 0.367 and 0.381, respectively. However, unsupervised admixture assay was able to discriminate seven different populations corresponding with the five historical lines, and the Black Merino and the Serena line. Among the white Merino historical lines, the percentage of individual adscription to their group varied between 100% in the Maesso, Egea, Lopez-Montenegro and Serena, and 50% in Hidalgo and Granda lines. Finally, the  $F_{st}$  values between the Black Merino and the rest of the strains varied between 0.089 and 0.123, while between the different strains the values varied between 0.047 and 0.084. In conclusion, we demonstrated the existence of a high level of variability in the Spanish Merino breed, even within the historical genetic lines suggesting a genetic influence of external populations. However, three of the historical lines were highly homogeneous, probably due to the occurrence of a closed mating since its creation. This study, which is the first carried out on the historical lines of Spanish Merino, could help to achieve a better understanding of one of the most important sheep breeds in the world.

## P108

### Genomic tools to support breed assignment in small ruminants

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Animal breed assignment is breed experts' responsibility. They identify the characteristics of different breeds described within the breed standard. Animals' registration to their respective herd-book takes place through verification of the genealogy and/or the correspondence to the breed phenotypic standards. When pedigree information is missing, an animal is assigned to a breed according to phenotypic information only. Today, genomic tools that support the traditional system of breed identification are available. We are presenting the results of the analysis of breeds' assessment with genomic tools of five Italian ovine breeds within 'Conservation, Health and Efficiency Empowerment of Small Ruminant' national project (CHEESR – PSRN 2014–2020). For the purpose of breed assignment, a total of 1122 animals belonging to five different ovine breeds (Comisana, Massese, Fabrianese, Delle Langhe, Gentile di Puglia) were genotyped with the OvineSNP50beadchip. The quality check was performed by Plink

software applying the following thresholds: minor allele frequencies ( $<0.01$ ), missing genotype ( $>0.05$ ), missing per individual ( $>0.05$ ). Furthermore, the individuals' probability of assignment to each K group (Q-values) was analysed with Admixture software and described with JMP software.

The analysis showed high median Q-values for Massese, Comisana, and Delle Langhe sheep, respectively equal to 0.98, 0.95, and 0.95 with an interquartile range (IQR) equal to 0.95–1, 0.91–0.98, and 0.88–0.95. On the other hand, Fabrianese sheep showed more variable values, with a median equal to 0.75 and an IQR equal to 0.61–0.91. Furthermore, Gentile di Puglia breed showed an intermediate median value equal to 0.85 with an IQR equal to 0.69–0.95. Finally, some animals showed low probability of assignment and the lowest values were detected in Fabrianese breed. The results indicate a good agreement of the breed's assignment, in particular for Massese and Comisana breeds, counting about 400 animals for each breed, while in the other breeds, counting 100 animals, also animals with a low probability of assignment were detected. With a properly constructed and updated reference panel containing all Italian ovine breeds or other breeds used in crossbreeding, it will be possible to combine the expert's evaluation with a genomic analysis that allows to discriminate doubtful cases.

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## P109

### Genomic inbreeding parameters in the autochthonous Reggiana and Modenese cattle breeds

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Reggiana and Modenese are autochthonous dual-purpose cattle breeds reared in the Parmigiano-Reggiano cheese production area. After the setting up of the herd book in the 1960s, inbreeding has been considered in mating plans. Inbreeding is traditionally estimated using pedigree information (FPED). Commercial platforms for high-throughput genotyping allow investigating inbreeding directly using genome information. One of the most effective methods to estimate inbreeding is based on runs of homozygosity (ROH), which can be defined as long homozygous genome regions detected with high density single nucleotide polymorphism (SNP) genotyping tools. The total proportion of



ROH on an individual genome is defined as FROH. In this study, we analyzed and compared the inbreeding rate of Reggiana and Modenese breeds with several genomic approaches. The study included 1684 Reggiana cattle and 323 Modenese cattle that were genotyped with the GeneSeek GGP Bovine 150K SNP panel. Filtering and data analyses were carried on with PLINK 1.9 and in-house scripts. FPED was calculated with Inbupg90 tools. Genomic inbreeding parameters calculated were: (i) F on ROH basis; (ii) observed versus expected homozygosity; (iii) genomic relationship matrix diagonal; (iv); correlation between uniting gametes. FROH was obtained considering different ROH length classes: ROH  $\geq 1$  Mb (FROH1), ROH  $\geq 8$  Mb (FROH8) and ROH  $\geq 16$  Mb (FROH16). Pearson's correlations between all inbreeding coefficients were calculated. FPED values in the Reggiana population ranged from 0.020 to 0.310 with a mean of 0.075 and FROH1 ranged from 0.001 to 0.380 with a mean value of 0.066. In Modenese, the minimum FROH1 was 0.003 and the maximum was 0.360 with a mean of 0.088, while FPED ranged from 0.00 to 0.270 and its mean value was 0.023. Correlations between FPED and FROH methods ranged from 0.170 to 0.195 in Reggiana and from 0.261 to 0.323 in Modenese. Despite the low correlations, both FPED and FROH depict a situation of low to moderate inbreeding. The high proportion of short ROH over the total number of ROH indicates no recent inbreeding events in both breeds.

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## P110

### Linear evaluation in Maremmano horse breeding value estimation

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Morphological scoring is a common evaluation method in domestic animals; assessors must be able to translate in numbers or words their judgement on a set of features of the animal body by comparing a subject to the 'ideal type' described in the breed standard. The study was carried out on a dataset provided by the National Association of Maremmano Breeders (ANAM) consisting of 600 horses (40 males, 560 females). For each animal, the variables considered were 4 biometric (cm) and 24 linear score (15 classes) traits; also, body condition score (5 classes) was

considered. The horses were grouped in three classes of age: CLASS 1 (young animals, 2–4 years), CLASS 2 (adult animals, 5–15 years), CLASS 3 (mature animals, > 15 years). A factorial analysis (PROC FACTOR, SAS) was carried out to obtain from the variables a few very informative factors and compute a unique suitable Best Linear Unbiased Prediction-Animal Model-Multiple Trait (BLUP AM-MT) index; the number of factors was fixed to 3. A mixed model analysis was carried out to estimate the genetic indexes from the three factors considered as fixed effects: sex, judge, BCS and class of age. The breeding value of each horse (BVH) was computed adding the genetic indexes of the three factors, each one multiplied for a coefficient indicated by ANAM according to the selection plan of the breed:  $BVH = (0.4 \times \text{Factor 1}) + (0.3 \times \text{Factor 2}) + (0.3 \times \text{Factor 3})$ . The practical feasibility of BVH was evaluated through Spearman correlations (R software) between the ranks of BVH and both the ranks of BLUP-AM-MT estimated through the 4 biometric measures and the rank of the morphological score (MS) assigned to each horse by ANAM's judges. The factorial analysis allowed to estimate three factors called 'Trunk Dimension', 'Legs' and 'Length'; because the explained variance was 32% only, the model was rotated (VARIMAX) and the heritability of the three factors were 0.57, 0.05 and 0.41, respectively. After the rotation, the Spearman correlations estimated between the new BVH and the biometric measures improved, ranged between 0.41 (BVH – Withers height) and 0.20 (BVH – Chest circumference); low (0.12), was the correlation of BVH with the morphological score. These results encourage estimating a breeding value index that takes into consideration factors derived from the traits observed in the morphological evaluation of Maremmano horses; farmers can use the index to choose the best animals for mating plans.

## P111

### Genome wide association studies for several morphological traits and defects in the Reggiana cattle breed

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Reggiana is an Italian autochthonous cattle breed reared mainly in the Emilia Romagna region (North of Italy), with a high density of farms in the province of Reggio Emilia, in the well-known worldwide Protected Designation of Origin Parmigiano-Reggiano cheese production area. Milk of this breed is almost exclusively utilized to produce a mono-breed Parmigiano-Reggiano cheese. Here, we took advantage from the phenotyping data collected in Reggiana cattle and investigated association of single nucleotide



polymorphisms (SNPs) and haplotypes with several exterior traits and defects, including different grades of red coat colour, coat colour patterns, muzzle colour, stature, presence/absence and number of supernumerary nipples and length of nipples. DNA was extracted from blood and SNP genotyping was performed with the GeneSeek GGP Bovine 150k Array. PLINK v1.9 was used for quality checks. Haplotype estimation was carried out with SHAPEIT v2. The final dataset comprises 1776 animals, 124,482 SNPs and 404,061 haplotypes. Single-SNPs and haplotype-based genome scans were performed with GEMMA v0.98 via linear mixed models. Animals presented three main intensity of red of the coat colour (red, 83.7%; light-red 10.7%; dark-red, 5.6%). Association analysis for this trait pointed out two main significant regions on bovine chromosome (BTA) 5 and BTA13. Piedbaldism was observed in 4.3% cattle and associated with KIT gene markers (BTA6). The association study for muzzle colour reported that this trait is highly dependent on alleles at the MC1R gene (BTA18). Stature of the animals (in the range from 130 to 156 cm) was associated with SNPs in two regions harboring genes that have been already shown to affect this trait in several species: LCORL (BTA6) and PLAG1 (BTA14). A suggestive peak of association for the number of extra nipples was evidenced on BTA10. A suggestive peak of association on BTA8 was identified for the length of nipples. Overall, the identified genomic regions not only can be directly used in selection plans but also contribute to clarify the genetic mechanisms involved in determining exterior traits in cattle.

#### Acknowledgments

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## P112

### Milk protein gene variability in Reggiana and Modenese cattle breeds

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Reggiana and Modenese are considered dual purpose cattle breeds that are however almost completely dedicated to the production of Protected Designation of Origin Parmigiano-Reggiano cheeses. It is well known that milk protein polymorphisms affect milk production traits and cheese making properties. The constant monitoring of milk protein genetic variant diffusion in cattle populations is essential to avoid the increase of the frequency of mutations with unfavorable effects on milk and cheese

production traits. Aim of the present study was to analyze variability of beta-casein (CSN2), k-casein (CSN3) and beta-lactoglobulin (PAEP, previously indicated as LGB) genes in the two local breeds. A total of 1684 Reggiana and 340 Modenese cattle were genotyped. Genotyping was obtained for single nucleotide polymorphisms (SNPs) causing missense mutations responsible for eight variants/variant combinations (A1 + A2, A3, B, C, E, F, H1, and H2 +I) within the CSN2 gene, four variants (A, B, C, and E) of the CSN3 gene and two variants (A and B) of the PAEP gene. At the CSN2, the A1 + A2 alleles (it was not possible to distinguish the two forms) resulted the most frequent in both breeds (Reggiana: 0.75; Modenese: 0.64), followed by B (0.19 and 0.25) and C (0.05 and 0.11) alleles. At the CSN3 gene, an SNP corresponding to allele B, well known to be associated with better cheese-making attitude (i.e. favorable renneting time and curd firmness), resulted the most frequent in both breeds (0.55 and 0.70). Allele CSN3\*C was not identified, whereas allele CSN3\*E, associated with negative effects on rheological traits, was detected at very low frequency (0.02 and 0.01). As for the gene encoding for the principal whey protein, the frequency of two alleles was opposite in the two breeds: PAEP\*A allele was the most frequent in Reggiana (0.61) and PAEP\*B allele was the most frequent in Modenese (0.68). This study reported the results of the most comprehensive analysis of milk protein gene variants in these two breeds carried out thus far. The results will be useful to implement selection strategies oriented to improve cheese production and quality.

#### Acknowledgments

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## P113

### Comparing signatures of selection in Reggiana and Modenese cattle breeds

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Reggiana and Modenese (the latter also known as Bianca Val Padana) are two local cattle breeds mainly raised in the Emilia-Romagna region, in the North of Italy in two close provinces from which their names come from. These breeds mainly differ by their characteristic coat colours: red in Reggiana and white in

Modenese. In this study, we compared at the genome-wide level these two breeds to identify signatures of selection that could outline genome regions that could contribute to genetically differentiate these two breeds. A total of 1109 Reggiana and 326 Modenese cattle were genotyped with the GeneSeek GGP Bovine 150k Array. Multidimensional scaling plot and ADMIXTURE analyses indicated that the two breeds are well separated. Pairwise  $F_{st}$  analysis using single markers identified several single nucleotide polymorphisms with the highest  $F_{st}$  values on bovine chromosome (BTA) 18, in the MC1R gene that is well known to affect coat colour. Averaged  $F_{st}$  analysis in chromosome windows of 1 Mb confirmed the results of the single-marker  $F_{st}$  analysis and identified several other chromosome regions that may contain genes that can explain part of the genetic differences between these two cattle breeds, including genes affecting stature and morphological structure, coat colour and milk production traits. By exploiting local genetic resources, this study provided information that could be useful to explain the genetic architecture of some contrasting features in cattle.

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## P114

### Analysis of genetic diversity and signature of selection in commercial and fancy rabbit breeds

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The European rabbit (*Oryctolagus cuniculus*) is a multi-purpose species used for meat and fur production and considered a fancy animal used for show purposes. For this reason, several commercial and fancy breeds have been developed, mainly over the last century, starting from recently domesticated genetic stocks. Fancy breeds can be mainly distinguished by many different coat colours and patterns, fur structure, and morphometric traits, i.e. body size, shape and position of the ears, the structure of the skull, among other characteristic features. This study aimed to identify signatures of selection in the rabbit genome that could highlight candidate genes whose variability might contribute to explain

the diversity of exterior traits that distinguish some commercial and fancy rabbit breeds. A total of 660 rabbits were genotyped using the Affymetrix Axiom OrcunSNP Array, which can analyse 199,692 DNA markers. These animals were from 12 fancy rabbit breeds (Belgian Hare, n. = 24; Burgundy Fawn, 6; Champagne d'Argent, 19; Checkered Giant, 79; Dwarf Colored, 20; Dwarf Loop, 20; Ermine, 20; Giant Grey, 27; Giant White, 20; Rex, 19; Rhinelander, 28; and Thuringian, 9) and three Italian commercial rabbit breeds (Italian d'Argent, 20; Italian Spotted, 93; and Italian White, 256).  $F_{st}$  analyses were performed using sliding genome window methods based on a single-breed approach (i.e. one breed against all other breeds) or by grouping breeds based on common pigmentation or morphometric features. Our results pointed out many genome regions harboring signatures of selection and including several coat colour genes already described to affect pigmentation in rabbits (ASIP, MC1R, and TYR) and coat structure (LIPH). In addition, several other regions contain genes involved in body structure/stature, fat deposition, growth and reproduction performances. This study added information that can contribute to clarifying the genetic mechanisms determining phenotypic differences among rabbit breeds.

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## P115

### Variability in genes affecting morphological traits in the Greek black pig breed and conservation of this genetic resource

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Autochthonous pig breeds of Mediterranean regions are genetic resources that are well adapted to the harsh and hot environmental conditions of this area. These breeds might represent important reservoirs of genetic variability that could be exploited in the context of climate change. The Greek black pig is a local breed mainly raised in Thessaly and Macedonia regions whose origin goes back to the ancient times. The desertification of Greek countryside occurred between 1955 and 1960 and the migration of the inhabitants to large urban centers caused a harsh extinction of this breed and the import of well-known foreign pigs. About 20 years ago, a conservation program for this breed started from

only ~100 animals found in the whole country. Pigs of this breed are characterized by a solid black coat colour phenotype. The current population is constituted by a few small nuclei of pigs bred in extensive or semi extensive production systems where genetic hybridization with wild boars could frequently occur. This breed has not been characterized at the molecular genetic level yet. In this study, we analysed polymorphisms in five genes affecting coat colour and colour patterns (MC1R and KIT), growth rate and lean meat/fat deposition (IGF2) and vertebral and teat number (NR6A1 and VRTN) in a total of 59 Greek black pigs (7 boars and 52 sows). At the MC1R gene, the most frequent allele was the European dominant black ED2 (0.70). Other alleles at this gene were identified but always in heterozygous state with the dominant allele that might confer the characteristic black coat colour of the analysed animals. A new ED2 variant was also identified in three pigs. We also detected the presence of the KIT gene polymorphism associated with the belted phenotype in two animals and the KIT gene duplication in one pig. At the IGF2 gene, allele g.3072A had a frequency of 0.86. The domestic allele g.265347265T of the NR6A1 gene was the most frequent (0.84). The wild type C allele at this polymorphic site was detected in 11 pigs. Finally, the SINE insertion of 291 bp in the VRTN gene, associated with an increased number of vertebrae and teats, had a frequency equal to 0.45. The genetic variability detected in the five investigated genes can confirm, to some extent, the occurrence of introgression events with both wild boars and other pig populations, further strengthening the need to develop appropriate conservation programs of this autochthonous Greek pig breed.

## P116

### A map of the distribution of *Apis mellifera* mitochondrial DNA lineages in Italy

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Two *Apis mellifera* subspecies have been described to be native and endemic to Italy: *A. m. siciliana* (A lineage), originally populating Sicily and surrounding minor islands, and *A. m. ligustica* (C lineage), known as the Italian honey bee and widespread along the Peninsula. Other two subspecies have been reported mainly in border regions: *A. m. carnica* (C lineage), mainly described in the North-East regions of Italy, and *A. m. mellifera* (M lineage), mainly reported in the North-West borders and in several Alp valleys. Movements of honey bee populations due to beekeeping activities and crossbreeding programs have contributed, more recently, to distribute different lineages and hybrids all over Italy. In this study, we produced a first comprehensive map of the distribution of the three main mitochondrial *A. mellifera* lineages

(A, C and M) along the Italian Peninsula, Sardinia and Sicily. The study took advantage of the possibility to analyse honey bee DNA recovered from honey. A total of 549 honey samples produced by different beekeepers in all Italian regions were included in this study. DNA was extracted from these samples and amplified using an optimized PCR protocol for degraded DNA. This PCR method was developed to detect a specific informative region of the honey bee mitochondrial DNA (mtDNA). Then, PCR products were subjected to gel electrophoresis to assign the corresponding mitotypes. Several DNA fragments were also sequenced to confirm the expected sequence. The most frequent mtDNA lineage in all Italian regions was lineage C with the exception of Sicily, where the most frequent mitotype belonged to lineage A. Sicily was also the region in which the A lineage was found with the highest percentage among all other investigated regions or provinces (>60%). This result confirms the African origin of the Sicilian original *A. mellifera* genetic stock. Lineage M was observed in most regions but it was never the prevalent mitotype. This work provided the most recent and updated assessment of the diffusion of different mtDNA lineages in Italy. The results obtained can be useful to support conservation programs of different honey bee subspecies in Italy.

## P117

### Mining shotgun sequencing of honey DNA to identify *Apis mellifera* genome variability

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Honey is a food matrix produced by honey bees (*Apis mellifera*) from the nectar of specialized plant structures. During their activities, honey bees get in close contact with different organisms present in their ecosystem, whose traces are transferred into honey. Therefore, honey represents an interesting reservoir of environmental DNA (eDNA), including *A. mellifera* DNA. This allows to study the genome variability of these insects directly using metagenomic data produced from honey DNA. With this aim, we specifically designed a DNA mining methodology of deep shotgun sequencing datasets produced from a few honey samples. DNA was extracted from honey, sequenced on a BGISEq500 machine and analyzed via a bioinformatic pipeline that selected reads belonging to *A. mellifera*. To extract information useful to assign the subspecies of *A. mellifera*, reads were mapped over the Amel\_HAV3.1 reference genome. We evaluated both the mitochondrial DNA (mtDNA) haplotype variability of the region NC\_0015661:3363–3447 (that discriminates the A, C and M honey bee mtDNA lineages) and the nuclear genome variability



considering a set of 144 ancestry-informative single nucleotide polymorphisms (SNPs) and the related allelic frequencies in different honey bee sub species and populations, including *A. m. carnica*, *A. m. ligustica*, *A. m. mellifera*, Buckfast, hybrids and artificial DNA pools. We produced more than 90 million reads assembled into 341,370 contigs (average size of 1222 bp). Honey samples showed an mtDNA coverage >99%, with a read depth in the range of 24–831×. Only reads compatible with the *A. mellifera* C lineage were identified at the mtDNA level. The nuclear genome presented a coverage and depth of sequencing in the range of 8–98% and 0.4–10×, respectively. For one sample it was possible to obtain information for 106 out of 144 discriminant SNPs. The clustering analysis placed the *A. mellifera* DNA obtained from this honey close to *A. m. ligustica* (C lineage). Information of variability at the complementary sex determiner (CSD) gene was retrieved from all samples. Overall, our study demonstrated that shotgun sequencing of honey eDNA can be useful to identify genome variability of the honey bees that produced the honey and for the identification of the honey bee subspecies.

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## P118

### PigPhenomics: metabolomics merged with genomics to develop novel breeding and selection approaches in pigs

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Filling the gap between phenotypes and genotypes is crucial for a proper understanding of biological complexity of an organism. This generates more interest when disentangling biological complexity can be directly applied to the development of genetic tools that can guide animal breeding, selection and management decisions for a more sustainable animal-production sector. A comprehensive and systematic collection of phenotypes, as well as the production of genomic data, will help to bridge the gap between genetic variability and the production traits of the animals. Omics techniques have changed the way to investigate the complexity of biological systems. In the last years, the availability of more and more sophisticated high-throughput sequencing (genomics, transcriptomics) and phenotyping technologies (proteomics and metabolomics) coupled with the massively amount of produced data, has spawned 'phenomics', the use of large scale approaches to study how genetic instructions translate into the full set of phenotypic traits of an organism. In addressing this

challenge, we applied a phenomic approach in two heavy pig breeds. We collected and generated phenotypic data including production traits (endpoint information) and blood metabolites (intermediate phenotypes). Metabolomics information was obtained using both mass spectrometry (MS/MS) and nuclear magnetic resonance (NMR) analytical pipelines. About 1000 small compounds belonging to different biological classes including amino acids, amines, carbohydrates, peptides, lipids and sugars were identified and quantified. Genomic data were obtained from the Illumina Porcine 60K e 70K BeadChips and from whole-genome sequencing data. Phenotypes and genotypes were linked together via univariate and multivariate statistics. Genome scans evidenced many genomic regions controlling metabolite levels as well as effects on production traits. Re-sequencing data were used to identify causative mutations. The metabolic network reconstruction and the related cluster analyses led to identify common and specific features characterizing the different pig breeds/lines. Overall, the obtained results not only can be useful to better understand the genetic architecture of physiological traits in pigs but also represent a first effort to establish genetic tools for precise pig breeding and selection programs.

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## P119

### Comparing morphometric data and mitotype information for the identification of *Apis mellifera* subspecies

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Honey bee subspecies can be identified using classical morphometry that in its complete and most precise analysis is based on measurements of 36 traits, which include the size of various body parts (forewings, abdomen and legs), colour and pilosity. Simplified morphometric analyses based on reduced number of measurements are routinely applied. Molecular analyses have been also proposed to complement morphometric classifications and eventually as alternative methods. Some specific mitochondrial DNA (mtDNA) lineages have been associated with morphometrically assigned subspecies, which almost completely overlap with the corresponding geographical lineages. Honey DNA can



be used to identify honey bee mtDNA lineages of the colony that produced this food matrix. *Apis mellifera ligustica* is an endemic honey bee subspecies native to the Italian peninsula, which might carry the C1 and, in a few cases, the M7 mtDNA haplotypes. Conservation of this subspecies is threatened by beekeeping activities and crossbreeding programs. Regional legislative initiatives have been recently developed to promote the conservation of this honey bee genetic resources. In this study, we evaluated the proportion of colonies belonging to *A. m. ligustica* in Emilia-Romagna region (North of Italy) using classical simplified morphometry and matched this information with the mtDNA lineages of the same colonies detected using different approaches: analysis of honey DNA with two amplified mtDNA regions; analysis of two informative mtDNA regions in one or more individual workers for each family. A total of 80 colonies have been morphometrically analysed and 40% of them could not be assigned to *A. m. ligustica*. Analysis of honey DNA reported that five of these colonies had mtDNA from the lineages A and/or M. Analysis of the mtDNA COI-COII intergenic region in individual workers identified six different mitotypes. Partially contrasting outcomes were obtained when the results of the applied methods were compared, complicating the final assignment of the colonies to the *A. m. ligustica* subspecies.

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## P120

### Whole genome sequencing analyses identified genomic regions affecting genetic defects in cultivated gilthead seabream (*Sparus aurata*)

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The gilthead seabream (*Sparus aurata*, Sparidae family) is an important aquaculture species of the Mediterranean region. One of the major problems of farmed gilthead seabream is the high frequency of morphological defects, which might have genetic causes which effects arise during the early developmental stages. In this work, we applied a whole genome sequencing approach based on DNA-pool seq to identify genomic regions affecting two abnormalities that occurred in a cultivated *S. aurata* population: a coloration defect and the lack of operculum. Three DNA-pools were constructed from offspring of the same broodstock nucleus using each equimolar DNA extracted from about 20–30 fry: one DNA-pool was constructed from fry having a reduced coloration of the whole body, another DNA-pool was constructed using DNA from fry that lacked the operculum and the last was composed by normal fry and used as control against the two other DNA-pools that constituted the cases. *Fst* analyses obtained comparing variability in sliding genome windows in the two case and control experimental designs identified an *Fst* peak on chromosome 6 containing markers associated with the discoloration defect and a region on chromosome 2 associated with the lack of operculum defect. The results of this study have practical applications, opening the possibility to eliminate these defects from the breeding stocks, with direct economic advantages derived by the reduction of discarded fry. Other studies are needed to identify the causative mutations determining these abnormalities in the cultivated gilthead seabream population.

## P121

### A genomic approach for herd management – the GENORIP project

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It is well known that the increase in inbreeding may lead to a variety of negative effects, such as inbreeding depression, loss of genetic variability, and higher frequency of homozygous and deleterious genotypes. The reduction of genotyping costs allows farmers to use genomic information, such as SNP chip data, to monitor inbreeding within their farms. 'GENOMIC tool for the management of REProduction in dairy cattle and for the control of inbreeding – GENORIP', a 3 years project funded by Regione Lombardia GO-PEI action, will release an innovative tool for the control of inbreeding, the maintenance of genetic variability and for facilitating the choice for animal replacement based on genomic information. Interconnected partners of GENORIP are farmers-researchers-industry. Six Holstein dairy farmers, ARAL and INSEME S.p.a. are directly involved together with the

University of Milan in the 'step by step' project development. During the first year of the project 2183 Holstein females of five farms were sampled using Flexoplus Geno biopsy ear tags and genotyped by the Neogen laboratory (UK) with the DNA chip GeneSeek Genomic Profiler (GGP) Bovine 100K. A principal component analysis (PCA) based on genotypes was performed using the SVS 8.9 software of Golden Helix to assess the genetic diversity within and among farms. For all bovine of each farm two genomic inbreeding coefficients were estimated based on the difference between the observed and expected numbers of homozygous genotypes (FHOM) and based on the Runs of Homozygosity (FROH), respectively. The FHOM values show that farmers pay attention in controlling inbreeding in their farms: the average values obtained in the first five farms were slightly negative in four of them ( $-0.01/-0.02$ ) and zero in the fifth one. The FROH represents the proportion of genome tracts of minimum length equal to 1 Mb with all markers in homozygous state over the total length of the genome. The average value of FROH is similar in all the farms, ranging from 0.11 to 0.13. This shows the homogeneity in the selection process of males and females in the Holstein breed across all farms. The PCA showed the existence of genomic variability in each of the farms and clusters of females clearly separating.

#### Acknowledgements

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## P122

### Genomic analyses for adaptation of Italian cattle breeds: preliminary results. The BOVITA Project

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Climate change will impact diverse geographic areas, including the Mediterranean region. Understanding the adaptive ability of livestock to climate variations has become a crucial focus in

biodiversity conservation and management. Cattle breeds are valuable genetic resources to understand and study the molecular mechanisms of adaptation to their specific environments. Several landscape genomics approaches have been proposed to detect adaptation to different environmental pressures including correlative genotype-environment associations. The BOVITA project aims at characterizing the genetic basis of adaptation for 755 individuals from 30 Italian local cattle breeds. Here we present the first results of a joint analysis of genetic and climatic data. By considering Bovine 50K SNP genotyping data and four climate variables data from Climond database (i.e. annual temperature, annual precipitation, annual mean radiation, and annual mean moisture index). To identify genomic regions harboring footprints of selection, a whole-genome scan for adaptive differentiation was performed using genotyping data with the XtX model implemented in the BAYPASS software. In addition, whole-genome scans for association with the population-specific climatic variables were performed using the AUX model. Footprints of selection were detected on BTA1, BTA4, BTA5, BTA6, BTA7, BTA12, BTA13, BTA14, BTA16, BTA20 pointing out several candidate genes (e.g. ST3GAL6, NUDCD3, CCND2, ABCG2, LCORL, VDAC1, MIR466B-2, CTNNB1, XKR4, CDC42BPA, SLC45A2, RXFP3); moreover, different genomic regions (on BTA7, BTA19, and BTA20) were associated with the annual mean moisture index. Ongoing analyses will specify candidate regions and genes involved in local adaptation.

#### Acknowledgements

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## P123

### Type trait evolution of the Italian Mediterranean Buffalo: preliminary results of the last 15-year selection

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The linear type trait evaluation for the Italian Mediterranean buffalo (IMB) was introduced by the Italian National Association of Buffalo Breeders (ANASB) at the beginning of the 2000s. The proposed chart contains 23 traits, and it has been used for the admission to the herd book of both buffalo cows and bulls. Despite this, the morphology was captured in the selective process only in 2018, with the new aggregate index IBMI which include udder

and limbs and feet scores. The aim of this study was to evaluate the trend of type traits in the last 15 years when only productive selection and improved management system have been applied. For this purpose, the ANASB dataset containing type trait evaluations of the female buffalo carried out between 2006 and 2020 was used. The dataset accounted for animals born between 2004 and 2017 and scored at 2–6 years of age. Data were analysed (IBM SPSS Statistics 26.0.0.1/2019) with a model that included the birth year, the type of mating (born from artificial insemination (AI) with progeny tested bull or from natural services with farm bull), the age at the evaluation as the linear covariate, and the experts as a random effect. The analysis showed that morphology changed over time, but not always linearly for many traits. Because the change was significantly more marked for some traits in buffaloes born from AI, this indirect selection process could probably be attributed the improvement of both the management system and the address of genetic selection toward milk yield for these traits. Some traits showed a positive trend for morphological traits, in agreement with the breeding objectives of the IMB selection process, but other traits presented changes in the opposite way, i.e. negative trends. The new aggregate IBMI index aimed at improving some of these traits, but the knowledge of indirect selection applied for type traits highlight important objective to be implemented in the future to optimize breeding goals in Italian Mediterranean buffaloes.

## PRECISION LIVESTOCK FARMING

### P124

#### Evaluation of a collar monitoring activity and rumination for detecting estrus and health issues in an organic dairy farm

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Early detection of estrus and health issues in dairy cattle are crucial for enhancing animal welfare and boosting profitability. Smart technologies have been recommended for monitoring heat and health minimizing labor requirements (Paudyal et al. 2018; Schweinzer et al. 2019). This study aimed to assess the suitability of the SCR HeatTime<sup>®</sup>HR system to detect estrus and health issues in dairy cows. The study was conducted on an organic dairy farm in North Italy, housing approximately 850 Holstein-Friesian cows; 200 SCR HeatTime<sup>®</sup>HR neck collars were used for continuously monitoring activity and rumination in the group of fresh

cow. The system applying algorithms to movement intensity patterns and rumination time generated heat and health alerts calculated based on a deviation in current data from historical data. From October to December 2020, 314 events were recorded concerning 146 cows. The events included clinical examinations and/or heat and health alerts. The events were treated as 'gold standard' for detection of estrus and health issues if clinical and behavioral manifestations of heat and a health issue were, respectively, revealed by the authors. The events were classified as true positive, true negative, false positive or false negative depending on concordances and discordances between gold standard and alerts. Statistical measures of the performance of a binary classification test were calculated. Sensitivity, specificity, positive and negative predictive values, and accuracy for estrus detection were 96.3%, 97.0%, 91.8%, 98.7%, and 96.8%, respectively. These values are in line with those recently found on a Slovakian commercial dairy farm (Schweinzer et al. 2019). The same measures calculated for health issue detection were instead 22.7%, 95.6%, 73.5%, 69.6%, and 70.1%, respectively. The system did not recognize issues related to respiratory and reproductive systems while alerted for lameness (28.6%) and diarrhea (64.5%). In Florida, a higher sensitivity for mastitis (53%) and lameness (59%) was found using rumination loggers (Paudyal et al. 2018). The reasons for this disagreement may be due to the different algorithms applied and to the greater accuracy of our clinical examinations and behavioral monitoring. Overall, the investigated system showed excellent performance in estrus detection while accurate clinical examinations and direct monitoring of the farmer seems to be still required for sensitive detection of health issues.

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### P125

#### Effects of precision diagnostics on mastitis control

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Bovine mastitis causes huge financial losses for dairy farmers, identification of etiological agents is essential to control the disease, reducing the risk of chronic infections and targeting antimicrobial therapy.

This research aimed to improve mastitis control into the University of Bologna dairy farm, through the application of a protocol for precision diagnostic, from June 2020 to February



2021. The study mainly focused on *Streptococcus uberis*, being this the main problem for this dairy farm. The herd (113 animals) was sampled at the beginning and at the end of this study, to evaluate the efficacy of the protocol.

Lactating cows were sampled, at mammary quarter level, 10 days after parturition, across lactation when showing clinical or sub-clinical mastitis symptoms, after mastitis treatment, and at dry-off. Moreover, an environmental microbiological survey for *S. uberis* was done by sampling bedding, drinking water, animal skin, feces from rectal ampulla, and milking parlor. Milk samples were struck onto blood agar (5% horse blood) and incubated at 37 °C in aerobiosis, while environmental samples onto Edward's modified agar for *S. uberis* count. After 24h, colonies were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). For some strains, isolated from animals and environment, antimicrobial susceptibility test was performed by disc diffusion method, testing the most used molecules for mastitis treatment.

*Streptococcus uberis* and coagulase-negative Staphylococci were the most frequent isolates from milk samples and *S. uberis* was isolated from bedding, drinking water, and animal skin with a higher rate in dry-off and post-partum areas. The results of the antimicrobial test were very similar between animal and environmental isolates and, a high rate of recovery in animals treated with amoxicillin/clavulanic acid and first-generation cephalosporine was observed. From this study appears, as already described by other authors, that dry-off and post-partum were the most critical areas.

Considering all the data, the occurrence of *S. uberis* went from 24.7% to 21.2% at the end of the study confirming that the reduction of the herd, the selective treatment of mastitis held by *S. uberis*, combined with improved environmental management, led to better mastitis control.

However, to evaluate the efficiency of this protocol a longer observation period is needed, and further studies by subtyping will be necessary for a correct epidemiological evaluation.

## P126

### A bioinformatic pipeline for image analysis of varroa related traits in honeybees comb images

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In honeybees, *Apis mellifera*, hygienic behaviour is the uncapping and removing of dead and diseased larvae and pupae from uncapped brood cells. In times of honeybee declining worldwide, beekeepers study hygienic behaviour manually quantifying removal of freeze-killed larvae from uncapped cells. Manually counting uncapped cells in comb images is time-consuming and prone to error.

Focus of this study is to design an automated pipeline for the segmentation of honeybee comb images.

For this purpose, honeybee comb images were acquired, selected, and analysed through digital image processing techniques, which must handle problems due to uncontrolled illumination conditions, differing colours, rotations, scaling, and comb sizes.

More precisely, for simultaneously handling poor illuminations and differing colour conditions several colour normalization algorithms have been experimented, ranging from unsupervised colour-enhancement models to colour normalization techniques used in digital histology. Next rough segmentation of the area of interest (AoI), and the cells in that area, have been obtained by clustering followed by Hough transform for finding the circular AoI, and by binary operations for detaching attached cells in the AoI.

Analysis of the histogram plots describing the connected components in the AoI allowed estimating the mean cell areas and therefore computing an estimate of the cell counts.

Among the 127 comb images, 80 images containing limited artifacts and acquired under acceptable illumination conditions were selected as test images and allowed obtaining a correlation with manual counted cells of 0.948. The remaining 47 images, containing strong artifacts and bad illuminations conditions, were used for development and resulted in a lower correlation. The hereby generated pipeline yields an estimation of honeybee comb cells correlating with manual counted cells.

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## P127

### LATTE DIGITALE (digital milk) and environmental sustainability

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Environmental sustainability is a main challenge of the livestock farming sector. Globally the contribution of the livestock farming sector counts for 10–14% of the total anthropogenic emissions



and ruminants are the major contributors (65–77% of the global livestock emissions). Farmers will need to adopt practices to low the environmental impact, such as manure management, efficient use of feed and high animal welfare. The LATTE DIGITALE (Milk production in Lombardy region towards digital and precision livestock farming) project aims to demonstrate that the digital technologies have positive effects on the farm efficiency and the environment by improving the performances and the welfare of dairy cattle. Precision livestock farming (PLF) may help to reach the goals because a farmer can monitor in real-time a wide series of parameters regarding animals and farm. He could then be able to early detect the outbreak of health issues by taking precocious actions, to verify the accuracy and the distribution of the diet, to simplify the reproductive management and to supervise the energy consumption of the farm. This may let the animal welfare and production and the efficient utilization of resources improve, thus avoiding economic losses. The PLF technologies monitor various aspects, for example food composition (NIR) and distribution (rail-guided feed wagons, feeding distributor wagons), animal welfare (videocameras, accelerometers, microphones, neck collars) rumination rate (neck collars), behaviour (automatic milking and feeding systems, videocameras), oestrus (neck collars, accelerometers), energy consumption, environment conditions and management. The project is coordinated by CREA and involves three innovative dairy farms in the Lombardy region. Three LCA analyses of the farms are carried out before, during and after the introduction of a variety of new PLF technologies. Primary data are collected by giving the farmers a questionnaire, while secondary data are obtained by databases and literature sources. All the data are then processed with CAP'2ER<sup>®</sup> in order to quantify the potential improvement of the global warming, the acidification and the eutrophication processes and the energy consumption. The estimated carbon footprints (kg CO<sub>2</sub>-eq/kg FPCM) of these farms in the first evaluation were 0.75, 0.74 and 0.83.

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## P128

### Monitoring calf body temperature by infrared thermography: preliminary assessment of environmental effects

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Pyrexia is one of most important non-specific symptoms of disease in animals. Among cattle, calves are very susceptible to get

sick, with often a high mortality rate. Traditionally, calf body temperature is measured using rectal thermometers. Besides being time consuming, this can be very stressful for animals. Recent techniques based on thermal imaging allow to estimate the body core temperature by measuring the surface temperature of different parts of the calf body. This study investigated the possibility to use thermal imaging of the calf eye to predict core body temperature and to diagnose pyrexia.

A total of 602 thermal images of the left eye were acquired on 113 female Holstein calves (0–90 days of age; mean  $29 \pm 20$  days) with FLIR C2 thermal camera over a period of 1 year. At the same time, rectal temperatures (RECT) of the calves were measured using a digital thermometer. Thermal camera emissivity, eye-camera distance and insertion depth of rectal thermometer were 0.95, 50 cm and 8.0 cm, respectively. Environmental temperature (ET) and relative humidity in calves' pens were measured with HOBO sensors. Image analysis was performed using the FLIR TOOL software, while statistical analysis was conducted using the Matlab Statistics and Machine Learning Toolbox. For thermal images, the maximum temperature point (EYET) was selected in the area of interest (the eye plus a 2 cm area all around it).

Over the study period, ET showed minimum and maximum values of 0.3 °C and 32.5 °C, respectively, and mean RECT was  $39.4 \pm 0.6$  °C. In newborn calves (up to 15 days,  $n = 172$ ), RECT showed moderate correlation ( $r = 0.6$ ) with the last 6-hr average ET. Mean EYET was  $36.2 \pm 1.5$  °C and the correlation with RECT was 0.50. The EYET and RECT ratio varied in the range 0.74–1.0 and was highly correlated with ET ( $r = 0.79$ ). The best equation obtained through a stepwise regression includes, as parameters, EYET, calf age and ET, and can predict RECT with an RMSE of 0.45 and  $R^2$  of 0.38; this equation shows, with a cut-off of 39.5 °C, a sensibility of 56%, 81% of specificity and 67% of precision.

The preliminary results show that thermal imaging eye temperature associated with environmental parameters is a promising technique for no-contact, rapid detection of pyrexia in calves. Further analysis will be devoted to improve the accuracy of the model by investigating the influence of additional environmental parameters.

## P129

### A bibliometric analysis in precision livestock farming

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The interest through the precision livestock farming (PLF), a concept discussed for the first time in a conference held in 2001,

has grown in recent years due to its important role in the development of sustainable livestock production system. To identify the global trends of PLF in scientific research, through a bibliometric analysis of the documents published between the years 2005–2020, was the aim of this study. The research was conducted searching and combining the words precision livestock farming (431 documents) OR precision fish farming (95 documents) OR automated milking system (529 documents) in the Web of Science database. A total of 1032 publications were found and sorted by relevance; 810 documents were retained after inspection of thematic pertinence and exported as a Bibtext file to be analysed with the bibliometrix package (version 3.0.3) for R software (version 4.0.3). The annual growth rate was 17.82% with a minimum of papers registered in the year 2008 (19) and a maximum during the year 2019 (139). Most of the documents were published by USA (337) whereas Italy was classified as the eighth country with 159 publications. An extensive collaboration between countries was found, especially between Belgium and Netherlands, Belgium and Italy, Canada with Brazil. The most relevant sources for publications were the Journal of Dairy Science (132), Computer and Electronics in Agriculture (75) and Applied Animal Behaviour Science (37) which were also the most local cited sources. The words ‘cattle’ (96), ‘behaviour’ (96) and ‘dairy cows’ (82) were the most frequent Keywords Plus, while ‘precision livestock farming’ (125), ‘dairy cow’ (41) and ‘animal welfare’ (29) were mostly used as Author’s Keywords. The analysis of the co-occurrence of the Keywords Plus showed a division in three different clusters; the items with the higher betweenness values for each cluster were Cattle, Management and Behaviour respectively. These findings showed that the PLF issues are arising a growing interest in the scientific community especially regarding bovine production systems, with a leading role of ‘behaviour’ and ‘cattle’ keywords that underlines the importance of applied behaviour studies in the development of many PLF tools. However, more studies are needed in other species linked to the food production.

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### P130

#### Freezing semen of wild trout with the use of commercial medium. Preliminary study

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Sperm cryopreservation in fishes can be an additional strategy to protect endangered species. Trout cryopreservation protocols tested use a specific basic freezing extender prepared in laboratory. The objective of this study was to evaluate the performance of commercial freshwater Fish Freezing Medium (FFM). Fresh semen from pure Mediterranean brown trout (*Salmo cettii*) and its hybrid with Atlantic strains were collected in January 2019 in two different rivers and transferred in laboratory at 4 °C. After evaluation with the CASA software system, the semen was diluted 1:4 with FFM kept at 4 °C and packaged in 0.25 mL straws sealed automatically. Cryopreservation was performed using programmable freezing equipment (–13.6 °C/min until –140 °C) and then plunged in LN<sub>2</sub>. After thawing (10 sec at 30 °C), 70 µL of semen of different samples were added to 600 µL of a commercial activation medium and 2 µL placed into Leja slides counting chambers for CASA system evaluation. After warming, in *S. cettii* semen had total motility ranging around 9% while hybrid trout semen had values around 16%. The duration of movement was around 71 seconds for the native trout and around 85 seconds for the hybrid. In conclusion, the commercial FFM seems to perform well, providing acceptable semen survival rates.

### P131

#### Particle size distribution of total mixed rations and feed sorting in Calabrian dairy herds

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The particle size of the diet is one of the most relevant factors influencing rumen function as well as the selection of the most palatable components of the total mixed ration (TMR). This important diet feature can be evaluated by the Penn State Particle Separator (PSPS), a simple sieving tool used to estimate the particle size distribution (PSD) of TMR according to the following 4 fractions: long, > 19 mm; medium, 8–19 mm; short, 4–8 mm; very short, < 4 mm.

The aim of this study was to evaluate the PSD of TMR for lactating cows by PSPS and to measure both the homogeneity and the level of diet selection as a function of particle size.

The research was conducted in 13 commercial dairy farms located in Calabria (Italy). Of the 13 herds, 8 fed TMR with hay (TMR-H) and 5 fed TMR with silage (TMR-S), all provided fresh feed once a day. In each herd, 6 TMRs were screened by the PPS in order to determine the PSD and to compare it with the one recommended by the Penn State and Bologna Universities; furthermore, homogeneity was assessed by comparing the PSD at the midpoint of three equal segments along the face of the feed, while the level of diet selection was assessed by comparing the PSD in two time-points (at fresh feed delivery, t0; 24 h after feed delivery, tf). Diet selection was considered tolerable if differences between t0 and tf samples were less than 3–5% for each class of particles; this threshold was set also to evaluate the homogeneity of TMR along the feed face.

None of the evaluated diet respected the recommended PSD showing an excess of the long fraction (11 TMR), of the very short fraction (7 TMR), or of both these fractions (5 TMR). Homogeneity was good except for 3 diets, but particle selection raised some concerns in 85% of the farms due to preferential consumption of the very short fraction by cows. A decrease in the proportion of this more palatable component was associated with the selective refuse of the coarser fraction which reached high levels in 6 TMR. This research shows that the proportion of the long fraction of TMR in Calabrian dairy herds is often higher than that recommended by current guidelines. This finding, together with the high incidence of cases of diet selection, seems to suggest the need for a more accentuated cut of the forage fraction to prevent the feed sorting.

experimental trials. Samples were analysed, using reference methodologies, for crude protein (CP), starch, amylase-treated ash-corrected neutral detergent fiber with addition of sodium sulfite (aNDFom), acid detergent fiber (ADF), acid detergent lignin (ADL) and ash. Moreover, the fiber undegradable fractions (uNDF) of TMR was quantified in vitro at different timepoints (24 h, 30 h, 120 h, 240 h). Spectral data in the range from 900 to 2500 nm were collected for all these samples using a TANGO FT-NIR spectrometer. Partial least squares regression was employed to calibrate NIRS prediction models through a cross-validation, but also to validate such models on a subset including a random 30% of the total observations which were excluded from the calibration set. Different mathematical pre-treatments were also applied to the spectra data in order to identify the transformation which provided the most accurate NIRS prediction model (in terms of maximised explained variance and minimised root mean square error of prediction). The coefficient of determination in external validation (R<sup>2</sup>P) was >0.80 only for starch, between 0.60 and 0.80 for CP, aNDFom, ADF and ADL, while <0.50 for ash content. The R<sup>2</sup>P for uNDF prediction models at different timepoints ranged between 0.56 (uNDF30) and uNDF240 (0.68). The residual prediction deviation (RPD) in external validation was <2 for all NIRS prediction models, with the exception of ADF model (RPD =2.20). In conclusion, NIRS can be a feasible and rapid method for the prediction of different TMR chemical components and digestibility measures, although large samples size and variability would be desired and could contribute to increase models accuracies.

## P132

### NIRs calibration model for chemical composition and digestibility of total mixed rations for Parmigiano Reggiano ration

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The quantification of feed composition parameters, as well as feed digestibility, requires time-consuming and expensive laboratory reference methodologies. In the context of precision livestock feeding, fast, accurate and on-line methods to quantify at a cost-effective the composition and characteristics of total mixed ration (TMR) are desired tools. Therefore, the aim of the present study was to develop prediction models using near infrared spectroscopy (NIRS) for a plethora of TMR compositional traits and digestibility. A total of 205 TMR samples were collected in herds located in the Parmigiano Reggiano area in different

## P133

### Analysis of feeding behaviour in pigs fed ad libitum in an automatic management system

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Feeding behavior in pigs during the fattening phase has been extensively studied under experimental conditions. However, the diffusion of automatic feeding systems, coupled with the technology of animal recognition, allows, today, to study feeding behavior also in groups of animals housed in the same box. This provides the opportunity to assess social influences and competition for access to the feed station. Our aim was to define normal



behaviour patterns in pigs fed ad libitum in an automatic management system.

Data from 261 growing boars housed in box equipped with automatic feeding systems were analyzed. Pigs were equipped with transponders for automatic recognition and the time of stay and feed intake was recorded at every access to the feeder. Experimental animals were grouped in box containing  $12 \pm 3$  performance tested boars. The most homogeneous fattening cycles were selected taking into account the starting weight ( $40.7 \pm 9.2$  kg), ending weight ( $134.6 \pm 16.4$  kg) and duration ( $110.8 \pm 15.2$  kg) of the trials.

Feed intake (kg), intake rate per access (kg/min), total time of feeder occupation (min/visit), were analyzed with a mixed model that contained: fattening cycle (23 levels) month, hour, cycle period ( $1 = < 125$  d,  $2 = 126-150$  d,  $3 = 151-174$  d,  $4 > 175$  d) and hour-by-period interaction as fixed effects, feed efficiency (total feed intake/weight gain during cycle) and weight gain per day as covariates, animal as random effect. The average number of accesses to the feeder was  $8.15 \pm 3.50$  visits/day, with an average duration of  $10.92 \pm 7.74$  min, for an average feed intake of  $0.311 \pm 0.227$  kg.

Access to the feeder was made throughout the day, with a greater frequency at 7 and 8 am and between 4 and 6 pm. This result partially agrees with the pattern of strong diurnal food intake with two peaks at the beginning and at the end of the lighting period, reported in the literature.

Feed intake increased progressively from period 1 to period 3, and it decreased in older animals (period 4). Total time of occupation per access was constant throughout the 24 h and it showed a clear descending trend moving from period 1 to 4.

On the other hand, the feeding rate increased in a specular manner with respect to the duration and presents higher rates during the night hours, especially in animals at the end of the cycle (period 4).

The indications obtained could be of great importance in food management, health and animal welfare status.

With the growing availability of technologies of precision livestock farming (PLF) for the continuous monitoring of animal health in intensive dairy cattle farms, there is the need for new on-farm approaches, capable to integrate the information from different data sources to improve animal welfare. The aim of this study was to experiment with a novel approach by using data from both infrared thermography (IRT) prior and after milking and PLF sensors to evaluate possible relationships among them. The study was conducted at the CREA Experimental Farm in Lodi. Thermal images of udders of all milked cows were captured on five dates over a 2-mo period (from August to October 2020) using a thermal camera (FLUKE T400). Four IRT pictures per cow were taken, two pre and two post the evening milking; IRT analysis was conducted on 434 images from twenty-four cows (selected to have three different SCC pattern at monthly milk controls). Statistical analysis was performed on the maximum temperatures of the udder area (MUT) pre and post-milking, milk yield (MY), milking duration, mean and maximum flow, activity, and rumination time collected during the IRT monitoring dates; milk composition was available for two dates. Analysis of variance was conducted by testing different linear models in the attempt to explain the relation between MUT pre and post milking and the available variables. Pearson's correlations between all collected data were investigated. Results showed mean MUT measured by IRT after milking increased linearly with MY ( $P = 0.0010$ ), with a fixed effect of the date ( $p = .0016$ ); on the contrary, MUT before milking did not varied. No MUT differences were found between the SCC classes of cows. Results from Person's correlation indicated that the relations between MUT measured before and after milking and other variables were weak and not significant. The reason for it could be that udder skin temperature is affected by several factors with high variability as ambient temperature, milk yield, other than cow health status. However, data analysis permitted to confirm such variables correlated with cow physiological stage, milking features, cow activity, and rumination. From our results we hypothesized a potential for an automatic monitoring system to collect IRT images of cow udder post-milking in combination with PLF sensor data, helping to improve the monitoring of cow health status.

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## P134

### Relationship between udder termography and milking features in dairy cows

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## OMICS TECHNOLOGIES

## P135

# Influence of mycotoxin detoxifying agents in animal feed on rumen and hindgut microbiomes of dairy cows

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Mycotoxins, especially aflatoxin B1 (AFB1), have become one of the most recognised feed chain contaminants, dangerous for humans and animals. In cattle, in addition to a specific symptomatology, they are able to modify the rumen functionality worsening the general health status. Therefore, the use of feed additives that reduce the exposure of the animals to mycotoxins is considered the most economical and practical large-scale method to decontaminate mycotoxin-containing feedstuffs and to improve animal welfare.

The aim of the present study was to evaluate the effect of two mycotoxin detoxifying agents, B1 and B2, individually included in the diet, on the rumen and hindgut microbiomes of dairy cows. Thirty clinically healthy dairy cows in mid lactation were divided in three groups of 10 subjects each: control (CTR) and treated (B1 and B2). Cows received the three experimental diets for 28 days. After a period of two weeks (T1), aimed to verify the potential influence of the feed additives alone, all cows were fed with total mix ration containing a level of AFB1 close to the maximum permitted (5 µg/kg) for 14 days. Milk yield was monitored at every milking, while health status and content of mycotoxins in feed were daily monitored. For metabarcoding analysis, as well as for microbiological and volatilome analysis, rumen fluid and rectal faecal matter were collected at T1 and at the end of the experiment (T2). The V3-V4 hypervariable regions of the bacterial 16S

rRNA gene were sequenced in two MiSeq (Illumina) runs with 2 × 250-base paired-end reads.

The 16S rRNA gene analysis revealed a sharp reduction in the alpha-diversity of the hindgut microbiota between T1 and T2 in CTR animals compared to B1 and B2 cows. Concerning beta-diversity, the hindgut microbiome clearly changed between treatments and time-points ( $p$ -value = 0.01). Minor differences were instead present in the rumen microbiota both at the alpha and beta diversity level ( $p$ -value between treatments and time-points = 0.079). Regarding volatilome analysis, some differences were revealed both between T1 and T2 as well as between B1 and B2. No significant differences were observed in the level of clostridia in fecal bacterial population. In conclusion, the dietary supplementation with the two mycotoxin binders seems to have a significant effect on both richness and evenness of the dairy cow gut microbiome respect to control diet.

## Acknowledgements

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## P136

# Effect of *Aloe arborescens* supplementation in dry cows on rumen and hindgut microbiomes

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The transition period in dairy cows is characterized by reduced immunocompetence, inflammations, oxidative stress with a higher risk of metabolic and infectious diseases. *Aloe arborescens* contains polysaccharides and exhibits anti-inflammatory, immunostimulant, antibacterial, and antioxidant properties. The aim of this study was to investigate the effect of this nutraceutical approach on the rumen and hindgut microbiomes. The experiment involved 30 multiparous dairy cows at the dry off, divided into three different groups: (1) control group (CTR) – dry cows following the typical antibiotic treatment and the application of teat sealant; (2) sealant group (SIG) – dry cows without antibiotic's treatment and with only teat sealant; (3) *A. arborescens* supplementation and sealant group (ASIG) – dry cow with teat

sealant and oral administration of 200 mL of homogenate *A. arborescens*. *Aloe arborescens* was administered in the morning during the distribution of the total mixed ration for 14 days (7 days before up to 7 days after drying). For 16S rRNA-gene sequencing and volatilome analyses, rumen liquor and fecal matter were collected fourteen days before (T0) dry-off, at drying-off (T1) and seven days after dry-off (T2, only fecal samples). The V3-V4 hypervariable regions of the bacterial 16S gene was sequenced in two MiSeq (Illumina) runs with 2 × 250-base paired-end reads. No significant differences were observed for alpha- and beta-diversity between treatments along the three timepoints in the rumen microbiome. Conversely, according to all indices except evenness (equitability, simpson\_e) the alpha diversity of the hindgut microbiome increased significantly (p-values in the range 0.002 – 0.011) in the ASIG group at T2. Regarding beta-diversity, the hindgut microbiome showed a statistically significant (p-value = 0.0479) separation between treatments. Independently from sampling time and treatments, the bacterial community of the hindgut was dominated by Bacteroidetes (~40%) and Firmicutes (~48%); rumen showed prevalence of Bacteroidetes (~45%), Firmicutes (~25%) and Proteobacteria (~12%). In rumen, due to the high variability for all the metabolites no significant differences were observed between T0 and T1. In conclusion, the dietary supplementation with *Aloe arborescens* seems to have a sizable effect on the composition of the dairy cow gut microbiome, but not at the rumen level.

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## P137

### Insights into the faecal microbial phenotyping of pig using a Biolog EcoPlate<sup>®</sup> method

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Gut microbiota plays a key role in the development and maintenance of health on livestock and contributes to their productive efficiency. Although gut microbiota taxonomy characterization has been increasingly investigated, little is known about its functional characteristics and phenotyping. To fill this gap, Biolog EcoPlates<sup>™</sup> could be applied, however, little is known about its application on pig gut samples. This study aims at evaluating the use of Biolog EcoPlates<sup>™</sup> to characterize the metabolic potential and activity of the microbial community and to investigate the effect of (1) different storage conditions (fresh vs. frozen) (2)

optimal cell concentrations (3) specific length of storage conditions of pig faeces.

Two assays were performed aimed at evaluating differences in the metabolic activities between fresh and snap-frozen faeces at different dilutions (approximately 9 × 10<sup>5</sup> cells/g, 9 × 10<sup>4</sup> and 9 × 10<sup>3</sup> cells/g) and at different times of storage at –80 °C [15 h (T0) and 15 (T1), 45 (T2) and 150 (T3) days after collection]. Furthermore, the V3-V4 regions of the 16S rRNA gene were analysed to describe the biodiversity of community composition and predicted functionality.

Metabolic capacity of microbial community was detected for 31 lyophilized relevant C substrates, that were grouped by chemical classes (8 carbohydrates, 8 carboxylic acids, 4 polymers, 6 amino acids, 2 amines and 3 miscellaneous substrates). Results highlighted that snap freezing of pig faecal samples preserved the metabolic activity of the microbial community compared with fresh faeces ( $p > .1$ ). Sample storage at –80 °C for 150 days did not affect the metabolism of the microbial community, whose activity remained stable throughout the study period ( $p > .1$ ). The carbon source utilization by pig faecal microbiota was significantly affected by bacterial cell density ( $p < .05$ ). A cell concentration of 104 and 105 cells/g allowed detecting the highest metabolic activity of the microbial community. Overall, after 96 h of incubation, carbohydrates were the most frequently metabolized carbon source, while amines were the least.

In conclusion, results evidenced that the functional metabolic activity of the pig faecal microbial community can be preserved without significant variation until 150 days of storage at –80 °C. The Biolog EcoPlates<sup>™</sup> technology represents a rapid and useful method to explore the metabolic capabilities of the microbial community in animal samples.

## P138

### Effect of diets containing *Hermetia illucens* on rainbow trout microbiota: DGGE and NGS approaches

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*Hermetia illucens* (Diptera: Stratiomyidae; H) larvae are commonly studied as aquafeed ingredient due to their nutritional composition comparable to that of fishmeal. Different dietary formulations are reported to have different effects on fish gut microbiota, which, in turn, modulate fish digestion, immunity, energy balance, social behaviour and more. The previous studies reported contradictory results regarding the effect of insect-based

diets on the microbiota of salmonids. Moreover, to the author's knowledge, the effect of dietary full-fat insects on fish microbiota has not been addressed up to now.

To fill the above-mentioned gap, the aim of this study was to elucidate the effect of a diet containing full-fat H larvae meal (H50, 50% substitution level of fishmeal with full-fat H larvae) on the gut microbiota of rainbow trout (*Oncorhynchus mykiss*) in comparison to a control diet containing fishmeal (H0). Microbial DNA from pyloric caeca mucosa (PC), pyloric intestine content (PIC), mid intestine mucosa (MI) and mid intestine content (MIC) from five fish samples per diet was extracted and subjected to denaturing gradient gel electrophoresis (DGGE) and high-throughput sequencing (MiSeq, Illumina; HTS). Irrespective of the diet, the analysis of selected DGGE bands (excision, sequencing and closest relative search on BLASTN, NCBI) showed that the microbial communities were dominated by *Bacillus* sp. and *Staphylococcus* sp., with sparse *Streptococcus* sp., *Mycoplasma* sp. and *Shigella* sp. Bacterial relative abundances resulting from HTS analysis showed the domination of Proteobacteria in all samples (up to 85% relative abundance), followed by Firmicutes, Actinobacteria and Bacteroidetes. Fusobacteria were almost only found in MIC and PIC extracted from fish fed H0 diet. The differences between dietary groups were not captured by alpha-diversity (observed OTUs, Shannon's entropy, Pielou's evenness, tested with a Kruskal–Wallis test) or beta-diversity indices (unweighted unifracs, Jaccard, robust Aitchison metrics, plotted on PCoA and tested with a PERMANOVA).

Further studies with a higher number of replicates might be able to find significant differences between dietary treatments and are needed to postulate a clearer hypothesis on the microbiota structure and diversity in fish fed dietary insects. That knowledge would pave the way to unravelling the functions of the microbiota and to understand the mechanistic laws at the root.

## P139

### Anthocyanins dietary supplementation in lambs: effects on gut microbiome

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In ruminants, the gastrointestinal tract is colonized by highly concentrated and variable populations of microorganisms, particularly bacteria, archaea, fungi, ciliated protozoa and viruses. Age, diet and management influence bacterial community both for proportions and for diversity. At the first stages after lambing, lambs are functional monogastric animals and low rumen

bacterial activity leads to a greater influence of the diet on the gut microbiome composition. The aim of the present study is to assess the effect of anthocyanins on the fecal bacterial microbiome and microbiota of lambs. A total of 44 Merino male lambs of 25 days were randomly divided in a control group (CG;  $n = 22$ ) and anthocyanins group (AG;  $n = 22$ ). All lambs were fed with alfalfa hay and starter ad libitum and only the AG received a red orange and lemon extract with an 85% anthocyanin concentration (90 mg/kg live weight calculated each two days). Lambs were slaughtered at 40 days and fecal samples were sterile collected from rectum and frozen at  $-20^{\circ}\text{C}$  until analysis. Analysis of fecal microbiome was carried out by metabarcoding analysis of 16S rRNA. After reads denoising, sequences were aligned against SILVA rRNA sequence database using MALT, and taxonomic binning was performed with MEGAN. Regardless of the dietary treatment, Proteobacteria and Firmicutes were the predominant bacterial phyla identified. The amount of Firmicutes was 10% and 15% in the AG and CG respectively. Moreover, the amount of Actinobacteria was almost two-fold in the AG than CG. At genus level, Acinetobacter percentage of number of reads recorded double values in the AG than CG, while an increase of Psychrobacter and Streptomyces was observed in AG compared to CG. Dietary supplementation of anthocyanins reduced the relative abundance of Enterobacteriaceae as Escherichia coli and Salmonella compared to the CG. These results are consistent with some studies carried out on lambs and using other phenolic compounds. Results indicate that the dietary supplementation with anthocyanins in lambs inhibits the growth of some potential pathogenic gram-negative bacteria. These outcomes encourage further studies aiming to deepen knowledge on this topic, as a potential way for reducing the use of antimicrobial substances, as well as improving animals' health and welfare status.

## P140

### Multi-omics approach to assess the effects of a dual mode synbiotic supplementation on gut health and performance of broiler chickens

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Feeding pre- and pro-biotics to broilers immediately after hatch and during growth has led to positive health and performance outcomes. Such strategy could synergistically affect gut health establishment and preservation. Hence, this hypothesis was tested by



comparing performance, caecal metabolomics, and caecal microbiota data of post-hatch and in-feed synbiotic-treated broilers.

1440 one-d-old male Ross308 were randomly assigned to 4 groups (9 replicates each): CON was fed a control basal diet (BD); PH, PH11, and PH21 received a post-hatch gel-droplet-based synbiotic (a lactic acid bacteria and fructooligosaccharides blend; 100 g/10,000 chicks) and then were fed BD or BD supplemented with the synbiotic in a microencapsulated formula during starter (0.1%, 0–11 d) or up to grower phase (0.1%, 0–11 d; 0.05%, 12–21 d), respectively. On a replicate basis, chicks were weighed at housing, while body weight (BW) and feed intake (FI) were recorded at 11, 21, 31, and 43 d (slaughter) to compute feed conversion ratio (FCR). Performance data was subjected to ANOVA and Tukey's post-hoc. From 1 bird/replicate, selected at 21 and 43 d according to similar BW, the caecal content was collected and analysed via  $^1\text{H-NMR}$  and 16S amplicon sequencing. Caecal metabolomes at 43 d were analysed through t-tests (CON as reference) and PCA: data at 21 d was modelled on this PCA to compare the two sampling points via Spearman's correlation.

Overall, PH and PH11 showed greater FI than CON (5281, 5277 and 5159 g, respectively;  $p < .05$ ). However, only PH11 reached higher BW than CON (3150 vs. 3031 g;  $p < .05$ ), while PH21 and CON performed similarly. No significant FCR differences were detected. At 43 d, caecal metabolomes of synbiotic groups had less formate and 2-hydroxyphenylacetate, whereas more glutamate, betaine, leucine, isoleucine, and valine than CON ( $p < .05$ ). PC2 scores at 43 d were significantly correlated with those at 21 d, displaying similar trends of the above molecules between the two sampling points.

This study confirms the beneficial effects of a synbiotic supplementation program combining post-hatch and in-feed treatments on certain performance traits of broilers. Variations of the caecal metabolomes, arising in the mid-cycle and then consolidating towards slaughter, as well as preliminary microbiota results indicate a cumulative and possibly desirable effect of the dual mode synbiotic treatment on broiler gut ecosystem.

## P141

### Assessment of transcriptional activity within isochores in healthy and subclinical cattle

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Isochores are large genomic regions characterized by homogeneous base composition divided into five families: L1, L2, H1, H2 and H3 (in increasing GC level order). The GC poor ones (L1, L2 and H1) are characterized by distinct and opposite functional and structural properties compared to the GC rich ones (H2 and H3). In this study, we have explored the transcriptional levels of the different isochore families using RNA-seq data, of white blood cells, from 15 healthy (APP-) and 15 subclinical stressed (APP+) Italian Holstein dairy cows, from a previous study.

IsoXpressor was used to identify isochores, to align RNA-seq reads to the reference genome (ARS-UCD1.2), and to calculate the RPKM score (Reads Per Kilobase of transcript, per Million mapped reads) within each isochore. Isochores with very low RPKM in both groups were excluded from further analyses, to avoid false positive results. The level of transcription was compared between APP+ and APP- animals, isochore by isochore, along the genome. Isochores having at least a fold change of 1.5 ( $\log_2\text{FCI} > 0.58$ ) were further investigated. The majority of the differentially expressed isochores are up-regulated in subclinical animals. Among these, the relative most frequent were from the GC-rich classes (H2 and H3), while GC-poor isochores were mainly down regulated. Subsequently, functional analysis of those genes included in the differentially expressed isochores was performed using PANTHER over-representation test. Genes within the L1 and L2 isochores were significantly enriched in the detection of stimuli and sensory perception GO terms and under-represented in H2-H3, confirming previous results.

The herein findings indicate that stress condition activates genes located in GC-rich regions and down-regulates the ones in GC-poor regions. Probably this is due to a combination of accessibility to transcription of GC rich regions, generally having open chromatin structure, and the function of the genes included in these isochores, in agreement with other studies reporting a correlation between genes isochore localization and function.

The obtained results reinforce a functional implication of isochores, and genes contained therein, in subclinical stress response, with GC-rich regions activation and GC-poor region silencing.



## P142

### Genome-wide association for semen quality traits in Italian Holstein bulls

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Semen quality traits have a direct influence on the fertilization process, on the viability of preimplantation embryo and, consequently, on the conception rate. Sperm motility is a complex trait influenced by pleiotropic gene effects and environmental factors such as season, temperature, nutrition, age, and intervals of semen collection. Genome-wide associations studies (GWAS) aim at identifying quantitative trait loci through statistically significant associations between genetic markers and phenotypic data. Genes associated with male fertility and sperm motility that might be used to improve reproductive performance in genetic programs have been previously identified in both crossbred and pure-breed cattle. In this study, we carried out a GWAS in Italian Holstein bulls to identify candidate regions and genes associated with progressive and total motility (PM, TM). After data editing, the final dataset consisted of 6558 records from 856 bulls having semen collected in 21 artificial insemination stations and 604,697 SNPs (Call rate >95%; Minor Allele Frequency >5%). (Co)variance components were estimated using single trait mixed models and associations between SNP and phenotypes were assessed using a ssGBLUP approach. P-values obtained using a PostGSF90 were adjusted for multiple testing using Bonferroni correction. A total of 141 markers across 11 chromosomes exceeded the significant threshold ( $p < .05$ ;  $P < 8.26 \times 10^{-8}$  after Bonferroni correction). Genes within 50kb flanking interval from each significant SNP were selected to carry out QTL enrichment and functional analyses using the Gallo and WebGstart R packages. Some of the genes identified in this study (DCC, PACRG, bta-mir-12055 and bta-mir-2285) have previously been associated with male fertility. The QTL enrichment analyses identified 14 QTL on BTA3, BTA7,

BTA22, BTA23 and BTA26 related to reproductive traits and immune response. Gene Ontology enrichment analyses, including biological processes and molecular functions, showed significant GO terms involved with hormone regulation and enzyme activity. These results may provide important information for understanding the genetic architecture of semen quality traits and offer information for molecular breeding in Italian Holstein bulls.

## P143

### Dietary supplementation with a polyphenolic extract from olive mill wastewater: evaluation of the potential effects on hepatic metabolism in rabbits through an RTqPCR approach

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Plant processing for the production of food generates a wide range of by-products and residues rich in biological active compounds including polyphenols. Their use as supplements may regulate performance, health status and animal welfare, obtaining functional foods for human consumption. This study applied a nutrigenomic approach to identify the effects induced by a diet supplemented with a phenolic extract from olive mill waste water (OMWW) on the expression of liver target genes (AGER, BAX, COX2, IL1B, PPARA, PPARG, SIRT1, TNFA), involved in oxidative stress response and inflammatory pathways. Twenty-four New Zealand rabbits were equally divided at weaning (30 days of age) in two experimental groups: CTR (conventional diet) and POL (OMWW-supplemented diet). The experiment lasted 60 days. Growth performance of the two groups of animals was not affected by the dietary treatments. Liver samples collected at slaughtering were snap frozen and total RNA was extracted using a commercial kit. Gene expression of specific target genes was evaluated through RT-qPCR normalizing the expression levels with two reference genes (18S – ACTB). Relative expression was evaluated using the  $2^{-\Delta\Delta CT}$  statistical method, comparing the expression levels of POL and CTR groups and assessing the statistically significance through Student *t*-test ( $p$ -value < .05 as threshold). A decrease of SIRT1, TNFA, AGER, BAX and PPARA expression was observed in POL group compared to CTR, suggesting a potential direct involvement of OMWW polyphenols in the hepatic oxidative stress-dependent metabolic pathways. Modulation of SIRT1 and PPARA gene expression is involved in the regulation of lipid

metabolism and energy homeostasis in response to nutrient availability. Dietary OMWW phenolics can mitigate negative effects exerted by oxidative stress and contribute to reduce the environmental impact of olive oil industry by-products.

## P144

### Influence of green forage on metabolomic characteristics of buffalo milk

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The aim of this study was to compare, with a metabolomic approach, different feeding strategies in lactating water buffaloes. The study was carried out on 32 milking buffaloes that were randomly divided into two groups: Group D ( $n = 16$ ) that were fed a total mixed ratio (TMR) and Group F ( $n = 16$ ) that had a isoen-ergetic and isoproteic diet as Group D, but with the inclusion of the 30% of alfalfa green forage. Milk samples were collected indi-vidually, once a month, for four months, from June to September and analysed by LC-MS. Statistical analysis was carried out by using Mass Profile Professional, version 13.1.1 (Agilent Technologies). Metabolomic analysis revealed the presence of several metabolites differentially expressed between Group D and Group F. Among these, a total of five metabolites were identified by comparison with an online database and a standard compound as L-Carnitine, Acetylcarnitine, Propionylcarnitine, Butyrylcarnitine and 2-Methylbutyrylcarnitine. Distinct separation between these two groups was evident in principal compo-nents (PC1 – PC2) of the variance in the LC-MS dataset. Moreover, hierarchical clustering analysis also showed a clear separation of Group D and Group F in all months taken into account. In the first month, we only detected three metabolites out of five and we founded an up-regulation ( $p < .05$ ) of Acetylcarnitine, Butyrylcarnitine and 2-Methylbutyrylcarnitine in Group D compared to Group F. In the other three months L-Carnitine, Acetylcarnitine, Propionylcarnitine were always down-regulated ( $p < .05$ ) in Group F compared to Group D. Butyrylcarnitine and 2-Methylbutyrylcarnitine were down-regulated during the sec-ond month and up-regulated the third and the fourth months in Group D compared to Group F ( $p < .05$ ). In conclusion, the inclusion of green forage in buffalo diet enhances the presence of functional molecules in milk with important antioxidant and

anti-inflammatory activities. The presence of these molecules should induce consumers to consider milk as a highly important source of specific nutrients with health-promoting properties.

## P145

### Insect meal and poultry by-products as innovative ingredients for rainbow trout feed: impact on intestinal microbiota and gut health

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Fish consumption and fishing levels rose sharply in the last decades leading to dramatic consequences on marine habitats. Aquaculture represents the fastest growing food production sec-tor although its sustainability is strictly linked to provenance of feed ingredients. In the search for fish meal (FM) and fish oil (FO)-free formulations, insect meal (IM) and poultry by-product meal (PBMs) ingredients have been proposed as candidate pro-tein sources. The aim of this study was to evaluate the effect of partial replacement of vegetable meal (VM) with IM and PBM in rainbow trout (*Oncorhynchus mykiss*) on gut health, by focusing on microbiota composition, markers of inflammation and gut barrier integrity. Animals were fed eight dietary treatments with different percentages of *Hermetia illucens*-IM or PBM to replace FM and VM. Test diets showed comparable growth performances to FM and VM. Microbial 16S rRNA analysis revealed that IM increased microbiota alpha diversity and led to a significant increase of chitin degraders *Actinomyces* and *Bacillus* genera. Analysis of feed microbiota revealed shared OTUs between IM feed and intestinal microbiota of IM fed fish supporting connec-tivity between food-chain microbiomes. IL-1 $\beta$ , IL-10, TGF- $\beta$ , COX-2 and TCR- $\beta$  gene expression in midgut and head kidney, together with plasma LPS as biomarker of gut permeability,

revealed that the experimental diets were well tolerated and did not compromise gut barrier function or induce inflammation. Both IM and PBM singly or in combination could be considered as valid alternatives to VM and FM in the aquaculture practice.

## P146

### Erythrocyte membrane lipidome profile of heifers during the transition period: a preliminary study

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The analysis of red blood cells (RBC) membrane lipidome is a powerful tool to assess the quantity and quality of fatty acid composition and for the follow-up of the membrane remodeling under physiological and pathological conditions. In dairy cows, the transition period is marked by nutritional, metabolic, hormonal and immunological changes that have an impact on the incidence of infections and metabolic diseases. The aim of this study was to evaluate RBC membrane lipidome profiles in healthy heifers 30 days before calving (T0) and at 7 (T1) and 30 days (T2) post calving. RBC membranes were isolated from EDTA blood of 13 Friesian heifers and a cluster of 10 saturated [SFA (palmitic; stearic)], monounsaturated [MUFA (palmitoleic; oleic; vaccenic)] and polyunsaturated [PUFA (linoleic; dihomo-gamma-linolenic (DGLA); arachidonic; EPA; DHA)] fatty acids was quantitatively evaluated by Gas-Chromatography. Relevant lipid parameters [SFA/MUFA, SFA/PUFA, n6/n3, PUFA balance, Peroxidation (PI) and Unsaturation (UI) indexes] were calculated. We observed an increase in the biosynthesis of SFA with higher value of Palmitic acid ( $p < .0001$ ) at T1 and Stearic acid at T2 ( $p < .001$ ) compared to T0. The n6, PUFA, DGLA and arachidonic acids ( $p < .0001$ ), decreased at T1 compared to T0 and DGLA decrease also at T2 compared to T1 ( $p < .001$ ). EPA levels were decreased at T2 ( $p < .0001$ ) while we register an increase in the n6/n3 ratio ( $p < .001$ ) at the same time. Also, UI and PI were significantly decreased after calving ( $p < .0001$ ). The increase in SFA could be due to an energetic role of SFA during post-partum period even if the increase in SFA should be avoided to the insulin resistance. The UI decrease at T1 ( $p < .0001$ ) underlines a change of the membrane properties, with increased rigidity and reduced permeability. The level of DGLA decreased at T1 ( $p < .001$ ) compared to T0 and was also lower at T2 compared to T1 ( $p < .001$ ). This result could be due to a reduction of anti-inflammatory defenses with possible onset of inflammatory

diseases during the post-partum. Moreover, we observed a significant decrease in the n6/n3 ratio after calving, that could be considered as valid indicators to monitor and predict inflammatory conditions in cattle during this critical period. In conclusion, an early knowledge on lipid membrane changes allows personalized nutritional intervention in order to promote a healthy status reducing metabolic stress and promoting anti-inflammatory activity.

## P147

### Effects of the MTNR1A gene polymorphism and month of ram introduction on reproductive recovery in Sarda sheep

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Small ruminants in the Mediterranean latitudes show a marked reproductive seasonality which, hence determines the seasonality of production, resulting in problems for marketing of these products. The photoperiod is the factor mainly involved in the regulation of reproductive seasonality through the secretion of melatonin by the epiphysis. Melatonin is secreted during the hours of darkness and acts through its binding to specific receptors involved in regulating reproductive seasonality in sheep. In particular, in different sheep breeds, the polymorphism g.17355452C>T of the melatonin receptor 1A (MTNR1A) gene is associated with an early reproductive recovery in spring. The aim of this study was to highlight the influence of this polymorphism on the reproductive recovery of Sarda sheep after the ram introduction into the flock in the months of March, April, May and June. In a farm that raised about 1000 animals, we genotyped 600 ewes. Among the genotyped animals, 60 animals were selected for each genotype. The selected animals were 3–6 years old, and had a body condition score (BCS) between 2.0 and 4.0. The animals were divided into four groups (A, B, C and D) of 60 sheep each, based on age, BCS and genotype. In group A rams were introduced on March 1, in group B on April 1, in group C on May 1 and in group D on June 1. In all groups, the lambing and the number of lambs born from 150 to 220 days after the ram introduction were recorded. In all the groups, the genotypes C/C and C/T of the polymorphism in position g.17355452 showed the greatest fertility ( $p < .01$ ) and the shortest distance between ram introduction to lambing ( $p < .01$ ), compared with the T/T genotype. In addition, sheep in groups A and B, carrying C/C or T/C genotype, showed higher fertility than those in group C and D. Sheep in group A and B, carrying A/A genotype showed the lambing peak 10 days later than those in groups C and D. We concluded that the polymorphism in position g.17355452C>T influences



the reproductive recovery and that its effect is more evident when exposed to rams in the months of March and April.

## COMPANION ANIMALS AND SOCIETY

P148

### Nutritional management with a soy byproduct of multiple acquired extrahepatic portosystemic shunts with hepatic encephalopathy in a dog

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Chronic hepatitis (CH) with acquired extrahepatic portosystemic shunts (PSS) can be complicated by hepatic encephalopathy (HE). Commonly, such cases are managed with commercial diets developed to supply a lower intake of protein, preferably of vegetal origin (e.g. soy), along with other beneficial nutrients. This case report describes the use of a homemade diet for CH complicated by HE.

Zoe is a 5-year-old, spayed female Yorkshire with a history of vomiting, weight loss, staggering gait, disorientation, restless attitudes and persistently ptialism. At the time of the first visit, the dog's body weight was 3.9 kg, the body condition score (BCS) 3/9 and a severe loss of muscle tissue was identified. Blood and urine tests, abdominal ultrasound, abdominal and chest CT scan were performed. A diagnosis of CH with multiple extrahepatic PSS complicated by stage 1 HE was made. The dog refused to eat the commercial diet prescribed and therefore a homemade diet was formulated. Zoe's ideal body weight was estimated at 5.5 kg and its daily energy requirement was estimated to be 466 kcal/day. Dietary protein intake was set at 45 g/Mcal and fat intake was set at 45 g/Mcal. The choice of the ingredients was also considered. The literature suggests a beneficial impact of soy protein compared to poultry protein in lowering the ammonia production. For this reason, tofu (a by-product of soy) was chosen as one of the protein sources of the home diet whose protein intake was supplemented with animal sources such as chicken, pork or codfish. One week after feeding the prescribed homemade diet, neurologic signs decreased and disappeared after further 10 days. After 9 weeks, Zoe weighed 5.1 kg and she had reached a satisfactory BCS and a normal muscle mass condition. Zoe was followed up for 2 months, during which neurologic signs never showed up and clinical conditions were stable.

A home-prepared diet based on tofu and covering the minimum protein requirement suggested by FEDIAF resulted able to control HE signs in a dog with CH and acquired PSS complicated by hepatic encephalopathy (HE). Protein restriction must be applied

carefully to avoid muscle tissue depletion. The ideal amount of protein should be able to control HE signs while keeping the patient anabolic.

P149

### Effects of *Saccharomyces boulardii* supplementation in breeding adult dogs

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The intestinal microbiota has several roles, including the defense against intestinal pathogens, supporting the development of a healthy epithelium and immune system. Dysbiotic conditions can be linked to gastrointestinal pathologies and increased intestinal permeability. For the restoration of intestinal eubiosis conditions, numerous studies report the benefits of *Saccharomyces boulardii* (*S. boulardii*) which is a yeast used as a probiotic in the prevention and treatment of gastrointestinal disorders. Group living conditions on the farm can expose dogs to psychophysical stress conditions, increase the spread of parasitic and infectious agents that cause dysbiotic conditions and lead to the use of antibiotics. *Saccharomyces boulardii* is considered a valid alternative to antibiotic treatment. The aim of this study was to demonstrate the efficacy of *S. boulardii* in intestinal eubiosis and dog welfare under farm conditions by focusing on nutritional and fecal parameters. A 35-day study was conducted on 24 healthy Staffordshire Terrier dogs, divided into two homogeneous groups: a control group (fed with a commercial basal diet) and a treated group (fed the same diet as the control with the addition of *S. boulardii* at a dose of  $1 \times 10^9$  cfu/kg/die). The effects of the treatment were evaluated, at weekly intervals (T 0, T 7, T 14, T 21, T 28, T 35) on the nutritional parameters (body weight, BCS) and fecal parameters (pH, consistency) of the subjects. In addition, the following parameters were measured on all weekly fecal samples: Calprotectin, Lactoferrin, Zonulin, Cortisol, IgA, SCFA, Indole / Skatole.

There were no significant differences between the groups in terms of body weight, BCS, fecal pH and fecal consistency. This suggests that the *S. boulardii* did not adversely affect these parameters. The positive effect of the treatment was supported by the significant decrease in calprotectin and fecal IgA, possible indicators of improvement in intestinal well-being. Moreover, fecal cortisol was found to be significantly decreased on dogs belonging to the treatment group; this result could be implicated to a less stress condition due to a better adaptation of the animal to the environment.



This study highlights that *S. boulardii* is a valid probiotic, useful for improving intestinal eubiosis conditions and the well-being of healthy dogs in breeding conditions.

## P150

### Runs of Homozygosity in 21 Italian dog populations

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The Italian peninsula is characterized by a high level of genetic diversity in all its indigenous domesticated animals, with the dog species being no exception to this. We characterized the length, abundance, distribution and evolution of the Run Of Homozygosity (ROHs) and genomic inbreeding (FROH) of 379 dogs of 21 Italian indigenous breeds and populations. Our aim is to provide insight in the homozygosity and inbreeding of the Italian canine populations. We estimated ROHs on a high density (230k) Dog SNP chip using the PLINK software considering ROH of length  $\geq 1$  Mb, in sliding window of 50 SNP with no heterozygous allowed, and a maximum gap of 0.1 Mb between markers. We calculated FROH as the proportion of the autosomal genome covered by ROHs. Lastly, to better describe the ROH distribution in the different populations, we subdivided them in five length classes: 1–2, 2–4, 4–8, 8–16, >16 Mb.

We observed a great variability in FROH values within and across the populations considered. For instance, the mean population FROH goes from the very high values of Lupo Italiano breed (0.40) and Neapolitan Mastiff (0.35) to a minimum of 0.05 in the Oropa shepherd dog, while the population with the wider range of internal variation is the Bergamasco shepherd dog (from 0.03 to 0.40). The ROHs length classes are well balanced in almost all breeds, with a prevalence of ROHs belonging to the classes 2–4 and 4–8 Mb. We also found a very low presence of extremely long ROHs (>16), apart from the Cirneco dell'Etna and the Mannara dog. Characterizing ROHs distribution and evolution could be a useful genomic tool to better understand today's structure and the past history of small dog populations. This could be applied to monitor and safeguard the conservation of the Italian canine diversity. Using ROHs information, breeders' associations could evaluate more efficiently the evolution of homozygosity level, the inbreeding within and across breeds and discover regions that host gene under selection for positive as well as negative traits. At the same

time, single breeders could improve the management of reproduction with a more accurate choice of the animals mating.

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## P151

### Fonni's dog: genetic variability and relationship with other breeds

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The Fonni's dog is an ancient Sardinian breed of livestock and property guardian dogs, known for its longevity, loyalty, and strong protective instinct that even made it suitable for use as war dog in the past. Despite not being officially recognised as such, different genomic studies have proven the Fonni's dog to be a full-fledged breed, a remarkable achievement considering the selection based only on behaviour. Fonni's dogs, in fact, show a considerable variability in morphology and physical appearance: the double layered coat is defined as coarse 'goat' hair with a woolly undercoat, but shorthaired dogs are admitted too; coat colours range from black to ash to honey; a natural lack of tail can be present. Nevertheless, this breed keeps some peculiar features, like the typical 'monkey face', with amber frontal eyes and an intense and unfriendly expression. This study aimed to assess the genomic background of Fonni's dogs. We sampled and genotyped 30 Fonni's dogs and 380 subjects of other 25 Italian and foreign breeds of shepherd dogs, hunting dogs, sighthounds and molossers using the Canine 230K SNPChip. All the animals have been photographed, underwent to morphological evaluation, and were judged to be eligible for registration as Fonni's dogs. A quality check was performed on genomic data with standard thresholds. The genetic background of the breeds was evaluated with ADMIXTURE 1.3 and the individuals' probability to be assigned to each cluster (Q-score) was calculated.

Quality check left 379 dogs (30 Fonni's dogs) and 120,853 SNPs. Mean  $\pm$  standard deviation of Fonni's dogs' Q-score for their own cluster was  $62 \pm 22\%$ , ranging from 19% to 100%. Particularly, it was  $\leq 25\%$  in 1 dog,  $>25\text{--}50\%$  in 10 dogs,  $>50\text{--}75\%$  in 11 dogs, and  $>75\%$  in 8 dogs. The most represented secondary breeds

(>5%) were Maremma sheepdog (in 11 subjects), Segugio italiano (5), Neapolitan mastiff & Cane corso cluster (2), and Mastiff (1). These results show that genomic tools can and should be implemented alongside the morphologic evaluations for the identification of dog breed membership, especially for breeds that show a large phenotypic variability such as the Fonni's dog.

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## P152

### ***Ascophyllum nodosum*-derived fucoidan modulates the intestinal expression of immune-inflammatory genes in a biopsy model of canine chronic enteropathy**

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Like terrestrial plants, algae contain a tremendous amount of bio-functional compounds, some of which may affect inflammatory and immune responses. As such, algal bioactive components might find use in the treatment of canine chronic enteropathy (CE), where persistent inflammation underlies clinical signs. In this investigation, we studied the effects of *Ascophyllum nodosum*-extracted fucoidans (ANFE) on cytokines gene and protein expression and on the morphology of intestinal tissue explants obtained by CE dogs. Duodenal biopsies from 22 dogs with CE were incubated for 24 h with or without ANFE. TNF- $\alpha$ , IFN- $\gamma$  and IL-15 proteins were measured in culture supernatants by ELISA whereas relative expression of *IL1B*, *IL6*, *IL10*, *IL15*, *TNFA*, *IFNG*, *FOXP3* and *IDO1* genes was assessed through real-time PCR. Inflammatory cell infiltrate and mucosal integrity was evaluated by light and transmission electron microscopy. Cytokine protein concentrations were all below the detection limit, and no differences were found regarding morphological features between the two groups. With respect to transcriptomic data, mRNA levels of the pro-inflammatory genes *IL15* and *TNFA* were significantly higher ( $p < .05$ ) in the control group, whereas a tendency ( $p = .08$ ) for a higher relative gene expression was noticed for *IL6* and transcription factor *FOXP3* in ANFE-treated samples. In conclusion, while failing to improve morphological outcomes, ANFE supplementation seemed to be associated with an overall positive effect on intestinal phlogosis and immune function. However, due to methodological limitations of the study, further research is warranted to confirm the present findings.

## P153

### **Biochemical parameters, oxidative stress and inflammatory status in English Pointers fed a citrus molasses-supplemented diet**

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Exercise has been shown to increase the production of reactive oxygen species (ROS) in canine serum causing oxidative stress. Citrus molasses is a by-product of citrus juice production containing phytochemicals substances that contribute to health maintenance. Naringin and Hesperidin are the predominant flavanones in citrus fruits with antioxidant, lipid-lowering, hypoglycaemic and anti-inflammatory properties. The aim of this study was to evaluate the effect of citrus molasses inclusion in the diet for hunting dogs on some biochemical parameters, oxidative and inflammatory status. Ten neutered adult English Pointers were divided into two groups homogeneous for sex (2 male, 3 females), age (>12 months old), initial body weight (CTR: 21 kg  $\pm$  1.50), BCS (4.25  $\pm$  0.5, score 1–9) and fed with the same commercial diet supplemented with (MOL group) and without (CTR group) of 2.5% as fed of citrus molasses (Antioxidant Pool Content: Hesperidin = 3.3 g/kg; Vitamin C = 0.4 g/kg; Pectin = 28.3 g/kg). The trial lasted 4 weeks during which the dogs were used to train hunting twice a week for 1 h. Blood samples were withdrawn from the cephalic vein at the beginning (Day 0) and at the end of the trial (Day 28). All samples were taken from fasting dogs, in the morning, to evaluate: total cholesterol and triglycerides by a chemistry analyser Catalyst Dx (IDEXX Laboratories), d-ROM, OXY and BAP tests using reagents from Diacron International s.r.l. (Grosseto, Italy) and some cytokines expression (IL-1 $\beta$  and IL-10) were detected using Elisa kits (Genorise, Philadelphia, USA). Data were analyzed using a mixed model with covariates of XLSTAT statistical package (Addinsoft, v. 2014.4.03). The model included the fixed effect of the dietary treatment (MOL vs. CTR), of the time (Day 0 vs. Day 28) and their interaction (Diet  $\times$  Time); the individual animal was considered as a random effect in the model. Diet significantly increased ( $p = .010$ ) the biological antioxidant potential (BAP) and reduced triglycerides ( $p = .05$ ) and lymphocytes ( $p < .001$ ), indicating a possible role of citrus molasses in plasmatic antioxidant, lipid-lowering and anti-inflammatory capacity. Time lowered significantly the IL10 levels ( $p < .001$ ) and IL-1 $\beta$  ( $p = .05$ ) levels and the interaction Diet  $\times$  Time reduced significantly the lymphocytes ( $p = .005$ ). Data confirm the positive role of functional compounds of citrus fruits to counteract the oxidative stress in working dogs.

### Acknowledgements

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## BEYOND FOOD: NON-MARKET BENEFITS OF LIVESTOCK SYSTEMS

### P154

#### Entrepreneurial horizons and sustainability of mountain livestock farming: the case of Lessinia (NE Italian Pre-Alps)

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The recognition of a historical-cultural landscape of an alpine region requires the description of the livestock systems, especially through their evolution. The mountain livestock systems of the summer pastures of Lessinia (Official Italian Rural Landscape, Verona, NE Italian Alps) were analysed. These pastures represent an area of about 6300 hectares on the top of Lessini Mountains, between 1200 and 1900 m a.s.l. The geological conditions have favoured the predominance of a pasture-based landscape on smooth morphologies ideal for cattle grazing. A survey on 169 breeders made it possible to know both the management methods and the expectations for future improvement scenarios. There are about one hundred alpine farms, mostly private, managed by one or more breeders, with 6800 cattle (of which 93% are dairy cows) kept on predominant extensive grazing during at least four months a year. The main cattle breed is the Holstein-Friesian (70%). The extensive road network has enabled breeders to move up only for milking because the milk is processed at dairies in the lowlands. Only four farms produce cheese directly. The individual high milk production and the relevant feeding rations require a considerable integration of grazing with concentrate feeds, averaging 4.6 kg head<sup>-1</sup> day<sup>-1</sup>. These more intensive management choices have determined the worsening of pasture quality and the reduction of plants' biodiversity. The economic return for milk and dairy products – processed and sold elsewhere – is poor as well as the connections with consumers and tourists. The interviews with the farmers (26% under 40 years, 56% 41–60 years and 18% above) tested their willingness not to change the production orientation: 67% of the farmers indicated that they do not intend to process cheese directly on the summer pasture in the future, and 61% are not interested in starting agritourism activities there. Nevertheless, 88% of the respondents plan to continue summer pasture farming in the

next 5 years. In view of these results, information and awareness actions have been launched to improve this static and not so resilient rural scenario. We believe that the results of the survey not only precisely describe livestock farming systems of the Lessinia region but also highlight the critical points for farmers who are willing to start improvement actions towards an economically and environmentally sustainable management for maintaining a historical landscape too.

### P155

#### Soil erosion patterns in Alpine and Apennine extensive farming system

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Soil erosion by water is one of the major threats to soils in the European Union, with a negative impact on ecosystem services, crop production, drinking water and carbon stocks. Much of the Italian soilscape is subject to water erosion because of the morphology, the soil parent materials, the climatic characteristics, and the anthropogenic activities. Within the agrarian surface (~12.6 million ha, 41.7% of the territory), arable land accounted for 56.7%, followed by permanent meadows and pastures (25.6 %); these two different types of management have a different impact on the soil conditions. Aim of this study was to evaluate the different soil erosion patterns in two case studies of Alpine and Apennine mountains, through the Revised Universal Soil Loss Equation (RUSLE) model. Both the farms adopted extensive systems with cattle grazing. For each farm and on the same soils, two hypotheses were compared: (i) presence of permanent grasslands used for cattle grazing and for the production of fodder (real condition); (ii) presence of arable land subjected to mechanical work (plowing, harrowing, etc.) (simulated condition). In a GIS environment, the farms boundaries have been georeferenced and digitized in .shp format. The hydrographic basin relating to the farms parcels was reconstructed through resampling (10 m grid) of the Digital Terrain Model (DEM) with regular mesh with a grid pitch of 25 m. A cleaning procedure ('fill') of false depressions was also performed. Rainfall erosivity (R-factor), Soil Erodibility (K-factor), Topography (LS-factor), Cover Management (C-factor) and Support Practices (P-factor) were estimated as suggested by the bibliography. The results showed that in both the farms there would be an average annual erosion risk higher in the case of plowed crops, while in the case of grassy meadows (as in the real situation) the average value would be significantly lower. Farming models that adopt extensive grazing practices contribute to the maintenance of multiple ecosystem services, including those linked to the hydrogeological and landscape structure. These



aspects should be considered in the marketing strategies that support the process of creating the value of mountain products.

## MONITORING IMPACTS ON ENVIRONMENT

### P156

#### Life Cycle Impact Assessment of different forage production system

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Life Cycle Impact Assessment (LCIA) can be used to quantify the carbon emissions within production processes and to support planning of effective mitigation practices. The aim of this study was to evaluate the environmental impact of different forage production techniques. Five different haymaking processes were considered: traditional hay, TH; early cut hay, ECH; baled and wrapped haylage, WBHL; chopped and wrapped haylage, CWHL; corn silage, CS. A life cycle inventory for each technique was performed on primary data at farm level in the year 2017, conducting a farm survey and collecting measures and samples from field operation during forage harvesting. Secondary data were used to determine off farm and secondary emissions from plastic, machine and equipment production. The study had a cradle-to-gate perspective, whereas consumption and final disposal were excluded. The system boundaries included land preparation, seeding, fertilization, and all the crop operations, comprising farm products storage. Total emissions were expressed per ton of forage DM. The SimaPro 9.0.0.35 software and the Intergovernmental Panel on Climate Change (IPCC) 2013 Global Warming Power 100a V1.03 were applied. Total emission intensities were equal to 2450, 2656, 2913, 2954, 3501 kg CO<sub>2</sub> eq/ton of forage DM for CS, WBHL, TH, ECH, and CWHL techniques, respectively. The variability of emission intensities was mainly attributed to differences in forage yields which were 3.5, 3.5, 4.8, 4.8 and 27.8 ton of forage DM for TH, ECH, WBHL, CWHL and CS, respectively. The contribution of haymaking process to total emissions were equal to 9%, 3%, 1%, 4% and 32% for CS, WBHL, TH, ECH and CWHL techniques, respectively. Highest haymaking emissions from haylage in respect to hays were due to the machinery used, harvesting equipment and fuel consumption for additional operations. In conclusion, mitigation strategies should focus on optimising utilization of inputs and improvement phases of haymaking considering that haylages and silages in respect to hays have usually higher nutritional value.

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### P157

#### Precision beekeeping systems as promising tools for the agro-environmental monitoring

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Precision beekeeping (PB) is raising interest by beekeepers but also by researchers involved in apidology. In the 2018–2019 period, six precision beekeeping systems have been used to evaluate, by continuous monitoring of the main honey bee (*Apis mellifera*) colony traits, the agro-environmental quality of the area where a non-hazardous wastes disposal plant (Ecosantagata S.r.l., Civita Castellana, Italy) is located. The PB systems included scales (HW), flight activity recorders (FA) and in-hive temperature sensors (IHT) (Melixa S.r.l., Trento, Italy). In early August 2018, the hives were populated by six *A. m. ligustica* Spin. colonies. The six-hive experimental apiary was located within the operative area where the ground excavation and non-hazardous wastes burial were carried out continuously. The in-hive mortality (IHM) was monitored through underbasket traps. The main mean apiary traits (HW, FA, IHT, IHM) including the 2019 honey production (HP) were compared with two reference apiaries (RA1 and RA2), located in areas not affected by industrial activities. The HW was characterized by a seasonal pattern, also recorded in the RA1, with the lowest value in late August 2018 (<30 kg) and the highest in late June 2019 (60 kg). The FA followed a bi-modal pattern with a peak recorded in the first week of June 2020 indicating a great foraging activity in both the experimental ( $6 \times 105/\text{week}$ ) and the RA1 ( $4.5 \times 105/\text{week}$ ). The IHT was held at 35 °C during the whole foraging season of 2019, but some inter-hive variability in the winter period when the minimum (>5 °C) was observed, as in the RA1. The IHM was generally low reaching critical values (i.e. above 18/day) in April and May 2019. In May 2019, a similar situation (43/day) was observed in the RA2, but the April data were unavailable for comparison purposes. However, the average year IHM for the two apiaries were comparable ( $p > .05$ ). The coincidence in the highest IHM value can be reconducted to the hive phenology or to the agrochemical usage in the surrounding areas of both apiaries. The average honey production calculated based on the HW recording was  $4.6 \pm 5.8$  kg with no difference with respect to the yield recorded in RA2 ( $6.2 \pm 10.8$  kg per hive) ( $p > .05$ ). From the results obtained for the 2018–2019 study period, the landfill activity did not affect the



hive functionality. Further investigations are needed to exclude any possible effect on the IHM recorded in 2019 spring.

## P158

### Nutritive value, in vitro fermentation kinetics, and methane production of hay produced in the internal area of Southern Italy

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In the internal area of Southern Italy, the small semi-extensive cattle farms producing high quality meat, milk, and dairy products commonly include a high amount of forage (about 80–70%) in animal feeding plans. When the grazing resources are inadequate (July–August, December–February), the hay locally produced is the most utilised forage, notwithstanding its nutritional value, it is not accurately evaluated. The use of local hay has economic advantages for the breeder and environmental rewards in terms of global warming potential (LCA study).

Eight mixed hays, collected in four different provinces (AV, BN, CB, PZ) were characterized. The chemical composition was analyzed, including carbohydrates fraction according to official protocol and the nutritive value was calculated. The in vitro fermentation trial was performed with dairy cow inoculum over 120 h of incubation. After 24 h of incubation CH<sub>4</sub> production was analysed using a gas chromatograph. The fermenting liquor was analyzed for pH and VFA. The influence of the different sampling areas was statistically evaluated using a Student's t test.

The sampling area significantly influenced most of the chemical composition parameters, excepted ADL. The protein level ranged from 7.92 to 14.20 % DM and NDF between 42.13 and 58.63 % DM, indicating forages of medium quality. Significant differences emerged for OM degradability and kinetics parameters ( $p < .001$ ) but not for gas production, VFA and pH. The highest CH<sub>4</sub> production (14.48 %;  $p < .05$ ) was measured in hays from the PZ area, associated with high acetate (58.5 mmol/g) and butyrate (7.56 mmol/g) values.

The forage produced in AV province was the best in terms of chemical composition and nutritive value (CP, NDF and energy content) and also showed the most interesting in vitro characteristics (high OM degradability, gas and VFA production) and favorable environmental aspects (low CH<sub>4</sub> production).

The variable chemical composition observed in the studied hay, depending on many factors (i.e. environmental condition during the plant growth and harvest, haymaking technique, etc.), has influenced the in vitro fermentation characteristics, including

CH<sub>4</sub> production. Data obtained from this study provide some useful information about forages produced in some internal areas of Southern Italy. The results underline the need to pay more attention to haymaking to improve hay quality.

## P159

### Life Cycle Assessment (LCA) and dairy cattle. A new perspective of allocation criterion

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In Europe dairy milk production shows the highest environmental impact (28–30%), along with beef (28–29%) and pork production (25–29%), in terms of greenhouse gas (GHG) emissions. In this study, the Life Cycle Assessment (LCA) principle was performed to estimate the environmental impacts in three different dairy farms, comparing organic (ORG), conventional (CON) and high quality (HQ) milk production, an Italian strictly regulated system (law decree no. 185/1991), commonly included in the conventional mode. The farms are not fully representative of each system, but they were compared because they share similar characteristics (family-owned company, geographical location, management of the same water resources), desirable for a LCA approach. LCA methodology, in compliance with the principles established by ISO standards, was applied to estimate global warming potential (GWP, kg CO<sub>2</sub>-eq), terrestrial acidification (TA, kg SO<sub>2</sub>-eq), freshwater eutrophication (FE, kg P-eq), marine eutrophication (ME, kg N-eq), agricultural land occupation (ALO, m<sup>2</sup>y), water depletion (WD, m<sup>3</sup>), metal depletion (MD, kg Fe-eq), fossil depletion (FD, kg oil-eq). The mass and economic allocation was also adopted to evaluate the effect of co-products in the Functional Unit (1 kg of FCPM, Fat and Protein Corrected Milk). The farms showed peculiar trends for farm management (somatic cell count, culling rate) and impact categories, i.e. the agricultural land occupation (ALO) and the fossil depletion (FD) of each system. The analysis of variance (ANOVA) confirmed different levels ( $p < .001$ ) of somatic cell count in the milk of the three farms, with the lowest values in the HQ farm. The ALO showed high values in the ORG farm, where the pasture-based system was also affected by low dairy yields. The

ORG company, with the lowest value of FD, would confirm the practice of minimum tillage, commonly adopted in organic farming. The culling rate highlighted that the non-dairy products mitigated ( $p < .05$ ) some impact's categories. Particularly, the mass allocation mode decreased all the impact categories of the ORG farm, except for the WD. The allocation method showed statistically different values than the unallocated output in the three farms, in several categories. The culling rate was considered a strategic issue of the study and its percentage is representative of each system (HQ, ORG and CON), with the highest value in the ORG farm.

## P160

### Experimental activities for the drafting of guidelines aimed at a sustainable exploitation of *Chamelea gallina* resource (L., 1758)

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Since 2009 there has been a gradual and inexorable decrease of the *C. gallina* resource along the coasts of Friuli Venezia Giulia-FVG (I), determined by the death of vast quantity and then aggravated over the years by the succession of adverse climatic events. In 2016 a productive reactivation activity was carried out in four macro-areas of the Maritime Compartment of Monfalcone (Go), with comforting results, which made it possible to contribute to the income of businesses. In 2017, a further initiative with cleaning of the seabeds and control of competing species (e.g. *Glycimeris* sp.) was conducted, but in 2018, the storm 'VAIA' occurred, which further worsened the criticalities of the natural banks of *Chamelea gallina*. The regional shellfish sector was affected by this situation, showing a 75% decline in turnover compared to 2009, with worrying repercussions on employment levels and consequent socio-economic problems. In order to identify guidelines for the recovery and subsequent sustainable environmental management of these resources, ERSA was entrusted by MIPAAFT with the task of carrying out a project aimed at environmental restoration and conservation of the *C. gallina* resource in the Maritime Compartment of Monfalcone.

Partnership: ERSA Agenzia per lo sviluppo rurale del FVG; ARPAFVG Agenzia Regionale per la Protezione dell'Ambiente del FVG; Dipartimento di Scienze della Vita UniTS; Co.Ge.Mo. Consorzio gestione pesca comp. Monfalcone. Planned activities: (a) bibliographic collection about the management of bivalve molluscs; (b) identification of suitable seabeds, cleaning, oxygenation of the substrates and containment of unwanted species; (c) dynamic management of the areas, with identification and characterization of *C. gallina* donor sites, productive reactivation

and subsequent monitoring and control; (d) drafting of operational protocols (Best practice), applicable in similar contexts. In December 2020 the productive reactivation was conducted, with the withdrawal of the resource (about 20 tons) from donor sites (Veneto-Italy) and its sowing in the experimental area. The monitoring phase has started in Jan. 2021. The productive reactivation foreseen for these coastal areas will be based on the need to restore a resource, guaranteeing its sustainability. The subsequent correct management, always in compliance with ecological balances, cannot ignore constant monitoring and periodic control actions, which must carefully verify the conservation status of the marine.

## P161

### Effectiveness of cover crops for supporting biodiversity and agronomic and ecosystem services in agricultural and rural landscapes

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Cover crops are assuming a central role in organic or conservation agriculture due to the many ecosystem services they can offer, not only in agriculture but also to the whole community. These plants are inserted in the rotation between two main crops to give adequate coverage to the soil, even in the intercropping period. Considering the cost of the seeds, the expensive necessary work, the required time to be dedicated and the equipment used, it is important to highlight their multi-functionality to encourage farmers to use these types of crops. The aim of this experimental trial was to estimate, in different field conditions, the effectiveness of the chosen cover crops in terms of agronomic success and support of ecosystem support services (improvement of soil conditions, nitrogen fixation, reduction of chemical fertilizers, improvement in the supply of organic matter, etc.) and regulation (containment of adventitious plants and pollination service to neighbouring crops). Overseeding (bulatura) was carried out on autumn-winter cereal of *Trigonella officinalis*, *Phacelia tanacetifolia*, *Lotus corniculatus*, *Onobrychis viciifolia*, *Trifolium incarnatum*, *Trifolium subterraneum*, *Medicago sativa*, *Trifolium alexandrardoinum*, *Trifolium repens*, and a sowing in late spring-early summer, in four distinct plots of *Trifolium alexandrinum*, *Phacelia tanacetifolia*, *Trigonella foenum-graecum*, *Vicia sativa*, *Sinapis alba* and *Fagopyrum esculentum*. In this first design phase, the following parameters were evaluated, also employing photo interpretation and multispectral remote

sensing by DRONE): abundance of weeds and cover crops; flowering intensity of cover crops; vegetation growth indices; CHN and isotopic values. In the field interested by the overseeding, it has been observed that *T. officinalis* positively affects the total plant biomass, while in the late spring-early summer sowing, *T. foenum-graecum* has developed the maximum biomass, usefully limiting the adventitious species. It has been noted that adventitious species are predominant in soils with low water capacity. *T. foenum-graecum* is the only species suitable for all soils, capable of maintaining a higher biomass value than adventitious species. In the next spring, further field activities and the collection of additional data relating to the performance of the investigated species will be carried out, aimed at identifying the best possible seed mixtures and the most suitable agronomic practices.

## P162

### The environmental impact of draught horses in Guatemala as assessed through Life Cycle Assessment

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The goal of this study was to assess the environmental impact of horses used in Guatemala as draught animals through Life Cycle Assessment (LCA). The functional unit was 1 MJ of work. Primary data on feeding regimen, wood load, age and body condition of the animals were directly collected on four farms managing 9, 9, 7 and 10 horses, respectively. Feeds included concentrates such as maize grains, wheat grains and afrecho (maize flour), hay (*Pennisetum clandestinum*) and tazol (maize plant without caryopsis). Horses were also allowed to graze on pastures mainly consisting of *Pennisetum* spp. Inputs and outputs were calculated for each crop. Each animal was loaded on the packsaddle with about 75 kg of wood. The track of the animals, including steepness, was gathered using a podometer linked to a free online recording system. The mean weight and body condition as assessed on a 5-point scale, were  $242.8 \pm 36.6$  kg and  $2.1 \pm 0.7$ , respectively. Five arbitrary categories of work expressed in MJ/year were identified (<599, 599–799, 800–999, 1000–1999, >1999 corresponding to category A, B, C, D and E, respectively). The ReCiPe Midpoint (I) module of SimaPro package was used. Total emissions were estimated for each work category. As expected, the categories producing more MJ/year were

less impactful. In particular, global warming was lower in categories D and E as compared with A ( $p < .05$ ), whereas in category E was lower than in categories B and C ( $p < .01$ ). Similarly, the use of non-renewable fossil was lower in category E than in categories A, B and C ( $p < .05$ ). Finally, the eutrophication potential was lower in category E than in category A ( $p < .01$ ). These results can be attributed to the dilution effect of the maintenance requirements which are divided across a higher number of MJ produced per year in the categories performing more work. However, all of the impacts assessed in this study were much lower than those produced by mechanical traction as reported in previous studies, indicating that horses impact less on the climate compared to substitutive motorised vehicles. In addition, no effect of the category of work on body condition was observed ( $p > .10$ ), suggesting that the welfare of the horses was related to other aspects (e.g. feeding, veterinary care) rather than to working load. We conclude that working equids in marginal areas may represent a way to contain the environmental impact of agriculture if the welfare of the animals is kept at satisfactory levels.

## P163

### Heritability of residual feed intake measured on young performance tested bulls belonging to Italian dual-purpose breeds

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Residual feed intake (RFI) is a measure of feed efficiency that is also related to the emission of greenhouse gases and pollutants. Therefore, improvement in feed efficiency has the potential to increase both cattle farms' profit and to decrease environmental footprint. For these reasons RFI has already been included worldwide in breeding programs, also considering its consistent heritability. The aim of this study was to estimate genetic parameters for RFI in young performance tested bulls of dual-purpose Pezzata Rossa Italiana (PRI, i.e. Italian Simmental), Alpine Grey (AG) and Rendena (RE) cattle breeds. A multibreed animal model approach has been applied to RFI data of 374 bulls (290 PRI, 47 AG, and 37 RE) recorded from 8th to 10th month of age. The model accounted for the following fixed effects: contemporary group (i.e. animals in the same pen, 73 levels) and breed, that produced a 0.489 of R<sup>2</sup> at the preliminary ANOVA. The animal in the pedigree file were 3250 animals: i.e. 1776 PRI, 746 AG, and 728 RE.



Variance and covariance components were estimated using a model implemented in a Bayesian framework using the software GIBBS3F90; 500,000 iterations were run discarding the first 200,000 samples as burn-in and storing samples every 100 iterations (i.e. 3000 chains were available for marginal posterior density analysis). RFI resulted  $0.002 \pm 0.759$  kg/d on average (min.  $-2.443$  kg/d, max  $1.985$  kg/d), with significant differences between breeds: i.e.  $0.091 \pm 0.768$  for PRI,  $-0.334 \pm 0.648$  for RE, and  $-0.278 \pm 0.641$  for AG. Genetic variance of RFI resulted  $0.1017 \pm 0.3189$  kg<sup>2</sup>/d<sup>2</sup>, while heritability was  $0.263 \pm 0.176$ . From these preliminary results, it seems that RE and GA resulted more efficient than PRI, but a larger dataset is needed for more definitive conclusions. Despite the large marginal posterior standard deviation of heritability, this preliminary analysis confirmed the presence of a genetic component for RFI.

## STRATEGIES FOR IMPACTS MITIGATION

### P164

#### Residual biomass from the oil sector as animal feed: preliminary data on chemical and microbiological contamination

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In recent years, the design of 'zero waste' supply chains has taken on incredible importance. In the past, residual biomass has been treated as a waste product. However, the perspective has changed: residual biomass is now considered a useful service for the ecosystem. The olive oil supply chain is certainly one of the most promising supply chains. In addition to oil for human consumption, there are numerous by-products, usually real waste, whose disposal is very expensive. Hence, it is necessary to identify a possible reuse of these by-products. Among these, the olive pomace, rich in antioxidant substances such as carotenes, anthocyanins, tocopherols and polyphenols, can be used in various contexts, including the food industry and the zootechnical sector. A significant aspect in this context is related to the safety of by-products. This study aims at evaluating residues of chemical and biological contaminants in samples of the totally pitted dried olive cake characterized by 16% (dry matter 93.85%) of olive oil content, for animal feed use. The samples were collected from 3

big bags containing 1500 kg of olive cake each. The content of 28 mineral elements, including the potentially toxic ones, was assessed via ICP-MS. The concentration of 142 organic contaminants (PCBs, IPA, pesticides) was assessed via GC-MS. In addition, hygiene indicators were determined. The concentrations of Pb, Hg and As ( $0.01$  mg/kg for each element) in the olive cake samples were lower than the maximum levels set by the UE 2015/186 Regulation. Among the organic contaminants, residues of very few contaminants were found, some of which not even mentioned in the current regulations. From the preliminary microbiological data, no value index of a possible contamination of this matrix emerged. To conclude, given the reassuring results obtained from the preliminary analysis and the almost total absence of chemical and microbiological contaminants, optimal use of dried olive cake as animal feed would be desirable. However, in the future, we will try to deepen, improve and optimize the quality of this matrix for the intended purpose.

#### Acknowledgements

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### P165

#### Introduction of innovative strategies in dairy sheep feeding on milk production and climate change

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The objective of the present study was to assess the effect of the introduction of innovative strategies in dairy sheep feeding on milk yield and quality and on the environmental impact. Three traditional dairy sheep farms (TF) were compared with three innovative dairy sheep farms (IF) in term of milk yield and quality and environmental impact. TF dairy farms adopted a semi-intensive system based on annual crop forages mainly composed of oat and clover, integrated with commercial concentrate feed composed by mix of cereals, and soybean meal and agri-industry by-products. IF dairy farms adopted a semi-intensive system based on the introduction of perennial forage crops (mainly legumes) and extruded linseed and soybean oil as ingredients of concentrate feed. Moreover, in the IF dairy farms, a precision feeding approach based on the Cornell Net Protein and Carbohydrates System was adopted, for the optimization of the



ration formulation, according to the amount and quality of pasture. Milk of TF and IF sheep was analyzed by Milkoscan to determine the milk fat and protein content. The milk production by the TF and IF farms was recorded once a week. The environmental impact of both farming systems was evaluated by using a cradle-to-farm-gate Life Cycle Assessment (LCA), considering 1 kg of Fat Protein Corrected Milk (FPCM) as functional unit. One-way ANOVA was used to assess statistically significant difference between TF and IF. Results showed that the milk production of IF farms was 50% higher than that of TF farms. No differences were found for milk fat content, whereas the protein content was lower in milk from IF farms compared to TF, probably due to the dilution effect. On average, climate change indicators per kg of FPCM were significantly lower for IF farms by 43% (like general consumption, manure management, grassland cultivation, concentrate, etc.). Estimated CH<sub>4</sub> emission from enteric fermentation was the largest contributor to the total carbon emissions for both systems. The introduction of innovative forage productions and precision feeding strategies improved the milk production and reduced climate change impact per kg of FPCM.

## P166

### Vermicompost from animal manure: characteristics and environmental impact

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Aim of this trial was to evaluate different animal manures as composting materials to produce vermicompost, investigating the chemical-physical characteristics of the final products and the environmental impact of the composting process. Four animal wastes (beef manure, BM; solid fraction from dairy slurry, DS; solid fraction from pig slurry, SS; digestate from anaerobic digestion of pig slurry, AD) were composted with earthworms (*Eisenia foetida*) into four fissured plastic bins (110 × 120 × 65 cm; 4 × 4 = 16 bins) for 1 year. During the composting process temperature, pH, weight of input (earthworms, manure, water) and output (earthworms, drained liquid, vermicompost) were recorded. Manure, vermicompost and earthworms were analysed for dry matter (DM), organic matter (OM), ether extract (EE), neutral detergent fibre (NDF), N, P, K, Cu, Mn, Ni, Pb and Zn content. In addition, vermicompost were characterised for N ammonium, nitrates, C/N ratio and apparent density and germination index (Ig) was evaluated. The manures have showed different initial characteristics: BM was higher for N, K, EE and NDF, AD was lowest for DM, P and EE. At the end of the composting process, the vermicompost showed good chemical and physical characteristics and no signs of phyto-toxicity (Ig > 78%). Total N, P and K content on DM ranged between 2.0 (BM) and 2.8 (DS), 0.1 (DS) and 0.4 (SS), 0.3 (SS)

and 1.2% (BM) respectively. N ammonium content was different for DS than BM and SS and than AD (67, 41, 37 and 23 mg kg<sup>-1</sup> on DM respectively;  $p < .01$ ). Nitrates were higher in DS, SS and AD than BM (751, 817, 697 and 420 mg kg<sup>-1</sup> on DM respectively;  $p < .01$ ). C/N ratio and pH were higher ( $p < .01$ ) in BM (13.5 and 8.1 respectively) and lowest in DS (C/N = 11.1) and SS (pH = 7.1). Apparent density (0.32 g cm<sup>-3</sup> as mean) was similar for all thesis. The OM losses involved in the processes were high for AD (36%), intermediate for AD and BM (12%) and low for SS (9%;  $p < .01$ ). N losses for leaching and volatilization were 2, 8, 17 and 27% for SS, DS, AD and BM respectively ( $p < .01$ ). Comparing the N content in manures and vermicompost, an average decrease of about 15% was recorded. In all cases, the heavy metals were well below the legal threshold. In conclusion, independently by the type of animal waste, the composting process could represent a useful strategy for livestock farms to better manage the manure and to reduce its N content, allowing an increase of the number of animals reared per unit of agricultural area.

## P167

### Salad crops leftover as feed ingredient for ruminant diets: nutrients content and microbiological quality

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In this study, selected salad crops (SC) have been researched for their nutritional composition and microbiological quality. Eighteen sample SC originating from a plant manufacturing have been collected in the summer season in two locations: at the factory plant and one-day storage (to mimic farm location). The samples were subsequently examined for pH, dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). The Total Viable Count (TVC) (ISO 4833) of eight selected samples were analyzed for Enterobacteriaceae (ISO 21528-1), *Escherichia coli* (ISO 16649-1), coagulase-positive Staphylococci (CPS) (ISO 6888), presumptive *B. cereus* and its spores (ISO 7932), sulphite reducing Clostridia (ISO 7937), Yeasts and Moulds (ISO 21527-1) and the incidence of *Salmonella* spp. (ISO 6579). Data were analysed using IBM SPSS Statistics version 21 software (SPSS Inc.). Salad crops at factory plant and at one-day storage presented pH value of 6.0 and 6.4, respectively. The SC nutrient composition was compared to conventional fresh grass; the results showed that the CP content was 210 g·kg<sup>-1</sup> DM, while the average NDF, ADF and ADL contents were 360, 238, and 74 g·kg<sup>-1</sup> DM respectively. The main variation between fresh grass and SC was for DM

content, that was lower ( $5.8 \pm 0.8 \text{ g} \cdot \text{kg}^{-1}$ ) in SC samples compared to literature grass values ( $25 \text{ g} \cdot \text{kg}^{-1}$ ). Total Viable Count was scant and *Salmonella* was never present. Salad crops are a prospective feed ingredient for ruminants' diets, albeit a full assessment of their potential requires further investigation.

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### SENSORY AND INSTRUMENTAL ORGANOLEPTIC PROPERTIES

#### P168

#### Mechanical strength of myofibrillar and connective tissue of Italian Mediterranean Buffalo meat

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Meat tenderness, or toughness, is a complex property determined mainly by the connective tissues and the muscle fibres. The amount of collagen, as well as the density and type of cross-links between collagen fibrils are directly linked to meat toughness. The aim of this study was to investigate the effect of sex and ageing time on the mechanical strength of Italian Mediterranean Buffalo meat using the Warner-Bratzler shear force (WBSf) test and the Texture Profile Analysis (TPA), which are the classic mechanical methods for estimating meat tenderness. Samples of *Longissimus thoracis* muscle, from 6 young males and 6 spent females ( $17 \pm 1$  and  $47 \pm 18$  months old, respectively), were aged at  $4^\circ\text{C}$  for 7 and 14 days. At each ageing time, meat samples were analysed for WBSf on raw and cooked meat, and for TPA on raw meat. WBSf of raw meat gives an estimation of the connective tissue toughness, whereas WBSf of cooked meat largely reflects myofibrillar toughness. For WBSf of cooked meat, the meat was vacuum packed and cooked in a water bath at  $75^\circ\text{C}$  until the internal temperature reached  $70^\circ\text{C}$ . In WBSf test, tenderness was measured as the maximum force required to shear  $1 \text{ cm}^2$  cross-section cores. In TPA test, samples were compressed twice at 20% and 80% of their original height. Hardness (H, maximum force required to compress the sample) at low and high strain values were used to measure the strength of the myofibrillar and connective tissue, respectively. Data were analysed by GLM procedure considering sex and ageing time as factors. As regarding connective tissue, no significant differences between sexes were observed in WBSf values of raw meat and H at 80%, probably also due to the confounding effect of age at slaughter. Similarly, no significant differences were detected

for the two parameters during ageing. Young males' meat was found to have significantly higher cooking loss percentages than that of spent females (26% vs. 22%;  $p = .004$ ). Concerning myofibrillar tissue, no significant differences between sexes were observed for WBSf values of cooked meat and H at 20%. Instead, ageing significantly reduced only the WBSf values of cooked meat ( $47.32$  vs.  $34.15 \text{ N}$ ;  $p < .001$ ) that is an estimation of myofibrillar toughness. The results indicate that H at 80% showed a lower coefficient of variation than that of WBSf of raw meat, and WBSf on cooked meat are more suitable to estimate the connective and myofibrillar tissue strength of meat, respectively.

#### P169

#### Muscle pigmentation in rainbow trout fed novel protein sources from microalgae and crustaceans: the image analysis approach

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The pink-red pigmentation of rainbow trout (*Oncorhynchus mykiss*) fillet determines consumer choice and economic value of the products. The main responsible for salmonid muscle pigmentation is astaxanthin, that is provided with diet, primarily as synthetic astaxanthin. However, the high costs of synthetic pigments and the consumers' concerns about their use in animal feeds are leading the research for natural carotenoids.

The pattern of fillet pigmentation was assessed in rainbow trout (*Oncorhynchus mykiss*,  $n = 63$ , mean weight  $260.5 \text{ g}$ ) fed for 15 weeks with six (in triplicate) iso-proteic (42%) and iso-lipidic (24%) pelleted diets deprived of fish meal where 10% of vegetable protein blend was replaced by microalgae dried biomass (*Arthrospira platensis*, AP, *Tetraselmis suecica*, TS, a mix of *Tisochrysis lutea* and *T. suecica*, MA) or red swamp crayfish (*Procambarus clarkia*, RC) meal. A commercial diet (CO, 3 replicates) was used in the trial. The feeding trial was carried out at the Edmund Mach Foundation (San Michele all'Adige, IT). All procedures involving fish

manipulation were carried out in accordance with the EU legal framework relating to the protection of animals used for scientific purposes (Directive 2010/63/EU). They were approved by the Animal Welfare Committee of the Istituto Zooprofilattico Sperimentale delle Venezie and authorized by the Italian Ministry of Health (permission n. 530/2018-PR). An automatic, repeatable, free, and objective image analysis tool for the integrated determination and direct visualization of fillet colour was applied to digital images. Colour of fillets was also measured with a chromameter ( $L^*$ ,  $a^*$ ,  $b^*$  indexes) and used to validate image analysis results. Fillet carotenoid content was determined, and the pattern of variation was compared to that obtained by image analysis.

Image analysis results and colorimetric data consistently indicated that, even if characterized by a significantly higher fillet carotenoid content respect to vegetable control ( $0.85\text{--}2.13\text{ mg kg}^{-1}$  vs.  $0.17\text{ mg kg}^{-1}$ ), fillets of trout fed microalgae-based diets displayed a yellowish colour instead of the desired pink-red one. *P. clarkii* meal was instead confirmed as a promising and sustainable integration in vegetable aquafeed for carnivorous fish, in view of its carotenoid content ( $0.49 \pm 0.07\text{ mg kg}^{-1}$ ) and coloring capacity. Further studies are required to evaluate the potential of this ingredient as a natural source of carotenoids, both testing the effects on flesh pigmentation of higher percentages of inclusion and longer administration times, or at different developmental stages.

## FOOD LABELLING AND VALORIZATION

### P170

#### Using Blockchain for animal welfare labelling: a case-study on dairy products

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This study aimed to explore the applicability of blockchain as an instrument to convey clear and unalterable information on the welfare of food-producing animals, in order to improve consumers' trust in farmers and in the livestock sector.

Dairy products were taken as a case-study, and parameters listed in the ClassyFarm welfare assessment protocol were used. An exploratory questionnaire was submitted to a small sample of Italian consumers ( $N = 112$ ) asking them to rate, on a 0-to-5 scale, the perceived importance of each of the parameters of the ClassyFarm checklist in determining dairy cows' welfare. Based on the ratings attributed by consumers, a prototype webpage was built, in which animal welfare parameters could be shown to consumers based on their perceived importance.

The Blockchain-based prototype was built so that consumers, through a QR-code and/or a batch number printed on the dairy product packaging, could access a specifically-created 'landing page'. The landing page allowed consumers to freely navigate among different animal welfare parameters and showed first those parameters which were rated as more important in the questionnaire. For each parameter, the page would provide specific information on the farm (or farms) from which the milk came from. Each parameter, classified as insufficient, improvable, or optimal according to the ClassyFarm protocol, would be visualized in the landing page using an intuitive traffic-light system (red, yellow, or green, respectively), and providing additional information upon request.

Our method allowed the creation of a prototype that would allow consumers to retrieve information concerning the product of animal origin they are going to buy. The prototype was meant to be intuitive and easy to understand also for those having no previous knowledge about animal production or animal welfare. This case-study could represent a first step towards using blockchain technology for animal welfare certification, fostering a direct information exchange between producers and consumers, thus enhancing the growth of new, diversified markets based on trust and transparency. A foreseeable weakness would be the need to feed the system with very heterogeneous data, originating from different data acquisition systems that are not always fit with automation. This issue could be overcome over the next years thanks to the progressive implementation of the integrated ClassyFarm system.

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### P171

#### A labelling scheme to communicate the health values and environmental sustainability of meat and milk from extensive farming

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Food from extensive agricultural systems may have positive peculiarities which can hardly be valued because not adequately perceived by consumers. Food labels let manufacturers to promote the value-adding qualities of their products and help consumers make informed choices about the food they buy. In the EU, the Regulation No 1169/2011 provides the principles governing mandatory food information on nutritional characteristics, allowing additional forms of expression and presentation only if based on sound and



scientifically valid consumer research. Aim of this study was to set up a labelling scheme to inform about healthy promoting nutrients and environmental sustainability of meat and milk obtained by extensive farming. In our case study, three farms were selected from the Apennine context (Umbria region) with similar marketing strategies (direct sales/long channels) and farming system (extensive). At a first step, a survey was conducted to map the perception of farmers about the added values of their products. Subsequently, the labelling scheme was conceptualized, and a multi-level system approach was adopted in order to transparently show that there are different production and quality standards for a food product. The labelling issues key issues were scientifically validated as follows: (i) health promoting nutrients (e.g. polyunsaturated fatty acids, n-6/n-3 fatty acids ratio, precursors of conjugated linoleic acid) were determined from meat and milk samples according to validated procedures; (ii) sustainable aspects were assessed estimating soil erosion patterns with the Revised Universal Soil Loss Equation (RUSLE), the territorial bipotential (BTC) and coexistence with wildlife (ascertaining the presence of wolves and other wild ungulates). As a final step, we proposed different graphic-text multi-level labels to be used packaging designed to minimize information overload and to emphasize the qualitative characteristics listed above. Further research is needed to focus on the effectiveness of the marketing communication around the labeling scheme proposed, also in order to identify what tools and forms of communication could be more relevant to consumers.

## P172

### Characterization of mountain cheese from cows fed Alpine pasture grass by fatty acids composition and multivariate analysis

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Fatty acids (FA) profile of dairy products is related to the livestock production system and to dietary regimen supplied to animals in the farm. Consumers perceive mountain cheese as more healthy and 'natural', is associated with a non-intensive production system linked to local/artisanal traditions. In this study, cheese samples from cows fed Alpine pasture grass collected in two Italian farms were characterized by means of multivariate analysis performed on feedstuffs composition and cheese FA profile.

Farm A was characterized by the exclusive employment of grazing in Alpine pastures set at 2000 m asl. In farm B cows were fed

daily-cut fresh grass harvested in an alpine valley set at 1300 m asl during the day and supplied with a mix of hay and concentrates during the night, in the ratio grass/mixed ration 65/35. Feedstuffs and cheese samples were collected once a week from July to September. Chemical and FA composition of feedstuffs were analysed by standardized methods. The FA profile of cheese was analysed by GC-FID. Samples collected from different farms were compared by means of non-parametric tests and then Principal Component Analysis was performed based on correlations.

FA profile of the diet consisting exclusively of Alpine pasture grass (farm A) showed a higher content of  $\alpha$ -linolenic acid and lower content of linoleic acid compared to the diet in farm B. Cheese collected in farm A showed a FA profile enriched in odd and branched chain FA ( $4.40 \pm 0.20\%$ ), MUFA ( $34.05 \pm 1.08\%$ ) and cis9trans11 CLA ( $1.49 \pm 0.07\%$ ), positively correlated to the amount of NDF, ADF, palmitoleic acid and  $\alpha$ -linolenic acid in the feedstuffs. Cheese collected in farm B showed a FA profile enriched in n-6 FA ( $3.18 \pm 0.15\%$ ), mainly linoleic acid ( $2.81 \pm 0.14\%$ ), and SFA ( $67.40 \pm 0.87\%$ ), particularly the medium chain lauric ( $4.03 \pm 0.15\%$ ), myristic ( $12.16 \pm 0.29\%$ ) and palmitic acid ( $29.60 \pm 1.23\%$ ), positively correlated to the amount of stearic, oleic and linoleic acid and negatively correlated to the amount of NDF in the ration.

Differences were detected among cheese samples collected in farms where cows were fed exclusively Alpine pasture grass or integrated with forage and concentrates. However, all cheese samples analysed were characterized by a high nutritional quality FA profile due to the bovine consumption of fresh grass, thus supporting consumers' appreciation toward mountain products.

## P173

### Nutritional profile of Ager cheese selection

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Food labels are the main method to ensure that consumers are appropriately informed as regards the food they consume. In Europe, the provision of food information to consumers is regulated by specific laws, including (i) the European Regulation (EU) no 1169/2011, which regulates the mandatory food information including a list of mandatory particulars and nutritional declarations and (ii) the European Regulation (CE) no 1924/2006, concerning the voluntary nutrition and health claims (NHC) made on foods. Nutrient profiling involves the classification and ranking of foods according to their nutritional composition to prevent disease and promote health. For this purpose, the following 14 cheeses were selected (Ager cheese selection) among the Natural Historic Cheeses of Southern Italy: Ragusano PDO; Provola dei Nebrodi PDO; Piacentinu Ennese PDO; Pecorino Siciliano PDO; Vastedda della Valle del Belice PDO; Caciocavallo Palermitano PAT; Maiorchino PAT; Caprino Nicastrese; Casizolu del Montiferru PAT; Pecorino di Filiano PDO; Canestrato di Moliterno PGI; Pecorino di Carmasciano PAT; Caciocavallo Podolico PAT; Cacioricotta PAT. A total of 173 cheese samples were analysed for moisture, protein, fat, ash, salt and fatty acid (FA) composition. On the basis of moisture content, the Ager cheeses may be classified into semi-hard, with moisture content varying between 35% and 45% (from Provola dei Nebrodi with 35.5% to Vastedda del Belice with 45.6%) and hard-cheese, with moisture content below 35% (from Pecorino di Carmasciano with 27.6%, to Maiorchino with 34.5%). Fat is an important component of cheeses and according to the general standard of cheese, the Ager cheeses can be classified as full fat, with a content of fat in dry matter above to 45% and less than 60% (from Pecorino di Carmasciano with 45.5% to Canestrato di Moliterno and Cacioricotta both with 46.5%) and medium fat, with content above to 25% and less than 45% (from Provola dei Nebrodi with 38.1% to Caciocavallo Podolico with 44.6%). As the saturated FA content, the AGER cheeses show the lowest content in Maiorchino cheese (65.4%) and the highest in Cacioricotta (73.3%). The chemical characteristics of each cheese are strongly linked to the milk species and breed, feeding system, production season and ripening stage. This nutritional information has been made available to producers for the labeling of their cheeses.

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## P174

### Evaluation of LAMP amplification and short enrichment time for the detection of pathogens in food

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The quick identification/quantification of foodborne pathogens is fundamental for ensuring food safety, containing and preventing pathogen spread and avoiding costly recall and destruction of food lots. *Salmonella* and *Campylobacter* spp. are among the most diffused pathogens responsible for foodborne infections in EU countries. Poultry often serves as a reservoir for these pathogens. *Salmonella* and *Campylobacter* screening is mainly accomplished using PCR or Real Time-PCR following 18–40 h-long incubation of food samples in specific enrichment media. In this investigation we compared the performance of Real Time PCR and Loop-mediated isothermal AMplification (LAMP) combined with colorimetric detection, in revealing the presence of these pathogens in experimentally contaminated poultry meat. Poultry meat was contaminated at different pathogen concentrations ( $10^1$ ,  $10^3$ , and  $10^5$  C.F.U./g) and submitted to short enrichments (0, 2, 4, and 6 h). Real-Time PCR was able to detect *Salmonella* even without enrichment (T0) at a concentration of 10 C.F.U./g for *Salmonella* and  $10^3$  C.F.U. for *Campylobacter*. Results from colorimetric LAMP was compared to RT-PCR results using McNemar's and binomial tests. The performance of LAMP was similar to those of Real-Time PCR but without the requirement of a thermal cyclor and with shorter amplification time. These characteristics make colorimetric LAMP a promising quick tool in food quality assessment when one-day results are needed.

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