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# **Book of Abstracts**

Guest Editors: Massimo Trabalza-Marinucci (Coordinator), Cesare Castellini, Emiliano Lasagna, Stefano Capomaccio, Katia Cappelli, Simone Ceccobelli, Andrea Giontella



### ANIMAL WELFARE, HEALTH AND BEHAVIOUR - CATTLE HEALTH AND METABOLISM

particular attention for their ability to modulate inflammation. The objectives of this study were to compare the effects of CIA and EFAs on the expression of pro-inflammatory cytokines and their protective efficacy against inflammatory status in mammary gland by an in vitro model based on bovine mammary epithelial cells (BME-UVI). BME-UVI cells were treated with complete medium containing 50 µM of cis-9,trans-11 CLA (c9,t11 CLA), trans-10,cis-12 CLA (t10,c12 CIA), (α)-linolenic acid (aLnA), (γ)-linolenic acid (gLnA) and linoleic acid (LA). After 48 h by fatty acids (FAs) administration the cells were treated for 3 h with 20 µM of lipopolysaccharide (LPS) to induce inflammatory stimulus. Reactive oxygen species (ROS) production after treatments was assessed by dichloro-dihydro-fluorescein diacetate assay to verify and to compare the potential protection of different FAs against LPS-induced oxidative stress. The mRNA abundance of bovine pro-inflammatory cytokines ( $TNF\alpha$ ,  $ILI\beta$  and IL6) and peroxisome proliferator receptor-α/γ (PPARγ/α) were determined in BME-UV1 by qRT-PCR. The results showed that cells treated with FAs and LPS increased ROS production compared with control cells. Among treatments, cells treated with c9,t11 CLA and t10,c12 CLA isomers revealed significant lower levels of ROS production compared with other FAs. All FAs reduced the gene expression of pro-inflammatory cytokines. Among FAs, t10,c12 CLA, LA and gLnA showed an homogeneous reduction of the three cytokines and this may correspond to more balanced and efficient physiological activity and may trigger a better protective effect. The PPARy gene expression was significantly higher in cells treated with t10,c12 CLA, aLnA and LA, whereas the PPARa gene expression levels were lower in cells treated with different FAs compared to the control. These results suggest that FAs inhibited the transcription of pro-inflammatory cytokines by the upregulation of PPARy expression and probably by PPARy activation.

### 0046

# Cows relocation affects exosomes and their cargos in bovine milk

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Extracellular vesicles in milk contains several compounds, as lipids, proteins, noncoding RNAs, and mRNAs. Among the different types of extracellular vesicles, exosomes have attracted researchers since their sorting cargos are a regulated, non-random process, which is heavily involved in cell-to-cell communication.

The aim of the study was to analyse the variations of RNAs in milk exosomes in response to the relocation of dairy cows between production groups. The study was carried out on 76 lactating cows during the relocation from high yielding group to low yielding group. The extent of stress was evaluated by means of milk cortisol in samples collected in the afternoon from 2 days before to 3 days after the change of the group. Mean cortisol concentrations significantly increase on the day of relocation (day 3) and on the days 4 and 5. Six cows were selected according to cortisol variations after the relocation. The 3 cows with the highest change and the 3 cows with the lowest change of cortisol after relocation were selected. Pools of Milk samples of days 1 and 2 (Before) and days 4 and 5 (After) were prepared for exosomes isolation using a commercial kit (ExoEasy Maxi Kit, QIAGEN).

Exosomes were visualized with TEM after immune labeling followed by negative stain and electron microscopy was carried out targeting CD63 and HSC70 and analyzed on Philips CM10. Total extracted RNAs were quality checked and processed with Illumina® TruSeq® Small RNA Library Prep protocol. Libraries were sequenced to 50 bp (average of 64 millions of reads per sample). Sequences were mapped against Btau 5.0.1 and analysed for differentially expression using Bioconductor Rsubread and DESeq2 software. Overall, 9 miRNA and 7 IncRNA resulted significantly differentially expressed (FDR<.05), suggesting a potential role of exosome cargos as a proxy for animal resilience. According to functional analysis, 5 differentially expressed miRNA were reported to be involved in the inflammation process in human. Further studies will be required to fully understand the regulatory activity of these miRNA in bovine in flammation.

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## Lameness in finishing beef cattle: distribution of claw disorders in 2716 hind feet

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Lameness impairs health and welfare of beef cattle, both reared in feedlot and indoor systems. This condition leads to an economic loss by the farmer, derived from costs for treatment or early culling of the animals that could result in

