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“Feeding behaviour and feed efficiency in cattle”

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“I think using animals for food is an ethical thing to do, but we have got to do it right.

We have got to give those animals a decent life and we have got to give them a painless death.

We owe the animal respect.”

Temple Grandin

ABSTRACT

The efficiency of farm animals is fundamental in the livestock sector to assure sustainability from the perspective of a planetary shortage of resources. One of the most relevant metrics of the whole animal efficiency is feed efficiency, and the information of feed inputs (e.g., feed intake) is required to compute feed efficiency. Unfortunately, in commercial beef and dairy herds, feed intake is difficult to measure due to several challenges, mainly economic and operational ones. Among several factors affecting feed efficiency, feeding behaviour is expected to be linked significantly with the total amount of individual ingestion. In light of this, the current thesis had the general aim to obtain experimental information about the association between feed intake and feeding behaviour of cattle, with the perspective of evaluating the feasibility of using feeding behaviour traits as proxy indicators of feed intake and consequently the feed efficiency.

Today, precision livestock farming is well-developed, with widespread use of technologies such as milking robots, automatic feeding systems, and accelerometers in commercial farms. These technologies produce large amounts of accurate data regarding animal performance, health status, and feeding behaviour. In this thesis the feeding behaviour in cattle, both dairy and beef, was studied across different breeds and production systems, using individual data obtained from different technologies.

The experimental section of the thesis comprises three contributions.

The first trial investigated and characterized feed efficiency and feeding behaviour of the main Italian dual-purpose breeds (Italian Simmental (IS), Rendena (RE), Alpine Grey (AG) and two strains of Valdostana breed, Valdostana Red Pied (VR) and Valdostana Black Pied and Chestnut (VB)). Data about dry matter intake (DMI), body weight (BW), growth performance, and feeding behaviour of 890 young bulls located at two Italian genetic centers were collected using automatic feeding systems. RE and AG bulls were slightly more efficient than IS bulls (-0.33 kgDM/d and -0.36 kgDM/d vs +0.09; $p < 0.01$). The VR strain differed significantly from VB for feed efficiency (-0.01 vs +0.13, respectively; $p < 0.01$) but not for daily growth capacity. Considering feeding behaviour, the only

factor affected by different levels of feed efficiency was feeding rate, which was higher for the less efficient breed (98.2 g DM/min for IS bulls vs 83.1–86.5 g DM/min for RE and AG bulls respectively; $p < 0.01$). Overall, dual-purpose breeds can achieve considerable growth performances and feed efficiency levels. Furthermore, feeding rate could be a useful behavioural trait in studying feed efficiency.

The second study focused on the association between the daily eating time of dairy cows, recorded with wearable sensors, and diet composition, feed management, and performance. 14 commercial dairy farms with a total of 819 dairy cows of two breeds (Holstein Friesian and Italian Simmental) were considered in the study. All animals were equipped with sensors capable of measuring eating, rumination, and other behaviours. Cows were split into five eating time classes (ETC, min/d) and a principal component analysis was performed. Eating time was linked to diet composition, particularly positively with neutral detergent fibre digestibility and mainly chemical constituents of the diet. Regarding feed management, a higher frequency of feed pushing per day increased ETC ($r = 0.30$; $p < 0.01$). Cows in higher ETC classes appeared more productive (31.91 kg milk/day for the ETC-5 vs. 27.04 kg milk/day for the ETC-1; $p < 0.01$) with lower urea and somatic cell content in milk but without differences in milk quality (fat and protein). There were also differences between the two breeds, especially in milk yield, eating length, and rumination length. In conclusion, eating time could be a useful indicator of the overall efficiency and health status of cows, pending further studies to better define its role.

The last study investigated how feeding behaviour (both eating and rumination time) changes during the whole lactation of dairy cows, using sensors available in commercial farms. For this purpose, 5 commercial farms, selected on the basis of similar characteristics (size, area of location, diet characteristics, etc.), and with a total of 350 Simmental dairy cows were considered in the study. Of these, 80 subjects for whom data from the whole lactation was available were selected and monitored for a year. Animals were split into two parity groups (primiparous, PP, and multiparous, MP), and then into three classes of eating time (ETC) and rumination time (RTC). Data was

statistically analysed with a factorial design with animals as repeated measurements. Parity was positively linked with milk production ($p < 0.01$), but not with milk composition or health status or somatic cells. PP were more active in terms of eating time ($p < 0.01$), while MP cows had a greater eating rate ($p < 0.01$) and rumination time ($p < 0.05$). Animals in the higher ETC were more productive, especially at both ends of the lactation curve ($p < 0.01$). A very interesting correlation emerged between ET and RT. The milk yield had a favorable correlation with RTC as well, but unexpectedly, there was no influence on milk fat ($p > 0.05$). The farmer could benefit greatly from the integration and combination of all this information in managing the herd.

In the appendix, there is a study that aimed to investigate the differences between two alternative diets, sorghum silage (SoS) and cover crop silage (CcS), on the performance, milk quality, feeding behaviour, and digestibility of dairy cows. Eight Holstein Friesian dairy cows in the last stage of lactation were considered. No significant effect was found on milk yield and quality ($p > 0.05$) between the two experimental diets. However, differences in feeding behaviour were observed. SoS had higher apparent digestibility of dietary protein ($p < 0.05$), lower rumination ($p < 0.05$), and tendentially lower eating chews ($p < 0.10$) than CcS. Despite this, CcS can be used as an alternative to SoS in the diets of dairy cows in late lactation.

Feeding behaviour can serve as a valuable tool for farmers to make informed management decisions. By monitoring feeding patterns, farmers can gain insights into various aspects of the productivity, health, and nutritional needs of the herd. Furthermore, feeding behaviour information could serve as a potential tool for the implementation of feed efficiency evaluation in cattle. The interest in this topic is spread also in the production realities of the livestock sector to achieve better management performance of precision livestock technologies in commercial farms. Further studies are needed to implement the knowledge on this topic and make the information more effective and applicable in the daily routine of commercial farms.

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INTRODUCTION

Efficiency of farm animals is fundamental in livestock sector to assure sustainability in a perspective of planetary shortage of resources. One of the most relevant metrics of the whole animal efficiency is feed efficiency (Bach et al., 2020). Animals that transform feed in the greatest amounts of products or eat less than expected while maintaining the same level of production are desirable in terms of herds' profitability. Feed efficient animals also produce less excretion in the environment per unit of feed consumed. In this context, measuring livestock feed efficiency and applying knowledge of the factors driving its variance is critical for providing with economic and environmental sustainability solutions.

For measuring feed efficiency, individual data on the daily feed intake of animals are requested. This is because feed efficiency is often measured by the difference between the measured feed intake and the estimated feed intake based on the animal performance (residual feed intake; Koch et al., 1963). Measuring individual feed intake is the main obstacle in computing the feed efficiency, particularly on commercial farms where dedicated feeding equipment, that can measure individual feed intake, are often unavailable. Under these conditions, the individual ingestion of animals can only be estimated and research is developing in the area of utilization of several types of animal data (e.g. metabolic, productive, behavioural, etc.) useful as proxy variables to predict intake and feeding efficiency.

The dairy sector is greatly involved in the technological process known as precision livestock farming (PLF), defined as the use of information and communication technologies for fine-scale control of the variability of animals and environmental resources to optimize the economic, social, and environmental performance of livestock farms (Kaur et al., 2023; Eastwood et al., 2012). The evolution of this sector has led to the integration of several advanced technologies in commercial farms (such as milking robots, automatic feeding systems, accelerometers, intestinal bolus, and so on), that allow the collection of a large amount of individual data daily. These data include milk production and composition, feeding behaviour, activity, health status, and more. These data can be

related to feed efficiency and can be useful for studying new ways to classify animals based on feed efficiency.

The main topic of the thesis is to define the importance of feeding behaviour as a potential tool for evaluating feed efficiency in cattle on commercial farms equipped with precision livestock technologies. Before the experimental section, an introductory chapter aims to clearly define the concept of feeding behaviour in cattle, the factors influencing it, and the current systems used to measure it.

CHAPTER I - STATE OF THE ART

1.1 Feed efficiency, excretions, and emissions

Farm animals use part of the feed ingested for their maintenance and the remaining part is transformed into animal products useful for humans. The process of feed utilization determines the elimination of substances derived from ingested feed into waste products in the environment because the processes of feed utilization in animal organisms are always inefficient. These are gases and volatile substances produced directly through respiration and belching, as well as solid and liquid materials in feces and urine, to which substances produced by the fermentative processes of manure during storage or retention in farms should be added. It is important to emphasize how the term emission is more suitable for gaseous components, while the term excretion is more used for solid substances eliminated with feces or dissolved in urine, but the term emissions is given precedence for all substances.

A simple and clear definition of efficiency is “the relationship between the amount of energy put into a machine or process, and the amount of useful work that it produces” (Cambridge dictionary). When applied to livestock, this concept can be translated as the relationship between input - the amount of feed ingested - and output - such as milk production, growth performance, and adipose tissue deposition.

Feed efficiency is a critical aspect in a continuously developing sector such as livestock production, and it is essential for achieving an economic, environmental, and social sustainability.

Several studies have compared the productive performances of animal with different feed efficiency levels, noting increases in milk production (Karlsson et al., 2022), milk quality (Xi et al., 2016), and beef production (Parsons et al., 2020; Lancaster et al., 2009) in efficient animals. Another important aspect concerning livestock farming is the impact this activity has on the environment in terms of greenhouse gases produced by the production process. Feed efficiency of animals is inversely related to the amounts of emissions/excretions released in the environment. Livestock farming is a

major contributor to greenhouse gas emissions in the atmosphere, accounting for 12% of total anthropogenic emissions, of which cattle farming is the main driver, responsible for 62% of this emissions (FAO 2023). The most significant emissions are methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), and to a lesser extent, P emission.

CH₄ is a potent greenhouse gas with a warming potential 28 times greater than CO₂ (Grossi et al., 2019), deriving from enteric fermentative processes and primarily emitted through eructation (only in minor part from storage of effluents). Its relatively short atmospheric lifetime makes it a significant contributor to short-term climate change, making it the main objective of climate mitigation strategies. CO₂ is produced during respiratory activity, and it is also emitted through eructation. Unlike CH₄, however, CO₂ emitted by animals does not increase its concentration in the atmosphere; it only returns the portion that is absorbed by plants during the process of chlorophyll photosynthesis.

The importance of feed protein efficient use is related to better performance of animals, less costs of the ration and reduced N emissions in water and atmosphere. Overall, ruminants are inefficient in the utilization of feed N, considering that approximately 70% of N contained in the ration is not utilized by animals and is excreted mostly as urinary N (Kebreab et al., 2001). N₂O has a global warming potential 265 times higher than CO₂ over a period of 100 years. Direct emissions come from the combination of nitrification and denitrification of N in the manure, while indirect emissions deriving from volatile N losses in the form of ammonia and NO_x (IPCC, 2019).

The P in cattle diets is negligible (around 0.5% DM). Moreover, the products used to add this component have very low costs compared to other raw materials. The very frequent risk is therefore to overdose the animals' diets without paying attention to their needs. This therefore leads to overfeeding and consequent economic losses, which are the main source of P emissions into the environment. P is excreted primarily with feces and contributes to the eutrophication of water sources (33% of the total P load into freshwater resources; Alvarez-Fuentes et al., 2016).

It is therefore clear how favoring efficient animals within farms, especially in terms of feeding, is advantageous in many ways.

1.1.1 Influencing factors

Feed efficiency is a complex and multifactorial trait. Research has delved into and is still delving into the factors that influence different feed efficiency levels.

1.1.1.1. Digestive function

Feed digestibility is a function of digestibility of ration ingredients and digestive ability of animals, and it is positively related to feed efficiency (Bach et al., 2020). Ruminants have a rich rumen microbiota, which helps break down feedstuff (also complex components such as cellulose and hemicellulose) to make them accessible for animal needs. Most of the energy precursors and many of the necessary nutrients that the animal needs are provided by the rumen bacteria (Clemmons et al., 2022). Thanks to the development of new technologies able to study the composition of bacteria and other microorganisms in the rumen environment (such as metagenomic), research was able to define the differences in rumen population between animals with different feed efficiency. Despite the conviction that differences in rumen microbiome are related to differences in the use of substrate and energy (which are mostly moved to host needs in efficient animals), some studies have found that there are minor but significant differences in the rumen microbiome in animals with different feed efficiency, while others consider the relation between microbiome and efficiency dependent on the type of the diet fed to the animals. Carmichael et al. (2024) found that in less efficient steers there was a greater relative abundance of methanogens (*Methanobrevibacter* and *Methanosphaera*) compared to efficient ones. Similarly, Lam et al. (2018) found that efficient cattle had greater total bacteria and lower methanogen concentrations compared to inefficient cattle. Efficient animals also differ in the morphology of ruminal papillae, having larger papillae density and shorter length values compared to inefficient ones (Romanzin et al., 2024), and this is the basis for a speculative theory of varied rumen absorptive capability.

1.1.1.2. Metabolic partitioning

Metabolic partitioning influences feed efficiency due to the different energy usage for physiological and production needs. Efficient animals have fewer requirements for maintenance compared to low efficient ones (Herd and Hartur, 2009). Additionally, it has been found that variations in feed efficiency reflect a different usage of metabolizable energy during growth, confirming that this aspect is not merely the passive result of the reduced intake by these animals but a determining factor of feed efficiency (Cantalapiedra-Hijar et al., 2018).

The loss of energy related to metabolic processes (urine, gas emissions, and heat) and in thermoregulation is another important source of variation in feed efficiency (Guinguina et al., 2020a). CH₄ production and emission accounts for 12% of total energy losses (Sakamoto et al., 2021). The relationship between CH₄ production and efficient animals is controversial, as the results found are conflicting (Romanzin et al., 2024; Sakamoto et al., 2021; Bonilha et al., 2017). From an economic and productive perspective, it would be more important to focus on reducing CH₄ yield (g CH₄/kg DM intake). In fact, lower CH₄ production in efficient animals is often associated with low or no significant differences in CH₄ yield between different feed efficiency levels, lacking even a physiological explanation (Fresco et al., 2024; Cantalapiedra - Hijar et al., 2018). The studies on the relationship between thermoregulation and efficiency, on the other hand, are more consistent with each other. Montanholi et al. (2010) studied heat dispersion in steers and found that more efficient animals had lower superficial body temperatures, suggesting that a smaller amount of energy is used in heat production. A similar result was found by Guinguina et al. (2020b) in dairy cows monitored in respiration chambers, with high-efficient cows that lost 114 MJ/day compared to 135 MJ/day in low-efficient cows ($P < 0.001$).

1.1.1.3. Physiological status

The physiological state of the animal can contribute to the variability in feed efficiency among individuals. Animals with health issues may exhibit a temporary reduction in feed intake and nutrient

absorption, that can lead to a reduction in feed efficiency. Severe mastitis, ruminal acidosis, ketosis are some of the issues that frequently affect cattle. Mastitis is the condition that most affects lactating cows, with negative effects both on the health and welfare of the animals, and economically due to the resulting milk losses Huhtanen and Bayat (2024) and Potter et al. (2018) found a reduction of feed efficiency in dairy cows with higher somatic cells in milk, due to the increased heat production related to the inflammatory state, which leads to the allocation of energy for immune purposes. Acidosis is another prevalent health issue in livestock (mainly in beef cattle where the diets are characterized by a very strong energy component), negatively impacting production (yield and quality), body condition, and animal welfare. While these conditions are well-known, their exact effects on animal health still need to be clarified. One significant consequence is the reduction of feed intake, which can lead to imbalances in the rumen's digestive processes and consequently a lack of essential nutrients. This contributes to the negative effect of acidosis on feed efficiency, which seems to be the most important economic consequence (Kleen and Cannizzo, 2012).

Feed efficiency varies depending on the age (young animals have higher energy demands for growth) and across lactation phases in dairy cows. During lactation, different energy demands follow variations in milk yield. In fact, early-lactation dairy cows use energy deriving from body reserves to support milk production and demonstrate higher feed efficiency than late-lactation cows, which allocate energy theoretically available for production needs to rebuilding body tissues (St-Pierre, 2008).

Heat stress is subject to particular attention, especially in relation to the issues related to global warming. Animals subjected to prolonged periods of heat stress must use a significant portion of their energy to dissipate the accumulated heat, resulting in repercussions on production and overall well-being. Several studies have investigated the relation between feed efficiency and heat-stressed animals, ending up different conclusions. Karimi et al. (2015) in dry cows found that heat-stressed cows tend to be also inefficient in feeding terms, while Chen et al. (2024) did not find any connections about this.

1.1.2 Evaluation methods

To gain a comprehensive understanding of feed efficiency, researchers have explored various indices. These metrics, sourced from American and European literature, assess different aspects of the animal to define its efficiency, and each one has its own strengths and weaknesses. While each index offers unique insights, combining multiple indicators can provide a more complete picture of an animal's overall efficiency (de Ondarza and Tricarico, 2017). Given the complexity of the topic, only the most relevant to date will be described below.

Residual Feed Intake (RFI) is the most important and widely used index for evaluating feed efficiency in both dairy and beef cattle. Koch et al. (1963) was the first to define feed efficiency in beef cattle as the gain adjusted for differences in feed consumption (deviation from the regression of gain on consumption), highlighting the significance of understanding the conversion of feed into body tissue for determining net income from beef cattle. RFI is calculated as the difference between the actual dry matter intake (DMI), and the expected DMI (eDMI) considering maintenance and production needs (growth in beef cattle or milk yield in dairy cows). eDMI can be calculated by performing a regression of DMI on average daily gain and metabolic body weight (BW) (Koch et al., 1963). This method is independent of phenotypic or genetic perspectives (if considering a genetic regression) from its predictors (metabolic BW, production level, etc.), which likely allows RFI to reflect digestive and metabolic variabilities (Martin et al., 2021). Efficient animals exhibit negative RFI values, meaning they consume less feed than expected for their group and production level or produce more with the same amount of feed.

Residual Energy Intake (REI) is defined as the difference between measured and predicted energy intake. It is calculated as the residual from the regression of net energy intake per day of lactation, accounting for sources of energy expenditure such as milk yield (MY), maintenance, BW change, and energy needed for pregnancy (Fischer et al., 2018). Efficient animals are characterized by negative values of this index (Hurley et al., 2016). The approach of using REI has the advantage of representing the variation in the diet's energy concentration, unlike RFI (because there is no

dependency with the energy sinks included in the model). Furthermore, as RFI, REI is independent of the variables considered in the predictive model, as shown by Seymour et al. (2020). Actual energy intake may be overestimated if adverse interactions between energy-enriched concentrates and forages with high fibre concentrations are not properly accounted for by the models. This is the main reason why RFI is more commonly used, as calculating energy intake requires frequent sampling and energy evaluations of ingredients in the ration. The *Feed saved (FS)* index, first introduced by Pryce et al. (2015) for the Australian dairy industry, combines RFI in growing calves and lactating dairy cows with feed required for maintenance predicted from BW. A higher, positive FS value (expressed as kg of feed saved per lactation) indicates greater feed efficiency, reflecting lower feed consumption and higher feed savings per lactation. The heritability of FS ranges from 0.35 to 0.41 in dairy cows (Khanal et al., 2022), demonstrating moderate genetic influence on this trait. Genomic reliabilities for FS are 38% for progeny-tested bulls and 28% for young bulls, offering opportunities for genetic selection (Gaddis et al., 2021). A key advantage of the FS index is its ability to distinguish between animals with similar RFI but different maintenance requirements. By accounting for BW, larger animals are not penalized for their higher metabolic needs. Additionally, including pre-lactation feed intake in the calculation is crucial, as it can constitute up to 25% of a herd's total feed consumption (Connor, 2015). *Residual Energy-Corrected Milk (RECM)* is an alternative approach to assessing feed efficiency in dairy cows. It calculates the conversion of feed energy into milk energy, considering respectively the energy content of feed ingredients and milk composition. Efficient cows have higher RECM values, meaning they produce higher MY with similar feed intake and metabolic BW compared to cows with lower RECM values (Guinguina et al., 2020a). RECM focuses specifically on feed efficiency, not production efficiency. In fact, animals of different sizes may consume different amounts of feed but still produce comparable MY, demonstrating similar production efficiency but different feed efficiency levels. *Gross Feed Efficiency (GFE)* and *Feed Conversion Ratio (FCR)* are simple parameters used to evaluate feed efficiency in dairy and beef cattle, respectively. They express the ratio between output (kg of energy-corrected milk or kg of weight gain) and input (kg DMI)

(Madilindi et al., 2022; Connor, 2015). While conceptually straightforward, this index has certain limitations. Firstly, it neglects growth and body tissue mobilization, leading to variations solely based on DMI levels, which fluctuate across lactation stages (St-Pierre, 2008). Secondly, it overlooks other crucial factors such as dietary nutrient composition (e.g., fat content in the ration). *Residual Solids Production (RSP)* was first defined by Coleman et al. (2010) as the difference between actual and predicted milk solids production for a given DMI, body change, metabolic BW, and body condition score (BCS). Positive values indicate efficient animals. RSP is independent of feed intake, which can be beneficial for increasing MY and milk component yield without negatively impacting energy balance, particularly in early lactation (Connor, 2015). *Income Over Feed Cost (IOFC)* and *Income Equals Feed Cost (IEFC)* translates feed efficiency into an economic perspective. IOFC, calculated as the difference between milk income and feed costs (Bach et al., 2020) is not ideal for short-term economic or nutritional decisions due to fluctuations in milk and feed prices (Bethard, 2013). However, it offers a more accurate indication of farm profitability compared to the simple estimate of the ration cost per cow (Buza et al., 2014). IEFC defines the milk production level required to cover feed costs (Pepin, 2009). It can aid in identifying unprofitable animals (candidates for culling or drying off), keeping in mind that varying production levels correspond to varying intake amounts.

Accurately measuring individual DMI remains a challenge due to the high costs and practical limitations. The simplest approach involves using electronic scales in feed bunks (Saar et al., 2022). In the late 1990s, more advanced individual intake recording technologies were developed. Growsafe (Growsafe Systems, Calgary, AB, Canada), SmartFeed (C-lock Inc., Rapid City, SD, USA), and RIC-system (Insentec B.V., Marknesse, The Netherlands) are just some of the most popular models. These systems, discussed in detail in Chapter 1.4 - Methods for the evaluation of feeding behaviour (section 1.4.4.- Automatic feeding systems), can collect valuable data on individual feed intake and feeding patterns. However, their high installation costs and increased demands for maintenance and cleaning of facilities limit their use in commercial farms and pasture-based systems. Researchers are exploring simpler, more practical methods for measuring DMI in commercial farms. One promising approach

is photogrammetry, a technique that uses multiple pictures of an object taken from different points to create a 3D model. Processing the model created with a specific algorithm, researchers can estimate the residual mass of the feed in the feed bunk and therefore the feed ingestion considering the volume and density of the feed. A study by Bloch et al. (2019) found an error rate of approximately 0.48 kg for heaps up to 7 kg in laboratory conditions, and 1.32 kg of estimation error for heaps up to 40 kg in the cowshed using this method. The most important advantages of this method are the use of less expensive equipment, the use of the original barn structure, and the relatively simple concept of the measurement. Considering the high difficulty in defining the intake of grazing animals, some authors have considered the possibility to use precision technologies (e.g., noseband pressure sensor) in estimating the herbage intake of grazing animals (Rombach et al., 2019; Leiber et al., 2016).

1.2 Feeding behaviour definition

Cattle behaviour is determined by instinct, sensory perception and experience. Cattle are social animals and form a herd with distinct patterns of behaviour and daily routines. Most of the cattle's day is spent on four primary activities, including eating (or grazing), ruminating, resting and walking (especially in pasture systems). Various factors, including breed, age, sex, health status, environment, and nutrition, can influence these behaviours. The feeding behaviours (eating and rumination) will be further explored in this chapter.

Feeding behaviour is simply defined as “any action of an animal that is directed toward the procurement of nutrients” (Encyclopedia Britannica). Various components make it up, and their combination allows for the differentiation of individual behaviour.

✓ Feeding and eating.

An essential distinction is that of feeding time (FT) and eating time (ET), which are frequently wrongly treated as synonymous. FT represents meals and includes periods of inactivity, while ET refers only to the time spent prehending, chewing, and swallowing feed (Beauchemin, 2018). In

general, during eating the animal use the lips, teeth, and tongue to prehend the feed and move it into the mouth. The following step is chewing, when a large amount of saliva is added to create the bolo. Typically, a single bolus requires 50–80 jaw movements during chew, however depending on the feed, this value might range from 20 to 400 (Rombach et al., 2018). The feed consumption process concludes when the chewed forage forms a cud that generates the stimulus to swallow it (Chelotti et al., 2024). Swallowing starts with a voluntary manipulation of the cud into the pharynx by muscles in and behind tongue, which follows an involuntary stage when the bolus is carried by peristalsis through the esophagus to the cardia (Campling and Morgan, 1981). Normally a bovine spends about 4.5 hours a day eating (Beauchemin, 2018).

✓ *Rumination.*

Rumination is the characteristic trait of ruminants and follows eating. Rumination time (RT) is defined as the time spent in the cyclical process of regurgitation, remastication, and reswallowing the feed to ensure the fermentation by ruminal microbiota to make nutrients accessible (Llonch et al., 2018). Regurgitation starts when feed particles sorted by the reticulum-rumen are gathered into a single bolus and regurgitated to the mouth. Remastication consists in the reprocessing of bolus into the mouth to decrease its size, by jumbling and binding the cud using molars and premolars in the middle region of the jaws (rumination chew) (Chelotti et al., 2024). Rumination is a crucial activity for cattle, occupying nearly 7 hours a day (Beauchemin, 2018). This behaviour has significant implications for animal performance, health, and welfare. As a result, changes in RT are often used as a proxy indicator for animal diseases or distress (e.g. onset of subclinical acidosis; Andreen et al., 2021). Additionally, rumination is a well-studied behavioural indicator to predict events like estrus, calving, and subclinical or acute illness (Beauchemin, 2018).

✓ *Meal characteristics.*

A meal is defined as the unit of feeding behaviour and consist of a distinct period when multiple bites take place, separate from the next period by a sufficient long nonfeeding interval. The bite is considered as the essential component of ingestive behaviour of grazing cattle, composed of the act

of apprehend and severing of forage (Chelotti et al., 2024). To describe a meal, it is possible to define meal duration, meal size (amount of feed ingested), meal frequency (number of meals per day), and eating rate (ER) (combination of grams per bite and bites per unit of time defining the speed of feed ingestion).

After delving into the specific definitions of feeding behaviour components, it is important to note that feeding behaviour is subject to complex mechanisms and is regulated by several factors.

1.3 Factors related to feeding behaviour

Improving the knowledge of bovine biology, particularly their feeding habits, can lead to enhanced animal welfare and improved production performance. Below, the main factors related to feeding behaviour will be discussed.

1.3.1 Physiology

The study of the structure of meal patterns (meal patterns analysis) defines that feeding could be influenced by sensory and metabolic stimuli, which define the meal frequency or size (Baile and Della-Fera, 1981). Ruminant feeding behaviour is downregulated by integrating sensory cues that initiate a meal, select feed, and determine intake quantity. Stimulatory signals from both central and peripheral brain centers initiate meals by increasing hunger (which is positively associated with energy balance). As energy status decreases, a series of signals are transmitted to the central nervous system via hepatic vagal afferents, serving as a primary stimulatory input. Among gastrointestinal peptides, ghrelin is the most important molecule known to stimulate feeding behaviour and meal initiation but not meal size (Allen, 2014). Secreted by cells in the abomasum, ghrelin's effect on feed intake is well-established in conditioned feeding (pre-meal peak and decline during eating). However, the effect on ruminants fed *ad libitum* remains uncertain.

Inhibitory signals, which include rumen distension, rumen osmolarity, gut peptides, pancreatic hormones, adipokines, and nutrient sensing by the central nervous system, contribute to meal

termination by increasing satiety (Allen, 2014). Ruminal tension receptors respond to the distention of the rumen during feeding by sending signals to brain centers, which are influenced by the mass and volume of the ingested material (which, in turn, depend on the chemical and physical composition of the diet). During meals, rumen osmolarity increases due to the ingestion of salts and the production of fermentative acids (volatile fatty acids), and this change is captured by the osmoreceptors present in the rumen wall. Acetate, a major contributor to increased osmolarity, can lead to intake depression. A similar action is performed by propionate, which also influences meal size by decreasing intake. The accumulation of these acidic components in the rumen fluid leads to a significant decrease in pH (below 5.5), potentially causing ruminal stasis. Cholecystokinin and glucagone-like peptide 1 are the most important inhibitors of feeding behaviour. These hormones are produced by intestinal cells, and the secretion is stimulated by fatty acids and some amino acids (Langhans and Holst, 2016). They decrease gastric emptying, increase rumen retention time, and promote the release of digestive enzymes, potentially improving digestibility (Allen, 2014). Metabolites that can stimulate a central inhibitory signal include non-esterified fatty acids, beta-hydroxybutyric acid, glucose, glutamine, and other amino acids. The ventromedial hypothalamus has a general inhibitory effect on feeding, while the lateral hypothalamus appears to be connected with the initiation of feeding.

As eating has both physiological and behavioural origins, then rumination is considered a physiological trait (Atashi et al., 2024), and the input starts when the gastric centers in the medulla integrate neural activity originating from receptors located in the reticulorumen (Beauchemin, 1991).

1.3.2 Management

Farm management practices are environmental factors that can significantly influence animal behaviour patterns. Grant and Albright (2001) suggest that farm management factors (such as animal grouping, social dynamics within the herd, feed bunk structure, diet composition, and others) can influence the feeding behaviour of dairy cows, particularly if they are strongly submissive to dominant subjects. Understanding social dynamics is crucial for defining individual and group

feeding behaviour patterns and designing feed bunk management plans. Cattle are social animals with a hierarchical structure (follow a leader-follower dynamic). The hierarchy is influenced by individual temperament, age, and size. When the dominant component has been defined, the submissive animals adapt to this organization of the group. Introducing new animals to a herd can disrupt the established hierarchy, leading to changes in feeding behaviour with consequent intake reduction and decreased milk production (Doyle and Moran, 2015). Moreover, it should be considered that frequent social regroupings could affect growth rate and endocrine profile associated with energy status particularly in young cattle (Fiol et al., 2024). Competition within the group affects how animals feed. In a non-competitive environment with free access to feed, animals tend to start eating quickly after feed delivery and slow down when they reach satiety. Otherwise, in a highly competitive environment, the ER tends to increase sharply, but the total ET per day tends to decrease (Proudfoot et al., 2009). This allows all animals, especially subordinates, to ingest enough feed in the shortest time and avoid the competitive pressure from dominant individuals.

Cattle are characterized by social learning, which is a way to take advantage of group living by observing their conspecifics and stocking with previous experience. When one cow starts eating, another cow is stimulated by sight or sound to eat as well, even though she is not hungry. This phenomenon is known as “social facilitation”, first described by Curtis and Houpt in 1983, and can lead to increased feed intake in group-fed cattle.

It has been seen how ration management (quality, frequency of distributions, and pushes per day) can influence feeding behaviour. Basically, feeding activity increases after fresh feed delivery or when it is pushed up to the feed bunk and when the cows return from milking (DeVries et al., 2003). The frequency of feed distributions is positively related to feeding behaviour, rumen health (digestibility of fibre), and production (milk fat, efficiency of production) (Hart et al., 2014; DeVries et al., 2005). On the contrary, single daily feedings can increase the risk of health issues (e.g., subclinical acidosis) because of the large pH fluctuation during the day.

1.3.3 Feed intake

The mechanisms governing animal feed intake are multifaceted and still need to be explored and fully understood. From a physiological standpoint, ingestion has a positive relationship with time spent eating (Allen et al., 2019). Understanding the interactions between DMI and feeding behaviour can lead to the possible integration of feeding behaviour into feed intake estimating equations that already consider other aspects such as size, age, and production yield of the animal (NRC, 2021). Several studies have tested how the inclusion of feeding behaviour can lead to a significant improvement in the estimation. Results are various, and this could be partly explained by the trial structure, like animals considered (different production categories, ages, or breeds) and behavioural data collection (visual observation, sensors, automatic feeding systems). Pahl et al. (2016) found a good relationship between feed intake and FT ($r = 0.89$) even with a limited number of animals, but this result does not lead to considering the inclusion of feeding time in the prediction of DMI. Clément et al. (2014) tried to include only RT in the DMI estimation but without any improvements in the prediction. A similar result was found by Davison et al. (2021) in beef steers, where adding feeding behaviour in the estimation of intake resulted in an error greater than 10%. Size is another link between feeding behaviour and feed intake. Bigger animals can ingest more feed in less time thanks to the different bite size, which simply derives from a different anatomical structure of the mouth. In fact, the amount of forage picked by each bite is actually determined by the bite volume, which depends on the animal (bite area and depth) along with the diet (forage density) (Ungar et al., 2006).

Different is the knowledge about the relation between feed intake and RT (e.g., Clément et al., 2014 and Schirmann et al., 2012 found no relationship between DMI and RT, while Byskov et al., 2015 found a relation between the intake of specific ingredients of the diet and RT). In the review of Beauchemin (2018), the indirect relation between RT and DMI (weak) is explained considering the positive relation between RT and milk production.

1.3.4 Diet characteristics

Providing animals with the appropriate ration is crucial to satisfying their energy demands and physiological needs and ensuring their welfare and behavioural patterns are respected. To achieve this, certain criteria of the ration must be met. Animals raised on commercial farms are provided with an *ad libitum* ration, a homogeneous and balanced mixture of all the components necessary to meet daily requirements accounting for physiological stage or production category. Animals raised in pasture systems are fed primarily on the pasture herbage with compound feed supplements. In general, chemical and physical characteristics of the diet are important drivers for feeding behaviour, in particular for RT (Beauchemin, 2018).

Fibrous components certainly play an important role in the diet of ruminants, considering that a minimum amount of forage must always be guaranteed (25% of fibre should derive from forages). The different quantity and quality of fibre regulate chewing, peristalsis, and digestive functions. Silage (corn or sorghum) is one of the most common ingredients in cattle rations. Increasing fibre digestibility and reducing the particle size and the neutral detergent fibre (NDF) content of this portion minimize the need for the animal to chew it (Grant and Ferraretto, 2018; Ambriz-Vilchis et al., 2015). Despite this, in the literature there are contrasting results on the correlation between ET and NDF content (Shi et al., 2023; Jiang et al., 2017; White et al., 2017; Dann et al., 2015), while RT is more highly correlated with NDF intake (Beauchemin, 2018) even if the values are still low to moderate (from $r = 0.15$ to $r = 0.46$; White et al., 2017; Zebeli et al., 2006).

The concept of physically effective NDF (peNDF) was first defined by Mertens in 1997 as the proportion of diet that stimulates chewing activity and saliva secretion, which in turn affect ruminal fermentation and digesta passage. Furthermore, a low peNDF is negative for rumen health, predisposing the animal to the risk of acidosis and liver issues. Kröger et al. (2019) investigate the effect of a moderate peNDF on dairy cows, finding that the animals altered eating, sorting, and chewing behaviour to reduce diurnal variations of reticuloruminal pH within the duration of concentrate feeding. Heering et al. (2023), Llonch et al. (2020) and White et al. (2017) found that

increasing peNDF concentration increased rumination and chewing intensity but not daily total feeding behaviour parameters.

A general association, although highly variable, exists between particle size of the diet and ET. Cattle are more inclined to start the meal when more fine particles are present, while they require longer particles during the meal to meet their peNDF needs. Increasing particle size of silage leads to a continuous increase of ET because cows require longer mastication to swallow the feed bolus (Beauchemin, 2018; Grant and Ferraretto, 2018; Zhijun et al., 2010). Particle size also influences RT, with a considerable drop when the feed particles are very tiny (lower than 5 mm) (Esmaeili et al., 2016; Nasrollahi et al., 2015; Zhijun et al., 2010). After a certain particle size (around 10 mm), RT did not increase with further increase in particle length, as happens with the ET. This is most likely due to the effect of longer mastication during eating that reduces the variations in the bolus's ingested particle size and limits the need for extra reduction during rumination (Beauchemin, 2018).

Digestibility of fibre increased interest for the relation with rumen fill and consequently influence on DMI. As of today, there are few studies that analyze the relationship between this aspect of diet and feeding behaviour, finding a positive relation between high digestible NDF diets and RT, DMI, and ruminal pH, suggesting that NDF digestibility and the relative rumen retention time could be a limiting factor for DMI (Fustini et al., 2017).

1.3.5 Individual variation and other factors

There is increasing evidence across many species that individual animals differ in their behavioural expression. This could be explained by different personalities and could be further exacerbated by environmental conditions, social interactions, and physiological changes. Despite this, a subject maintains consistency over time (Schwanke et al., 2024), particularly in the characteristics of individual meals (frequency, size, time spent; Beauchemin, 2018), in the frequency of jaw movement, and in the number of times the bolus is chewed (Juhás et al., 2019). Dado and Allen (1994) found that the individual coefficient of variation for ET is around 17%, whereas for RT it is 16%.

Breed could be another important factor that can lead to differences in the feeding behaviour patterns of animals. For example, Aikman et al. (2008) found no differences in ET between Holstein and Jersey dairy cows, but Jerseys spent more time eating per unit of ingested feed, maybe due to their different size.

1.4 Methods for the evaluation of feeding behaviour

Monitoring animal feeding behaviour can provide valuable insights into their productivity, health, and welfare. A variety of methods can be used to collect individual information on these aspects in modern farms.

1.4.1 Visual observation

Visual observation, directly or by means of video recording, is the traditional method for data collection, involving an expert observing animals during and after feeding to gather information about different behaviours. This approach has some limitations due to the number of animals that can be watched, the time necessary for observation or video review, and the possibility of subjectivity and human mistakes. Observing animals raised in extensive systems poses additional challenges. For these reasons, sensors are a more efficient alternative, reducing data collection time, minimizing errors, and allowing for the collection of a larger amount of data on the entire herd in a short time.

1.4.2 Sensor technology

The widespread adoption of technology in commercial farms has resulted in a growing number of available measurement and monitoring devices and systems, which could provide valuable daily data for farmers to help in the farm management and support research efforts to improve the comprehension in different fields.

A sensor is a simple and costly device that can be used to detect data. There are **animal-based sensors** and **non-animal-based sensors**. The firsts are mounted directly on the animal's body to measure and collect data (e.g. ear tags, halters, neck collars, reticulo-rumen bolus sensors, leg tags, tail tags, tailead tags, and vaginal tags; Lee and Seo, 2021) (**Figure 1**). Non-animal-based sensors are technologies located near the animal.

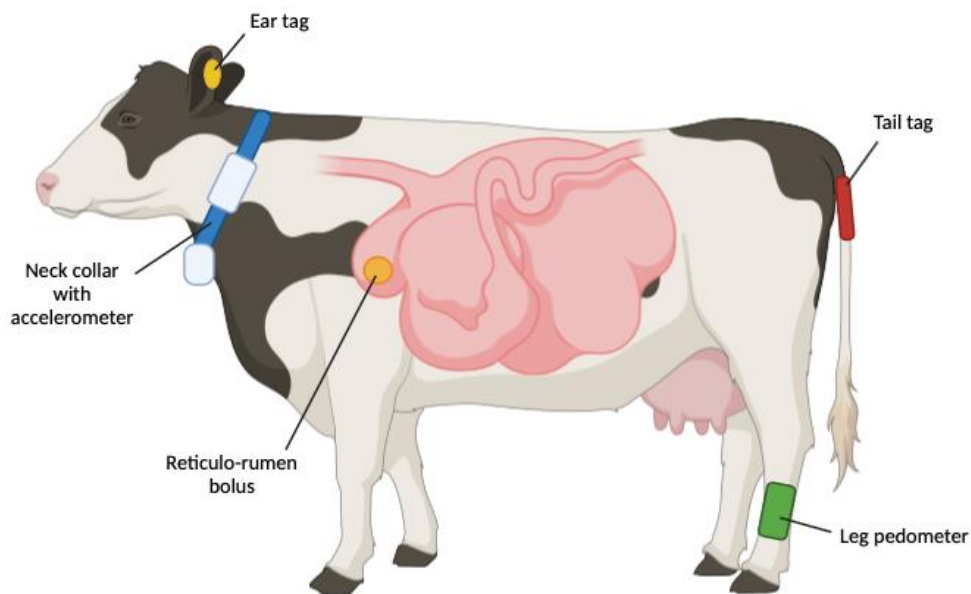


Figure 1. Animal-based sensors available for individual monitoring (adapted from Chelotti et al., 2024).

In commercial farms, sensors have been integrated into various stages of the production process for several years. Sensors can monitor milk quality at each milking and the individual animal production, supported by RFID technology (radio-frequency identification) that identifies animals and associates them with their respective data. Additionally, sensors are able to monitor the reproductive and health status, promptly alerting the farmer in case of ongoing heat, imminent calving, or potential health issues. More recent sensors focus on monitoring animal activity, including rumination activity, heartbeat, respiration, body position, and so on.

Below, only the sensors used in the detection of feeding behaviour will be presented.

1.4.2.1. Accelerometers

Ear tags and neck collars are the most commonly used sensors in commercial farms. Equipped with accelerometers, these devices can detect feeding behaviour and other activity patterns in real time for extended periods.

An accelerometer is a piezoelectric sensor that generates a wave-like voltage signal proportional to the acceleration it experiences over time. The acceleration derives from a gravitational deformation (posture change) or an inertial acceleration (animal movement). Triaxial accelerometers consist of three distinct accelerometers integrated into a single unit, each aligned with the orthogonal X, Y, and Z axes. The acceleration is measured along these 3 axes. Specifically, X-axis acceleration represents vertical movement of the head, Y-axis acceleration indicates the horizontal movement of the head, and Z-axis acceleration reflects the lateral movement (Tamura et al., 2019) (**Figure 2**).

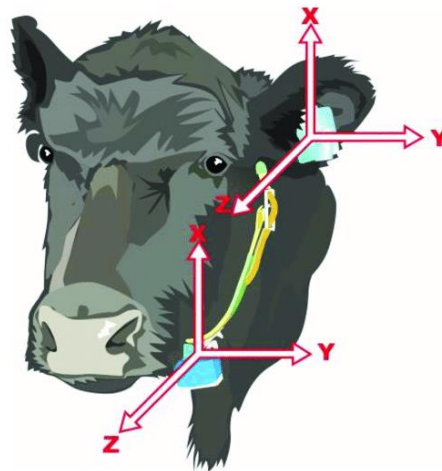


Figure 2. Specific orientation of the three axes of an accelerometer mounted on ear tag or neck collar (Simanungkalit et al., 2021).

Information are recorded using various window sizes: short (3-10 s), medium (20-30 s), and semi-long (32-96 s) (Nogoy et al., 2022). To classify the different behaviour patterns of the animals, raw data must be analysed. These are made up of wave-like signal with units of voltage which can be used to define the behaviour in a specific time point (Hendriks et al., 2020). To do this, the manufacturer provides an automatic software which directly returns the total daily count for each

behaviour of the animal. When cattle eat (or drink) the head is placed above the feed bunk to search, ingest or sort the feed. Differently, during rumination it can be lying down or standing up with the head always held in an upright position (Nogoy et al., 2022). Accelerometers must be validated to ensure the accuracy of their data in relation to actual behaviour. This can be done through various methods, such as visual observation, video recording or by comparing data from two different devices (Hendriks et al., 2020).

1.4.2.2. Noseband pressure sensor

This system allows for the continuous monitoring of animals even in open spaces and distant from farm structures, as is common in pasture systems. Even in this case, like others seen previously, the high costs of the equipment make it applicable only in experimental conditions.

RumiWatch (Itin +HochGmbH, Liestal, Switzerland) is the most widespread system, validated on various species (cattle, Norbu et al., 2021; equines, Weinert et al., 2020; sheep, Werner et al., 2019), productive categories (dairy cows, Pollock et al., 2022; beef cattle, Raynor et al., 2021), ages (calves, Eslamizad et al., 2018), and number of animals, besides different raising conditions (pasture, Pereira et al., 2020; Werner et al., 2018; stable, Zehner et al., 2017, Ruuska et al., 2016).

The sensor consists of a silicone tube filled with vegetable oil and equipped with a built-in pressure sensor housed within a fully adjustable polyethylene halter placed over the bridge of the cow's nose (Zehner et al., 2017) (**Figure 3**). When the animal moves the mouth, the pressure generated causes the bending of the tube. This change in mechanical pressure alters the electrical

resistance in the sensor, which is recorded as a signal (Braun et al., 2013). Jaw movements are registered with a frequency of 10 signals per second.

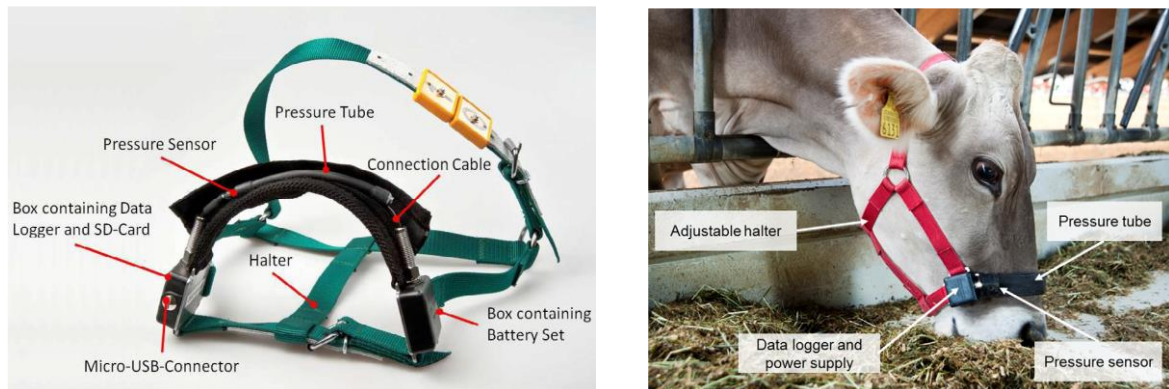


Figure 3: RumiWatch sensor components (www.RumiWatch.com) and his application on the animal (Zehner et al., 2017).

Feeding behaviour is classified according to the frequency of individual pressure peaks (peak rates and peak intervals) during eating, rumination, drinking, and other activities, as every single behaviour is distinctly different from the others (Figure 4). During eating, peak rates are homogeneous with irregular interruptions and peak frequencies that differ among animals due to changes in eating rate or feed selection. Differently, rumination is characterized by numerous sequential peaks interrupted by the regurgitation of the bolus (Zehner et al., 2017). Raw data are elaborated with algorithms, which can generate summaries of every single behaviour.

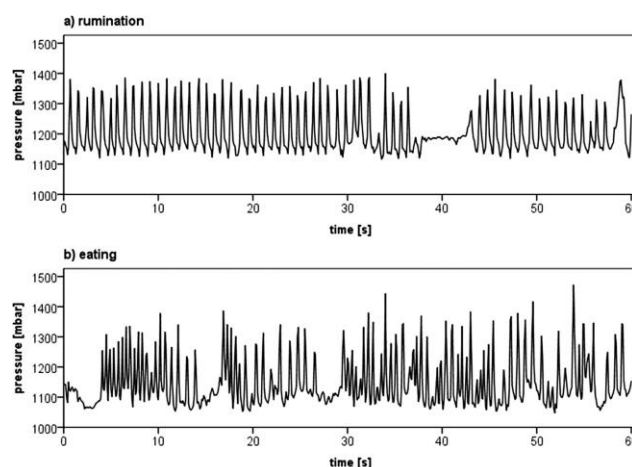


Figure 4. Pattern of pressure peaks in the different behaviours detected with the noseband pressure sensor (adapted from Zehner et al., 2017).

1.4.3. Sound system

Acoustic biotelemetry is a relatively new field of research that has gained significant attention in recent years. Biting and chewing sounds can reveal important features of the foraging behaviour of free-ranging or stall-fed animals (Galli et al., 2018). As seen with jaw movements, the acoustic patterns are also different among the various behaviours, allowing for accurate discrimination. In fact, eating chews and bites sounds are characterized by high energy and amplitude and long duration, while rumination chews are characterized by low energy, low amplitude, and middle duration sounds (Chelotti et al., 2020) (**Figure 5**).

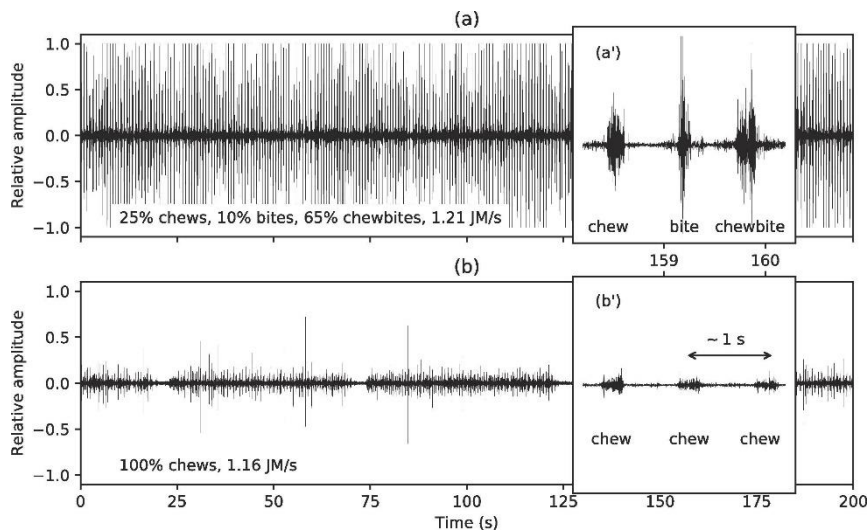


Figure 5. Sound patterns of eating and rumination (Chelotti et al., 2020).

The system is composed of a microphone, which could be positioned facing inwards to capture the bone-transmitted vibrations and pressed against the forehead of the animal, while the other microphone faces outwards to capture the sounds produced by the animal (Martinez-Rau et al., 2023) (**Figure 6**).



Figure 6. Recording system used to measure acoustic signals (Martinez-Rau et al., 2023).

Another possibility is to combine the microphone with a neck collar, positioned to hold the microphone on the left side of the cow's neck (e.g., rumination collar, SCR Engineers, Netanya, Israel). Ambriz-Vilchis et al. (2015) compared the rumination activity measured with this system against that obtained from direct observation and by analysis of video recordings, finding that it can correctly determine rumination activity in housed animals, while it could not be an alternative to direct observation in grazing animals.

Sound analysis has become more widely used in studying grazing animal behaviour because it is less susceptible to the typical barn noises that can interfere the previously described system (noseband sensor). Monitoring ingestive behaviour with the use of sounds can prove to be very useful in forage management, as forage species and forage heights influence key acoustic characteristics of feeding sounds (amplitude and duration) (Li et al., 2021). Moreover, it has proven to be low-cost, relatively easy to compute, and with good performance rates (combining the field of sound analysis with machine learning was found a total precision of cattle behaviour classification ranging from 90% to 95%; Abdanan Mehdizadeh et al., 2023).

1.4.4. Automatic feeding systems

Automatic feeding systems are primarily designed to measure the amount of feed consumed by animals. They have indeed been included within the ICAR (International Committee for Animal Research) guidelines as recognized systems for assessing the individual feed intake of animals to evaluate feed efficiency for selective purposes. These systems are often reserved exclusively for experimental farms or research institutions (small pens for a limited period) due to their high cost and the necessary modifications to the breeding structure.

The most widespread systems are GrowSafe 4000 System (GrowSafe Systems, Ltd., Airdrie, AB, Canada; validated by Basarab et al., 2003; Schwartzkopf-Genswein et al., 1999) and the Roughage Intake Control system (RIC, Insentec B.V., Marknesse, The Netherlands; validated by Chapinal et al., 2007). They consist of an electronic trough with neck bars that limit entry to one animal per visit (**Figure 7**). They can detect an animal's presence in the feed bunk by identifying the RFID tag when the head was properly within the hood. If an animal approach one of the feeding troughs, the system can identify it and open the gate.

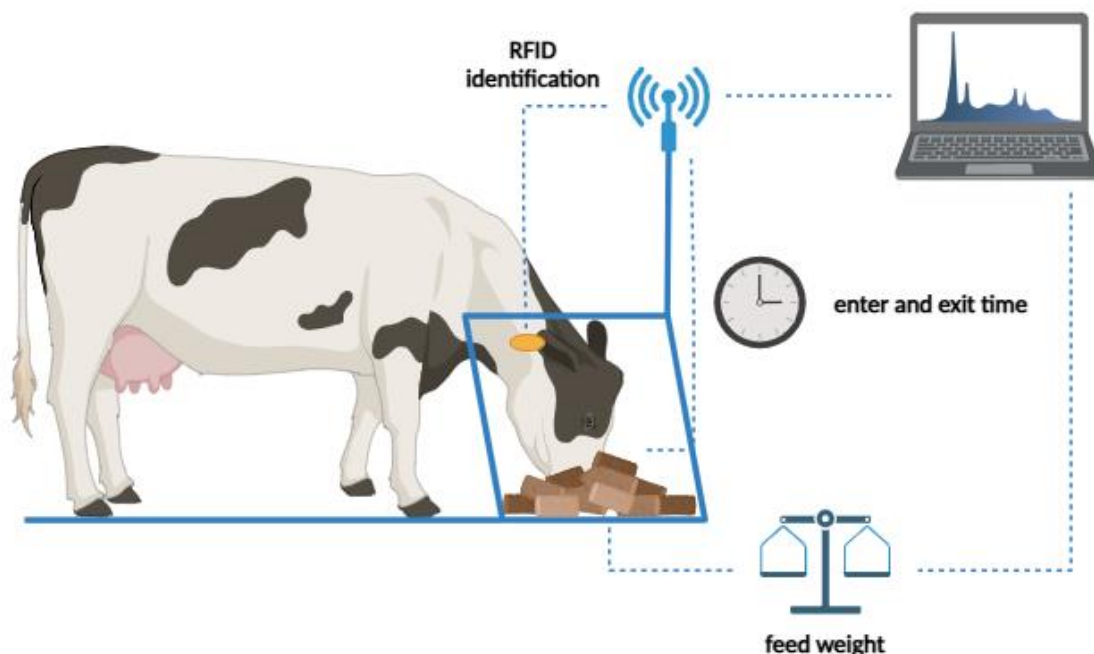


Figure 7. Schematic representation of an automatic feeding system.

Automatic feeding systems allow the collection of data regarding individual feed intake and feeding patterns (**Table 1**). This type of equipment is widely used in studies evaluating the feed efficiency of cattle, both dairy (Reyes et al., 2023; Brown et al., 2022; DeVries et al., 2003) and beef (DelCurto-Wyffels et al., 2021; Parsons et al., 2020; McGee et al., 2014).

Table 1. Definitions of feeding behaviour traits evaluated with automatic feeding systems (adapted from Brown et al., 2022 and Parsons et al., 2020).

Item	Definition
Bunk visit (n)	Number of times per day the animal gained access to the feed bin and resulted in at least 0.1 kg of as-fed feed disappearance
Bunk visit frequency (n/day)	Number of bunk visit events recorded daily
Bunk visit duration (min/day)	The sum of the durations of bunk visits events recorded each day
Meal frequency (n events/day)	Number of meal events recorded each day
Meal duration (min/d)	Sum of the duration of meal events recorded each day
DMI	Feed disappearance for a 24-h calendar period
Feeding rate (g/min or kg/min)	DMI divided by meal duration
Missing event number (n/day)	Number of times per day the animal gained access to the feed bin and resulted in less than 0.1 kg of as-fed feed disappearance
Missing event duration (min/d)	Duration of missing event per day

THESIS AIM AND OUTLINE

In the livestock sector, high feed efficiency is fundamental for maintaining and improving the economic and environmental sustainability of farms. Feed efficiency is generally directly measured in experimental conditions, while in commercial contexts feed intake, which is required to compute feed efficiency, is difficult to measure due to several challenges, mainly economic and operational ones. From a physiological standpoint, it is well known that the amount of feed an animal consumes daily varies greatly based on several factors, such as animal type, age, BW, stage of production, behaviour, and the quality of the ration offered. Among these factors, feeding behaviour which is the sum of different phenomena (ingestion, rumination, number of feeding events, and so on), is expected to be linked significantly with the total amount of individual ingestion. In light of this, the current thesis has the general aim to obtain experimental information about the association between feed intake and feeding behaviour of cattle, with the perspective of evaluating the feasibility of using feeding behaviour traits as proxy indicators of feed intake and consequently the feed efficiency.

The experimental part of this thesis can be divided into two main sections. A first part was carried out under experimental conditions, with individual measurements of intake (and feed efficiency). In the first study (**Paper 1, in Table 2**), the feed efficiency of growing bulls of dual-purpose breeds was assessed using a specific technology capable of tracking individual feed intake and feeding behaviour. The aim was to explore potential links between different degrees of feed efficiency and both performance and behaviour, with a specific focus on the relationship between feed efficiency and feeding behaviour. Always under experimental conditions, a feeding trial was conducted with late-lactation dairy cows where feed intake was measured and feeding behaviour data were collected using a noseband pressure sensor (**Appendix paper, in Table 2**).

Given the challenges of replicating experimental conditions in commercial farms, the attention turned on the potential of on-farm technologies to gain deeper insights into feeding behaviour and individual animal variation. This is driven by the recognition that traditional research settings often fail to capture the complexities of real-world farm management.

Considering the operational difficulties in applying experimental study conditions in commercial farms, the interest has focused on the possibilities that technologies integrated within commercial farms can offer to increase knowledge about feeding behaviour and its link with the diversity among animals. This also considering the fact that most of the knowledge currently in possession is derived from experimental studies or research facilities, which cannot be compared to the normal management of commercial enterprises.

The second part consists of two specific surveys conducted on commercial farms using lactating dairy cows equipped with modern precision farming sensors. One primary objective (**Paper 2, in Table 2**) was to determine whether differences in individual eating behaviour were associated with productive aspects, diet composition, and different diet management practices. Moreover, the last study (**Paper 3, in Table 2**) aimed to investigate how feeding behaviour varied throughout the whole lactation period, with the main objective of expanding the knowledge of this aspect in all phases of bovine lactation and assessing the potential application of this knowledge as a useful management tool for farmers.

The thesis concludes with a general discussion of the findings, suggestions for further studies in this field, and the potential practical applications of these learnings to better define feed efficiency in livestock.

Table 2. Key points of the papers included in the thesis.

Item	Work 1	Work 2	Work 3	Appendix paper
Title of the paper	Feeding efficiency and behaviour of growing bulls from the main Italian dual-purpose breeds	Eating time of dairy cows: a study focusing on commercial farms	Whole lactation feeding behaviour of Simmental cows in commercial dairy farms	Sorghum silage and cover crop silage in diets for late-lactation cows: Effects on feed intake, digestibility, feeding behaviour, and milk yield
Journal	Italian Journal of Animal Science	Italian Journal of Animal Science	Applied Animal Behaviour Science	Journal of Animal and Feed Sciences
Status	Published	Published	In peer review	Accepted
No. animals	890	819	80	8
Breeds	Italian Simmental, Alpine grey, Rendena, Valdostana strains	Italian Simmental, Holstein Friesian,	Italian Simmental	Holstein Friesian
Category	Growing bulls	Dairy cows	Dairy cows	Late lactation dairy cows
Aim of the study	Relation between feed efficiency and feeding behaviour	Investigation relationship between feeding behaviour, animal individual traits and diet	Investigation effect feeding behaviour on individual traits in the whole lactation	Influence of diets differing in silage type on intake, digestibility, feeding behaviour, performance
Data collection	Automatic feeding system	Accelerometers on neck collars	Accelerometers on neck collars	Noseband pressure sensor
Feed efficiency evaluation	Yes	No	No	No

CHAPTER II

Feeding efficiency and behaviour of growing bulls from the main Italian dual-purpose breeds

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ABSTRACT

To characterize the main Italian dual-purpose breeds in terms of feed efficiency and behaviour, the dry matter intakes (DMI) of 890 young bulls were collected using automatic feeding systems in two Italian genetic centers. In Experiment 1 Italian Simmental (IS), Rendena (RE), and Alpine Grey (AG) young bulls were fed total mixed ration for 46 d. In Experiment 2 Valdostana Red Pied (VR) and Black Pied and Chestnut (VB) young bulls were fed hay and concentrate for 60 d. The IS and RE bulls grew faster than AG (1.6 vs 1.3 kg/d, $p < .01$). The DMI was highest for IS and lowest for AG while all breeds had similar values for DMI expressed as relative to body weight. Overall, RE and AG bulls were more efficient than IS.

Regarding feeding behaviour, the number of feeding events (FEn) for IS and AG was higher than for RE, while the feeding time (FT) was not different between breeds. The IS bulls showed a higher value of feeding rate (FR) than RE and AG (98.2 vs 83.1-86.5 g DM/min, $p < .01$). Overall AG breed, despite its high feeding activity, was particularly efficient. In addition, efficient animals showed a low FR compared to inefficient ones. In Exp. 2, VR and VB strains were quite similar in terms of growth capacity but VR was more efficient despite its higher daily activity. In conclusion, dual-purpose breeds can reach notable growths and feed efficiencies and FR is well related to feed efficiency.

Keywords: dual-purpose breed; growing bull; residual feed intake; residual concentrate intake; feeding behaviour.

Highlights

- Rendena and Alpine Grey bulls are slightly more efficient than Italian Simmental bulls
- Valdostana strains differ for feed efficiency but not for growth capacity
- Feeding rate is a useful behaviour trait to consider in the study of feed efficiency

Introduction

The cattle dairy system in Italy is rich in biodiversity, with some important specialized dairy breeds in the lowlands and many dual-purpose breeds in mountain areas (Sturaro et al., 2013).

Alpine Grey (AG) is a small-medium-sized Alpine breed, widespread in the Austrian and Italian Tyrol where it is raised in small herds with summer transhumance in high mountain pastures (Mancin et al., 2021). Rendena (RE) is a limited-spread breed, native to the Province of Trento, where it is raised in small Alpine farms. It is also well adapted to lowland farms where it reaches good milk yields (Guzzo et al., 2018). Italian Simmental (IS) stands out among the Italian dual-purpose breeds for its wide diffusion and high production. It is a cosmopolitan breed, as it belongs to the Simmental strain, which is raised with different breeding systems from the alpine pastures to the large farms of the Po Valley (Romanzin et al., 2013). The Valdostana breed is a small-sized Alpine breed, widely distributed in the homonymous mountain region, and is the only breed that can be used to produce the well-known Fontina cheese. Within this breed, two strains are recognized: Valdostana Red Pied (VR) and Valdostana Black Pied and Chestnut (VB) (Mazza et al., 2015; Strillacci et al., 2020).

The characteristic type of breeding of these animals leads to important benefits in the Alpine areas, both social and ecosystemic (local products, landscape, biodiversity, animal welfare, etc.), with notable cultural, environmental, and, ultimately, tourist implications (Battaglini et al., 2014; Zuliani et al., 2017; Gianelle et al., 2018). Interest in the conservation of dual-purpose breeds has promoted the development of focused breeding programs, in order to preserve resilience and adaptability to different husbandry environments. In addition to production (meat and milk), feed efficiency has become increasingly important, not only for its economic implications (e.g. impact on costs per unit of animal product) but also for its environmental relevance (e.g. reducing methane emissions) (Madilindi et al., 2022).

The most common index used to state the level of feed efficiency is residual feed intake (RFI), which results from the difference between actual dry matter intake (DMI) and the DMI predicted from body size (e.g. live weight) and performance (e.g. growth or milk yield). Considering comparable

production level, efficient animals (negative RFI values) are characterized by lower feed intake and reduced emissions compared to inefficient ones.

Measurement of RFI on growing bulls selected for reproductive purposes is widely used as it has a fairly good heritability (0.33 ± 0.01 , with a range of 0.07 to 0.62; Berry and Crowley 2013) and in dairy breeds the transmission of the trait to the female progeny is expected to improve milk yield efficiency. However, the effects of the selection of this character on other traits (milk composition, fertility, health) are under study to avoid any undesirable correlated response (e.g., insufficient fat reserves to contrast negative energy balance in early lactation of dairy cows; Madilindi et al., 2022). Manca et al. (2021) proposed a new feed efficiency indicator for cattle that is only based on concentrate intake, named residual concentrate intake, (RCI). Despite the drawback of not accounting for forage intake, this index has the significant benefit of making it easier to monitor individual consumption using new feeding equipment (e.g., auto feeders with identification systems).

Feed efficiency is a multifactorial trait, with different factors influencing the divergences between animals (e.g. digestibility, rumen parameters, body composition) (Cantalapiedra-Hijar et al., 2018). Among them, feeding behaviour probably makes an important contribution also considering the significant differences in dry matter intake (DMI) between efficient and inefficient cattle (Cantalapiedra-Hijar et al., 2018; Fregulia et al., 2021). In literature, only a few studies compared different dual-purpose cattle breeds, among them Cozzi et al. (2009) monitored, with direct but partial observation (scan-sampling technique), feeding behaviour and diet selection without finding differences between breeds.

This study aims to compare feeding behaviour, feed efficiency, and performance traits of growing bulls from the main Italian dual-purpose breeds during performance tests. Evaluation of the relationship between feeding behaviour traits and performance metrics was given special attention.

Materials and methods

In this study, body weight (BW) and DMI data from performance tests conducted from 2018 to 2021 at the Italian genetic centers of Fiume Veneto (PN, Italy; for IS, RE, and AG; Experiment 1) and Gressan (AO, Italy; for VR and VB; Experiment 2) were used.

Animals, housing and animal management (Exp. 1)

Measures for DMI and feed efficiency were performed at the Fiume Veneto genetic center and have been reported in detail previously by Romanzin et al. (2021), so only the main points will be briefly described in this study. Calves born from the best sires and cows of the Italian populations of IS, AG, and RE were selected by the respective National Breeders Associations, transferred to the genetic center, and subjected to growth performance test. Calves arrived at the center at the age of about 30 days, were fed a milk replacer until weaning at the age of about four months, and then were allocated to multiple pens of six subjects. After an adaptation period of about 30 days, the performance test began and continued until the bulls were 12 months old.

During the performance test, at the age of about nine months, bulls from the same pen were moved to a pen in the same barn equipped for the RFI test, which lasted 46 days on average. The equipment for monitoring intake and feeding behaviour was composed of two electronic troughs (RIC system; Hokofarm Group, Marknesse, The Netherlands; Chapinal et al. 2007) which allows the identification of bulls through their ear tags and the measurement of weight changes of the ration in the feed bunk at each feeding event (FE). During each day of test the number of FE (FEn) and the length of each FE (FE_d) were recorded and the missing events number (MEn) and length (ME_d) were considered whenever a bull entered the trough without causing a change in the feed weight.

Animals were fed with a total mixed ration (TMR) prepared daily and distributed twice a day ad libitum in the electronic troughs. Every 42 days, after a 12-hour period of feed restriction, bulls were weighed in the morning. The effective length of the RFI test for each bull was calculated excluding the first 5 days (adaptation time) and days per subject with DMI falling into the highest or lowest 1%.

Animals, housing and animal management (Exp. 2)

Every spring, the National Breeders Association of Valdostana breed transfers calves born in winter from the best sires and cows of both strains (VR and VB) to the genetic center of Gressan to undergo further growth performance tests. Calves of this experiment arrived at the center at an age of about 30 days in homogenous groups of 20-30 animals, during three subsequent months. After a 30-day adjustment period, the performance test started, and it lasted until the bulls were 12 months old. They were fed a milk substitute until weaning at the age of four months. The feed control test was run over this time for an average of 60 days.

The bulls were equipped with a pedometer to measure the activity time and divided into pens of six-eight animals. All the pens were set up with an automatic feeder (AfiFarm system, SAE Afikim, Kibbutz Afikim, Israel) for the individual administration of concentrate ad libitum, whereas the meadow hay was provided daily per each pen in an amount of 1.86 kg DM/animal/d on average. The residue was assumed to be equal to zero. The feeding program adopted during the feed intake control of bulls was based on a specific fibrous compound feed with minimal amounts of hay (approx. 20% of total DMI) and was specifically studied to allow the calculation of the RCI index as proposed by Manca et al. (2021). Bulls were weighed at the beginning and end of the feeding period, in the morning after a period of feeding restriction of about 12 h.

Chemical analysis and prediction of nutritive value of diets

In Exp. 1 TMR was sampled weekly and samples were predried at 60 °C for 48 h and then milled through a 1-mm screen (Pulverisette; Fritsch, Idar-Oberstein, Germany). Analysis of residual DM was performed by heating at 105 °C for 3 h (method 930.15; AOAC 2000). Ash was measured by incineration at 550 °C for 2 h (method 942.05; AOAC 2000) and neutral detergent fiber (NDF) was determined using a fiber analyzer (Ankom II Fiber Analyzer; Ankom Technology Corporation, Fairport, NY) following the procedure of Van Soest et al. (1991) without correction for residual ash.

Finally, N content was determined using the Kjeldahl method (method 976.05; AOAC 2000) and the crude protein (CP) content as $N \times 6,25$.

In Exp. 2 concentrate and hay were sampled during the feeding control period of each group of bulls and were analysed with the same methods as Exp. 1.

The energy and protein nutritional values of both rations were calculated according to the French evaluating system (INRA 2018) adopting tabulated values of UFV (fattening feed unit for maintenance and growth) and intestinal digestible protein (PDI). The INRA protein system sums the by-pass digestible protein with estimated microbial protein synthesis, according to fermentable energy and rumen degradable protein, to predict total intestinal digestible protein and rumen protein balance.

Calculations

Three weights of each bull were used in Exp. 1 with the condition that the intermediate weight was taken during the RFI period. The BW and dates of measurements were used to obtain a linear regression equation for each bull, which was then used to estimate the average daily gain (ADG, as a slope of regression) as well the initial and final BWs of all bulls. Feed: gain ratio (F: G) value was obtained as the ratio between the DMI and the ADG. Concerning feeding behaviour, FEn and FEd data and daily DMI allowed calculation of feeding time (FT), the quantity of DMI of each feeding event (DMI/FE), and feeding rate (FR) as the ratio between DMI/FE and FEd for each bull.

In Exp. 1, RFI was computed as the difference between actual DMI and expected DMI (eDMI) from the linear regression of mean DMI on mid-test $BW^{0.75}$ and ADG, as described by Koch et al. (1963):

$$eDMI = \alpha + \beta(ADG) + \gamma(BW^{0.75}) + \varepsilon$$

where α , β , and γ are parameters of the regression equation and ε is the error term.

In Exp. 2 the BWs data were used to obtain the ADG of each animal. The RCI was calculated as the difference between actual concentrate DMI (cDMI) and expected concentrate DMI (ecDMI), which was estimated by using the same linear regression model of Exp. 1.

Enteric methane production (CH₄) and nitrogen (N) excretions were also calculated in both experiments. CH₄ was predicted using the following equation (adapted from IPCC 2006):

$$CH_4 (g/d) = \frac{DMI \times 18.45 \times (6.5 \times 10)}{55.65}$$

where 18.45 is the average dietary gross energy content (MJ/kg DM), 6.5 is the extent to which gross feed energy is converted to CH₄ (%), and 55.65 is the energy content of CH₄ (MJ/kgCH₄).

The N intake was calculated from the CP content of the diet and the DMI, and N excretion in urine (NU) and feces (NF) was estimated using prediction equations for beef cattle (Dong et al. 2014):

$$NU (g/d) = -14.12 + 0.51 \times N \text{ intake } (g/d) \text{ (RMSE } \pm 4.07)$$

$$NF (g/d) = 15.82 + 0.20 \times N \text{ intake } (g/d) \text{ (RMSE } \pm 2.68)$$

Statistical analysis

All statistical analyses were performed using SAS (SAS Institute, 2019, release 9.4) and data were checked for normal distribution by using UNIVARIATE procedure.

In Exp. 1 BW and age of bulls were statistically analysed as a factorial design using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

where: μ is the overall mean, α_i is the fixed effect of the breed (i=1,3), β_j is the random effect (block) of the cycle (group of animals moved simultaneously at the RFI test) (j=1,57) and ε_{ijk} is the random error. Performance data, predicted emissions, and feeding behaviour of bulls were covaried for initial BW and analyzed with the same model.

Correlations between performance (DMI, RFI, F: G) and feeding behaviour traits of all bulls in Exp. 1 were also calculated using the CORR procedure. After a visual examination of the data using the GPLOT procedure, the two variables with the highest correlation coefficient (DMI/FE and FEn) were fitted using the following nonlinear model and the NLIN procedure:

$$Y_i = A \times x_i^{-b}$$

where: Y_i and x_i are the average DMI/FE and the average FEn of each bull in Exp. 1 ($i=1,640$) respectively, while A and b are coefficients.

To study the feeding behaviour throughout the day in the three breeds DMI, FT, and FR (y) were analysed with the following model and the GLM procedure:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where μ is the overall mean, α_i is a fixed effect of breed ($i=1,3$), β_j is the random effect of time (in hours) throughout the day ($J=1,24$), $(\alpha\beta)_{ij}$ is the fixed interaction effect of breed and hour, and ε_{ijk} is the random error. The PDIFF option was used to evaluate pairwise comparisons between breeds' means, which were considered to be significantly different when $p < .01$.

In Exp. 2, BW and ages of bulls were statistically analysed with the same model as Exp. 1, whereas performance data, feed efficiency, predicted emissions, and measure of activity with the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijkl}$$

where: μ is the overall mean, α_i is the random effect (block) of the cycle ($i=1,3$), β_j is the year of control ($j=1,4$), $(\alpha\beta)_{ij}$ is the interaction between cycle and year of control, γ_k is the fixed effect of the Valdostana strains ($k=1,2$) and ε_{ijkl} is the random error.

Results

Diets

The two diets (Table 1) were different in terms of ingredient composition, since the corn silage and straw represented one-third of the DM in Exp. 1, while these ingredients were not used in Exp. 2 where the fiber was mainly supplied by meadow hay. In addition, soybean meal in Exp. 2 assured most of the protein intake, while in Exp. 1 different meals were used, such as soybean, sunflower, and rapeseed meals. According to the differences in diet formula, NDF was quite higher in Exp. 1 due to the important contribution given by forages. In general, both diets would have supported adequate ruminal function in terms of rumination and buffering capacity. CP was moderately different

between diets, with a head in Exp. 2 compared to Exp. 1 (14.1 vs 13.5 % DM). Based on the INRA (2018) evaluating system the concentration of PDI was adequate, with an expected slight imbalance at rumen level (less than 5%). Overall, the energetic concentration of both diets was similar (0.96 vs 0.94 UFV/kg DM).

Table 1. Ingredients, chemical composition, and nutritive values of the rations fed to the growing bulls in the two feed efficiency tests, RFI test (TMR ad libitum) and RCI test (restricted use of forage and concentrate ad libitum).

	Experiment 1 RFI test	Experiment 2 RCI test
<i>Ingredient, % DM</i>		
Ground corn	28.2	32.3
Barley	5.8	-
Wheat bran	5.8	12.9
Soybean meal	1.9	11.3
Sunflower and rapeseed meals	14.6	-
Dried beet pulp	5.8	6.5
Corn silage	27.2	-
Wheat straw	8.7	-
Meadow hay	-	30.6
Mineral and vitamin mix	1.9	6.5
<i>Chemical composition</i>		
DM (%)	61.8	90.0
NDF (% DM)	37.1	30.8
CP (% DM)	13.5	14.1
Ash (% DM)	6.1	9.2
<i>Nutritive values</i>		
UFV (UFV/kg DM)	0.96	0.94
PDI (g/kg DM)	88.2	98.4
Rumen protein balance (g/kg DM)	0.60	4.8

Vitamin and mineral mix (in 1 kg) (Exp. 1): vitamin A, 100,000 IU; vitamin D₃, 12,000 IU; vitamin E, 450 mg; choline chloride, 1000 mg; FeCO₃, 1076 mg; KI, 39 mg; Ca(IO₃)₂, 21.6 mg; Mn₂O₃, 1161 mg; CuSO₄·5H₂O, 275 mg; ZnO, 620 mg; ZnSO₄, 2055 mg; Na₂SeO₃, 3.1 mg; *Sacch. Cerevisiae* MUCL 39885, 120 * 10⁹ CFU; *Sacch. Cerevisiae* MUCL 39885, 120 * 10⁹ CFU.

Vitamin and mineral (in 1 kg) (Exp. 2): vitamin A, 250,000 IU; vitamin D₃, 25,000 IU; vitamin E, 2000 mg; vitamin B1 100 mg; nicotinic acid 10,000 mg; Ca(IO₃)₂, 30.0 mg; Mn₂O₃, 1800 mg; CuSO₄·5H₂O, 600 mg; ZnO, 4350 mg; Selenomethionine 2250 mg, *Sacch. Cerevisiae* CNCM I-1077 15 * 10⁹ CFU.

DM = dry matter, NDF = neutral detergent fiber, CP = crude protein, UFV = fattening feed unit for maintenance and growth for ruminants (1 UFV equals to 1760 kcal of net energy for maintenance and growth, INRA 2018), PDI = proteins truly digestible in the intestine (INRA, 2018).

Experiment 1

Results of performance, feed efficiency, predicted emissions, and feeding behaviour of the dual-purpose breeds considered are shown in Table 2.

The IS and RE bulls grew faster than AG, but the growth relative to BW was higher for RE than IS and AG. The DMI was highest for IS, intermediate for RE, and lowest for AG (10.2, 9.6, and 8.7 kg DM/d respectively, $p < .01$), while the intake expressed relative to BW was similar for all three breeds. Overall, RE and AG bulls were more efficient than IS in terms of RFI (-0.33 and -0.36 vs +0.09 kg DM/d, $p < .01$).

Emissions of CH₄ and N were significantly different between breeds. IS bulls had the highest values (220.5, 98.3 and 59.9 g/d of CH₄, NU and NF, respectively), while AG bulls had the lowest values ($p < .01$, at about 15%, 16%, and 11% respectively less than IS), while the RE bulls always had intermediate values among the other breeds.

Regarding feeding behaviour, the number of FEn for IS and AG (51 per day, on average) was significantly higher ($p < .01$) than in RE (45 per day). The total FT, on the other hand, was no different between the breeds (114 min/d on average). The average FE_d had no different duration between the three breeds (2.6 min/event) but resulted in a higher ($p < .01$) DMI per visit for IS and RE (0.23 kg DM/event) compared to AG (0.19 kg DM/event). In terms of FR, IS bulls showed a significantly higher rate than RE and AG (98.2 vs 83.1-86.5 g DM/min, $p < .01$). AG bulls had a higher MEn (11 per day) compared to IS and RE (on average 8 per day, $p < .01$) but MEd was similar between breeds (around 7 min/ME).

Table 2. Growth, feed efficiency, predicted emissions, and feeding behaviour of growing bulls in RFI test.

	Italian dual-purpose breeds			RMSE
	Italian Simmental	Rendena	Alpine Grey	
n	495	69	76	-
<i>BW and age</i>				
Initial age (d)	281.1	283.4	281.1	10.57
Initial BW (kg)	369.4 ^A	349.2 ^B	324.1 ^C	42.02
Final age (d)	326.9	328.1	327.2	10.53
Final BW (kg)	456.6 ^A	433.4 ^B	397.4 ^C	44.09
Mid-test BW ^{0.75} (kg)	91.5 ^A	87.8 ^B	82.7 ^C	7.12
<i>Performance</i>				
ADG (g/d)	1549 ^A	1606 ^A	1317 ^B	231.1
ADG (% BW)	0.38 ^B	0.41 ^A	0.37 ^B	0.052
DMI (kg DM/d)	10.20 ^A	9.57 ^B	8.71 ^C	0.749
DMI (% BW)	2.49	2.47	2.44	0.174
F: G (kg DM/kg)	6.76 ^A	6.16 ^B	6.89 ^A	1.083
RFI (kg DM/d)	0.09 ^A	-0.33 ^B	-0.36 ^B	0.655
<i>Emissions</i>				
CH ₄ (g/d)	220.5 ^A	206.9 ^B	188.3 ^C	16.18
NU (g/d)	98.3 ^A	90.3 ^B	82.1 ^C	8.27
NF (g/d)	59.9 ^A	56.8 ^B	53.5 ^C	3.24
<i>Feeding behaviour</i>				
FEn (number/d)	50.24 ^A	44.92 ^B	51.76 ^A	14.579
FT (min/d)	110.44	117.65	112.64	25.209
FEd (min/event)	2.50	2.92	2.49	1.137
DMI/FE (kg DM/event)	0.23 ^A	0.23 ^A	0.19 ^B	0.067
FR (g DM/min)	98.23 ^A	86.47 ^B	83.05 ^B	21.742
MEn (number/d)	8.68 ^B	7.71 ^B	11.42 ^A	4.467
MEt (min/d)	6.72	6.71	7.42	4.771
MEd (min/MEv)	0.84	0.92	0.74	0.561

^{A,B,C} means in the same row with different superscript are significantly different ($p < .01$).

BW = body weight, BW^{0.75} = metabolic body weight, ADG = average daily gain, DMI = dry matter intake, F: G = feed: gain ratio, RFI = residual feed intake, CH₄ = methane, NU = N urinary, NF = N fecal, FEn = number of feeding events, FT = feeding time, FEd = duration of feeding event, DMI/FE = DMI per feeding event, FR = feeding rate, MEn = number of missing events, MEt = time of missing events, MEd = missing event duration.

Pearson correlation coefficients between performance and feeding behaviour traits of the breeds considered in Exp. 1 are presented in Table 3. While there were strong correlations across behaviour traits, there were also significant but weak correlations between behaviour and performance traits. Most notably DMI/FE had a positive correlation with FEd (+0.80) and a negative correlation with FEn (-0.87), meanwhile, the correlation between FEd and FEn was negative (-0.77). In Figure 1 the graphic representation of the exponential fitting between DMI/FE and daily FEn is reported ($R^2=0.96$). A similar outcome was obtained for the relationship between FEd and FEn (the graph has not been included in this paper).

Table 3. Pearson's correlation (r values) between performance and feeding behaviour traits in RFI test.

Traits (unit)	DMI	RFI	F: G	FEn	DMI/FE	FR	FEd	MEn
<i>Performance</i>								
DMI (kg DM/d)								
RFI (kg DM/d)	0.69*							
F: G (kg DM/kg)	0.15*	0.38*						
<i>Feeding behaviour</i>								
FEn (number/d)	0.04	0.22*	0.01					
DMI/FE (kg DM)	0.28*	0.03	0.04	-0.87*				
FR (g DM/min)	0.33*	0.24*	0.25*	0.36*	-0.20*			
FEd (min/event)	-0.02	-0.14*	-0.13	-0.77*	0.80*	-0.62*		
MEn (number/d)	-0.19*	0.08	-0.07	0.53*	-0.49*	-0.06	-0.30*	

* $p < .01$.

DMI = dry matter intake, RFI = residual feed intake, F: G = feed to gain ratio, FEn = number of feeding events, DMI/FE = DMI per feeding event, FR = feeding rate, FEd = duration of feeding event, MEn = number of missing events.

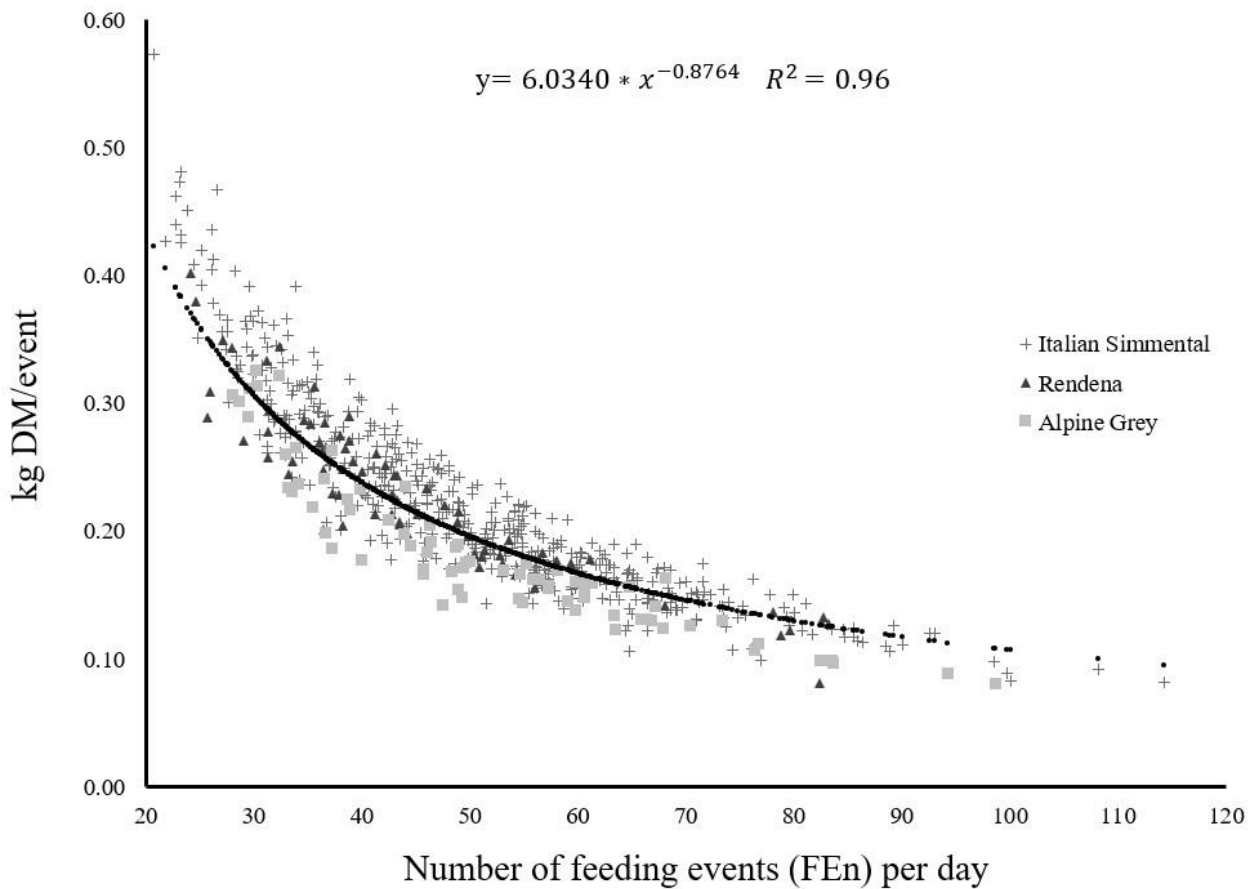


Figure 1. Average feed intake (kg DM) per feeding event (y) plotted against the daily average number of events (x) of each bull (i = 640) in RFI test and interpolation of the data with a nonlinear model ($Y_i = A * x_i^{-b}$).

A comparison between the three breeds in terms of DMI, FT, and FR throughout hourly intervals of the day (e.g. hour 1 is equal to the interval between midnight and one o'clock) is graphically reported in Figure 2. Two feeding activity peaks match to the two moments of the day when the TMR was delivered, while there was a sharp decline of DMI during nocturne hours. IS e RE showed greater DMI than AG ($p < .01$) at 09, 10, 11, 18, e 19 hours. FT had a similar trend but with less marked peaks and also without significant differences between breeds probably due to the high data variability (Figure 3). DMI and FT were divided to get FR (Figure 4), which was always a higher for IS compared to the other two breeds but reached the statistical significance only at 09 and 10 hours.

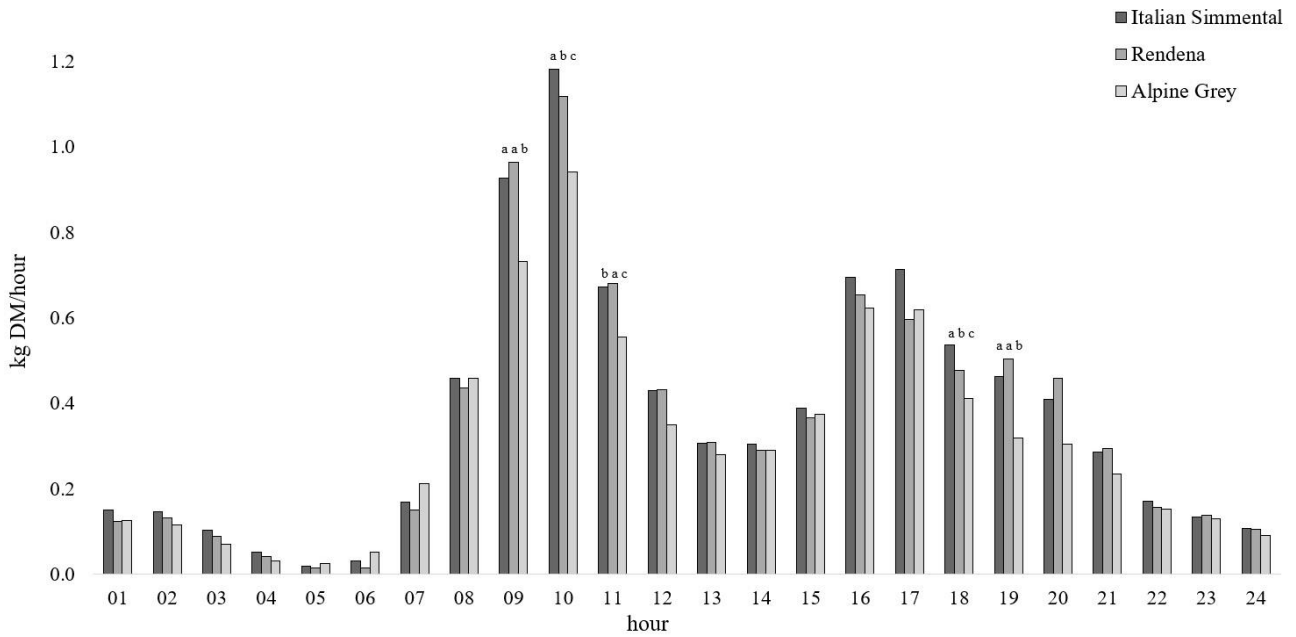


Figure 2. Feed intake (kg DM) at 1-hour intervals throughout the day in RFI test (different letters over the bars within each hour of the day denote statistical differences among breeds, $p < .01$; RMSE = 0.517).

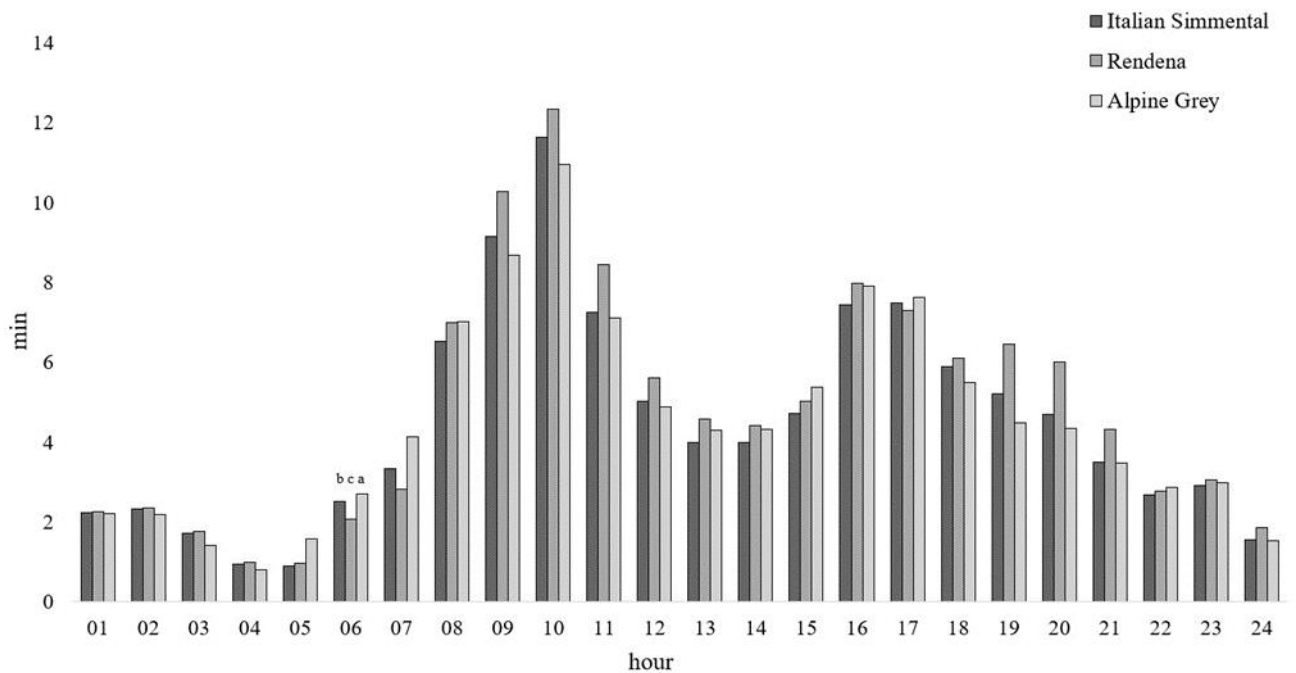


Figure 3. Feeding time (min) at 1-hour intervals throughout the day in RFI test (different letters over the bars within each hour of the day denote statistical differences among breeds, $p < .01$; RMSE = 11.882).

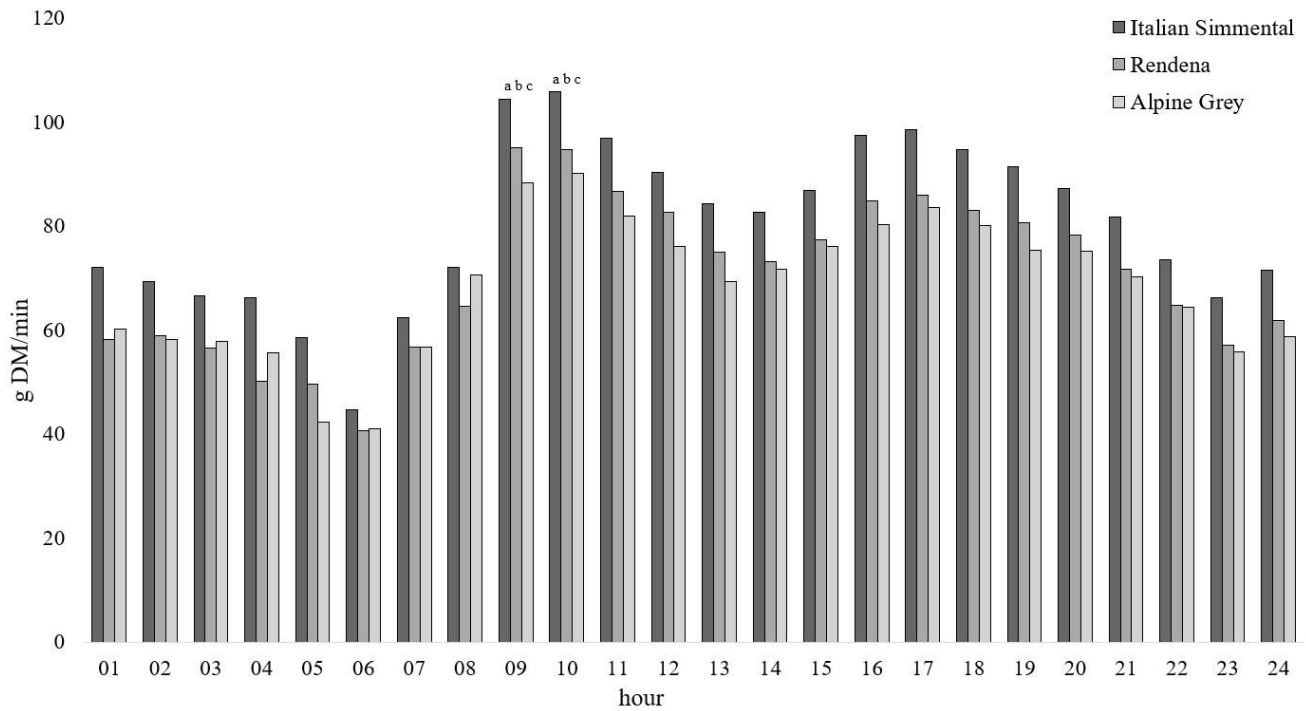


Figure 4. Feeding rate (g DM/min) at 1-hour intervals throughout the day in RFI test (different letters over the bars within each hour of the day denote statistical differences among breeds, $p < .01$; RMSE = 48.664).

Experiment 2

The main results of performance, feed efficiency, predicted emissions, and measure of activity obtained in Exp. 2 on two Valdostana strains are shown in Table 4.

The VB bulls had a numerically higher ADG than VR (0.89 vs 0.82 kg/d), which became significant when expressed as a percentage of BW. However, cDMI was also slightly higher in VB than in VR (4.28 vs 4.10 kg DM/d, $p < .01$), resulting in a similar F: G ratio between strains (6.19 vs 5.47 kg DM/kg respectively for VR and VB). The VR bulls had a neutral mean RCI value while the VB bulls were slightly inefficient (+0.13 kg DM/d). Emissions of CH₄ and N were always slightly higher in VB than in VR (+2-4% approximately). The activity index, recorded by the pedometers, was higher in VR than in VB (+ 7%, $p < .01$).

Table 4. Growth, feed efficiency, predicted emissions, and activity time of growing bulls in RCI test.

	Valdostana strain		RMSE
	Red	Black	
n	151	99	-
<i>BW and age</i>			
Initial age (d)	203.3	205.3	13.23
Initial BW (kg)	179.9	180.7	32.10
Final age (d)	263.5	265.7	13.85
Final BW (kg)	229.3	234.7	40.31
Mid-test BW ^{0.75} (kg)	54.0	54.5	7.05
<i>Performance</i>			
ADG (g/d)	816	887	215.1
ADG (% BW)	0.40 ^B	0.43 ^A	0.095
cDMI (kg DM/d)	4.10 ^B	4.28 ^A	0.300
cDMI (% BW)	2.06 ^B	2.12 ^A	0.187
F: G (kg DM/kg)	6.19	5.47	3.121
RCI (kg DM/d)	-0.01 ^B	0.13 ^A	0.302
<i>Emissions</i>			
CH ₄ (g/d)	88.6 ^B	92.4 ^A	6.47
NU (g/d)	68.0 ^B	70.4 ^A	3.94
NF (g/d)	37.0 ^B	37.9 ^A	1.54
<i>Behaviour</i>			
Activity (min/d)	825.5 ^A	766.4 ^B	126.57

^{A, B} means in the same row with different superscript are significantly different ($p < .01$).

BW = body weight, BW^{0.75} = metabolic body weight, ADG = average daily gain, cDMI = concentrate dry matter intake, F: G = feed: gain ratio, RCI = residual concentrate intake, CH₄ = methane, NU = N urinary, NF = N faecal.

Discussion

In general, the three dual-purpose breeds considered in Exp. 1 differ in size and, even if in a less pronounced way, in the production capacity (both in terms of milk and meat) as described by Cozzi et al. 2009. However, the juvenile phase considered in this trial reduces BW and ADG differences, in particular between IS and RE. The growth performances of IS confirmed the preliminary results discussed in Romanzin et al. (2021) in a trial on a more limited number of animals. RE had shown excellent growth performance with rather low consumption even compared to the few data that can be found in the literature (Corazzin et al. 2017; Guzzo et al. 2019). A separate discussion must certainly be made for AG, a breed with small size but good muscularity that had shown high growth

and very low feed consumption. In a study performed with Italian Alpine cattle breeds fed a diet similar to that of our study, Cozzi et al. (2009) obtained lower values of DMI and ADG. This is likely due to the genetic progress in the last decade and the larger growth phase considered in that trial. However, the breed comparison reflected what was found in this study: IS had higher DMI and ADG than AG, while RE had intermediate values. Our study showed that AG and RE were particularly efficient breeds throughout the growth phase and had favourable RFI values. Moreover, these breeds showed low expected emissions of N and CH₄, which reflected differences in DMI.

Data on feeding behaviour showed how FT was not different among efficient and non-efficient breeds (Table 2) and these results were consistent with those proposed in other studies on high and low RFI bulls (Menezes et al. 2020). On the other hand, Benfica et al. (2020) argued that the FT can be a strong indicator of feed efficiency. Regarding the FEn, it was thought that a high FEn was associated with less efficient animals that had a more intense activity, as reported in a prior study (Romanzin et al. 2021) and also supported by other authors in the literature (Lancaster et al. 2009; Kelly et al. 2010). In this study, the breed effect (e.g. AG breed) outweighs a possible influence of feed efficiency. The MEn also support this assumption. AG was a breed with restless behaviour, manifesting in a high number of FE and ME, despite being a breed with a low RFI. The FR data, on the other hand, proved to be more reliable. Bulls with a slow FR were more efficient in terms of RFI. In a review, Kenny et al. (2018) report that low-RFI growing steers, heifers, and pregnant beef females had a slower FR than their high-RFI counterparts. Even so, the correlation between FR and RFI was not particularly strong but highly significant (Table 3).

As already reported by Azizi et al. (2009), the correlations between FEd and FEn and between DMI/FE and FEn followed a non-linear regression model. Although the breeds had significant differences in daily average data (e.g. FEn and DMI/FE; Table 2) the distribution was similar (Figure 1). In all breeds, there were many subjects with high FEn (> 70) and DMI/FE around 100 g, while on the other side subjects with low FEn (< 30) and DMI/FE between 300 g and 500 g.

As expected, the visual representation of differences in DMI and FT between the dual-purpose breeds showed a strong reduction during the nocturne hours, while peaks in eating activity occurred in the hours immediately next the feed delivering throughout the day (Figures 2 and 3). This was also confirmed by numerous studies (Aikman et al. 2008; Cozzi et al. 2009), where the peaks occurred after the fresh feed was placed in the feed bunk. By focusing on breeds that differed for feed efficiency, the FEn and FT did not follow RFI values, whereas DMI does. Otherwise, IS bulls ate more in a time similar to that of AG, according to the values of FR and this is probably due to the greater dimensions of the bite-size, which increased the eating speed (Beauchemin 2018). In any case, the quantity of DMI BW-related was similar to the three groups. Fitzsimons et al. (2014), in a study on pregnant beef cows divergent for RFI, found that in the first period after the ration delivery (up to three hours later) there were no differences in DMI, whereas successively the high-RFI group had significantly higher ingestion than low-RFI group. Unlike this, we found that the less efficient breed had a higher DMI during feeding activity peaks.

As concerns FR (Figure 4), IS had the higher values during all the day, whereas RE and AG had similar behaviour. In general, feeding behaviour is known to be strongly linked to RFI, although the literature is equivocal about which behavioural traits are the most significant (Cantalapiedra-Hijar et al. 2018; Kenny et al. 2018). From our results, it would seem that even considering different behavioural traits, this effect was well distributed without particular changes over the 24 hours. In a recent work (Holló et al. 2022), in which the TMR was distributed once a day, a single rather protracted dietary peak was noted. However, the differences in terms of feeding behaviour between efficient and inefficient bulls were very few. It was therefore conceivable that even if the ration distribution frequency increased there would be no change in this indicator.

In a recent study, Brown et al. (2022) investigated the relation between feeding behaviour and RFI in mid-lactation dairy cows. The interesting outcome regarding the link between FR and RFI, which was inversely proportional as is also reported in the results of our study (inefficient cows has a higher FR, and vice versa). Therefore, this behavioural trait could be an opportunity for the identification of

efficient or inefficient animals. In addition, a genetic correlation between FR and RFI in steers was investigated in many studies (Chen et al. 2014, Kelly et al. 2021). The inclusion of FR in cattle selection can rely on an acceptable heritability (0.44 – 0.56) and on data generated with the use of smart technologies.

The VB strain (which includes Black Pied and Chestnut) is characterized by lower milk production and greater muscularity (Mazza et al. 2015). This was confirmed by the better growth performance compared to VR (Table 4). However, in a recent study on grazing heifers, Kreuzer et al. (2021) found no differences in growth performance between the two Valdostana strains. The higher cDMI recorded for VB determines a little bit lower efficiency in the growing phase, limited to the RCI. The difference in feed efficiency between strains was significant but numerically limited, as previously seen in Exp. 1. A study that compared 4 cattle breeds, both dairy and beef (Bureš and Bartoň 2018), found stronger differences in RFI, especially between Holstein (+1.55 kg DM/d) and Gascon (-1.36 kg DM/d) breeds. Valdostana strains had moderately different breeding goals, which do not much affect their feed efficiency. Surprisingly, the Chestnut strain, also selected for fighting ability (Sartori and Mantovani 2012), had a lower activity index. As previously stated for AG, even the feed efficiency of VR was not affected by its higher activity.

Conclusions

In general, the Italian dual-purpose breeds involved in this study do not differ markedly in terms of growth rate, or feeding efficiency (measured in terms of RFI and RCI) and behaviour, and they attain relatively high levels of performance. In general, these results show that, when properly fed, dual-purpose cattle breeds - which are not under intensive breeding selection for meat production - can achieve notable growth performances with good feed efficiency levels. This result should encourage a further diffusion of these breeds.

The relationships between feeding behaviour traits and performance metrics show that FR is well related to feed efficiency (e.g. AG bulls were the most efficient with the lowest FR), while FT does

not show significant differences between breeds. These findings support a research strategy that emphasizes the study of mechanisms that regulate feeding behaviour traits, notably FR.

Ethical approval

The experimental procedures followed the EU Directive 2010/63/EU, Italian legislation on animal care (DL no. 26 - 4 March 2014), and the guidelines of the Ethics Committee of University of Udine (Prot. no. 11/2018, no. 1/2021 and no. 2/2022).

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Disclosure statement

The authors declare no real or perceived competing interests.

Data availability statement

The data that support the findings of this study are freely available upon request.

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CHAPTER III

Eating time of dairy cows: a study focusing on commercial farms

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ABSTRACT

The association of daily eating times (ET) of dairy cows, measured by wearable sensors, with diet composition, feeding practices, and performance was investigated. About 800 lactating cows of two breeds (Holstein Friesian – HF and Italian Simmental - IS) reared on 14 commercial farms were considered. Cows were grouped into ET classes (ETC, min/d): ETC-1 ≤ 180 ; ETC-2 from > 180 to ≤ 220 ; ETC-3 from > 220 to ≤ 260 ; ETC-4 from > 260 to ≤ 300 ; and ETC-5 > 300 . Low ETC was positively associated with ether extract, crude protein, nonfiber carbohydrates, and neutral detergent fiber digestibility, and negatively associated with acid detergent lignin and particle size. A higher frequency of feed pushing per day seemed to be able to increase ETC. The relationship between ETC and performance, adjusted for breed, diet composition, days in milk, and parity, showed that cows with the highest ETC were more productive compared to those with shorter ETC (31.9 vs. 27.0 kg/d of milk) without significant changes in milk composition. Moving from short to long ETC, there was a linear reduction in urea, somatic cells, and body condition score. On the contrary, there was a positive association between ETC and time spent ruminating. Considering differences between breeds, HF had a higher milk yield, ruminated 20 minutes more, and had a higher ET (255 vs. 228 min/d) than IS. The study provides preliminary results for future research to better define the role of ET in the overall feed efficiency and health status of cows.

Keywords: eating time; feeding behaviour; dairy cow; commercial farm.

Highlights

- Eating time is linked to the composition of the diet
- Increased frequency of feed pushing lengthens eating time
- The most productive cows have a longer eating time

Introduction

Maintaining an efficient dairy livestock system is necessary due to the increasing demand for foods of animal origin. Precision dairy farming equipment allows the continuous monitoring and management of individual productivity and health issues (Lovarelli et al. 2020), reducing costs and exploiting the potential of the cows. Changes in behaviours could provide relevant information about the nutrition, reproduction, health, and overall well-being of dairy cows (Benaissa et al. 2020). Nowadays, wearable sensors have been widely tested and validated to automatically assess cow behaviours every day such as feeding time, activity, and other parameters helpful in herd management. Some studies (Borchers et al. 2021; Leso et al. 2021) found substantial correlations between visual observation and sensor-detected feeding behaviour ($r = 0.85$ to 0.93 for eating and $r = 0.83$ to 0.92 for rumination time, respectively). Rumination time (RT) is widely used in farms as an indicator of animal health (Magrin et al. 2022). As reported in a review by Beauchemin (2018), there is a negative relationship between RT and ruminal acidosis, and other studies have highlighted changes in RT in relation to the oestrous phase or in response to difficult calving (Reith and Hoy 2012; Mammi et al. 2021).

On the other hand, eating time (ET), which is defined (Beauchemin 2018) as the sole time spent prehending, chewing, and swallowing feed, is occasionally used on commercial farms as a sign of animal conditions. It is strongly affected by feed management, dry matter intake (DMI), diet composition, and environmental conditions and shows a high variability among animals (Grant and Ferraretto 2018; Corazzin et al. 2021).

At an individual level, few studies identified a relationship between performance and feeding behaviour. Johnston and DeVries (2018) found a relationship between ET and milk yield (MY) (+ 1.74 kg/d of MY for every hour of ET increased), as well as RT and MY (+ 1.26 kg/d of MY for every hour of RT increased). Similarly, in the study of Dado and Allen (1994), higher-producing cows ruminated longer each day with an increase in feed intake. ET and feeding rate have also been studied as factors influencing feed efficiency in cattle (Ben Meir et al. 2019; Romanzin et al. 2021).

Presently, most studies on the feeding behaviour of lactating cows are conducted on research farms with permanent fixed stations, which are very expensive and not suitable for commercial farms. Besides that, these systems allow the gathering of data such as feed intake and feeding time (which differ from eating time and represent meals, which comprise eating bouts interspersed with periods of noneating; Beauchemin 2018). The usage and application of herd monitoring with wearable sensors allow a relatively simple collection of data at the individual level on all sizes and types of livestock farms, both dairy and meat.

This study aimed to explore the association of different daily ET of dairy cows, as recorded by wearable sensors, with diet composition, feeding practices, and performance on commercial dairy farms.

Materials and methods

Data and samples collection

The study was conducted in May 2022 in 14 dairy farms of the Friuli Venezia Giulia Region (Italy), where dairy cows were equipped with a specific sensor (SenseHub™ Dairy, Allflex® Livestock Intellgence™, SCR Engineers Ltd., Netanya, Israel; described by Merenda et al. 2019) able to detect eating, rumination, grazing, resting, and other activity parameters. Dairy cows were kept in a cubicle housing system and milked with an automatic milking system (AMS). Overall, 819 lactating dairy cows were considered, and farms were visited once (within the same month). A questionnaire was created to gather qualitative information about the diet (ingredients and daily amount administered) as well as farm management (e.g., feed administration).

Data on cows' performance (days in milk (DIM), lactation number, MY, milk composition, and somatic cell count (SCC)), and feeding behaviour (ET and RT) were all collected by Lely time-for-cows and Horizon management software (Lely, Maassluis, the Netherlands) once for each farm, considering the 15 days prior the farm was visited. On the same day the completion of the

questionnaire, body condition score (BCS) measurement, and diet sampling were performed. A trained expert evaluated the BCS in all subjects, relying on the 5-point scale method described by Edmonson et al. (1989) with increments of 0.25 points. Partial mixed ration (PMR) samples were collected immediately after feed delivery following the procedure described by Robinson and Meyer (2010). In addition, a sample of the compound feed distributed by the AMS was taken. Samples were labeled and stored frozen at -20°C until chemical analysis.

Chemical analysis

The PMR samples were divided into two sub-samples. The first sub-samples were predried at 60 °C for 48 h, and then, together with the compound feed samples, were milled through a 1-mm screen (Pulverisette; Fritsch, Idar-Oberstein, Germany). Analysis of residual DM was performed by heating at 105 °C for 3 h (AOAC 2016). Ash was measured by incineration at 550 °C for 2 h (AOAC 2016). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using a fiber analyzer (Ankom II Fiber Analyzer; Ankom Technology Corporation, Fairport, NY) following the procedure of Van Soest et al. (1991) without correction for residual ash and by applying a pre-treatment of samples with α -amylase. The N content was determined using the Kjeldahl method (AOAC 2016), and the crude protein (CP) content was calculated as $N \times 6.25$. Ether extract (EE) was determined using the Soxhlet method (AOAC 2016). The NDF digestibility (NDFd) was measured by the ANKOM DaisyII Incubator, following the procedure described by the manufacturer (Ankom Technology Corporation, Fairport, NY). Dry feed samples, approx. 0.5 g, were weighed into filter bags (Ankom F57). All samples were contemporarily tested in quadruple in two fermentation runs of 48 hours. In each run, the four bags of each sample were distributed in four incubation flasks, which were filled with filtered rumen inoculum (400 mL) and buffer solution (1600 mL), according to Robinson et al. (1999). The rumen liquid of each fermentation run was collected at the slaughterhouse from four cows and pooled. At the end of each incubation, the bags were removed from each bottle, properly rinsed with cold tap water, and then dried for 24 hours in a 105°C

heater. The bags were weighed and then analysed for their NDF content, as previously reported. Compound feed samples were analysed with the same methods as stated prior for the first sub-sample of PMR.

The second sub-sample of PMR was fractionated according to the procedure of Kononoff et al. (2003) using a particle separator (NASCO[®], Pennsylvania State University) composed of three sieves (mesh diameters: 19, 8, and 1.18 mm) and a bottom pan. Samples were spread out on the top 19 mm sieve. The sieve set was shaken five times horizontally in one direction, rotated once, and then shaken five more times. The process was repeated for eight sets of five replications, for a total of forty shakes. Four PMR fractions were then obtained. Each fraction was weighed and combined to form two fractions: a "Short" fraction with particles confined in the bottom pan and a "Long" fraction with particles ≥ 1.18 mm in size. The long fraction was then submitted to the NDF analysis to define physical effective NDF (peNDF). The peNDF was calculated by multiplying the NDF content by its physical effectiveness factor, which was defined as the proportion of particles retained by the 1.18 mm sieve according to Cotanch and Grant (2006).

Data processing and calculations

The questionnaire data was converted to digital form using a spreadsheet (Excel, Microsoft Corp.). Individual data about performance and feeding behaviour taken from farm management software were exported and statistically examined together with questionnaire data.

Fat- and protein-corrected milk (FPCM) was calculated using the formula reported by Kok et al. (2016) as follows:

$$\text{FPCM (kg/d)} = \text{milk (kg/d)} \times [0.337 + 0.116 \times \text{fat content (\%)} + 0.06 \times \text{protein content (\%)}]$$

The weights of the wet fractions obtained from the particle separator were used to calculate the average particle size using the Particle size spreadsheet made available by the producer (Penn State College of Agricultural Sciences 2022). The nonfibre carbohydrates (NFC) content was calculated as $100 - (\text{CP} + \text{ash} + \text{EE} + \text{NDF})$. The CP, acid detergent insoluble nitrogen (ADIN) (determined from

the protein analysis on the ADF residue), NFC, and NDF contents (in %DM), as well as the NDFd, were used to calculate the truly digestible amounts, according to the equations proposed by NRC (2001). The digestible energy (DE) content at the maintenance level was calculated using equations 2 – 8a (assuming the fatty acid content equal to EE - 1; NRC 2001). This value was then used to determine the DE at production levels by applying a discount factor (equations 2 – 9; NRC 2001). The DE at production levels was used to calculate the net energy for lactation (NE_L) values for each diet, using the equations 2-10, 2-11, and 2-12 (NRC 2001).

Statistical analysis

Data was analysed using SPSS (ver. 17, SPSS Inc., Illinois) with the exception of Principal Component Analysis (PCA), which was performed with R software (4.1.2 version, R core team). The normality of the data distribution was assessed by the Shapiro-Wilk test. Outlier removal for feeding behaviour traits included daily mean values that were smaller than 5% or larger than 99%, according to Jaeger et al. (2019). In the second step, animals with less than 7 days of available data were deleted. The result was a total of 779 lactating dairy cows, respectively, 540 Italian Simmental (IS) and 239 Holstein Friesian (HF), of whom were considered the last 7 complete days before the date of sampling.

Cows were distributed in five classes of ET length (ETC): ETC-1 ≤ 180 min/d; ETC-2 from > 180 to ≤ 220 min/d; ETC-3 from > 220 to ≤ 260 min/d; ETC-4 from > 260 to ≤ 300 min/d; and ETC-5 > 300 min/d (Grant and Albright 2000). In the statistical models, parity was coded as 1 (primiparous), 2 (multiparous with 2 parities), and 3 (multiparous with more than 2 parities). To explore the relationship between the ETC and the main variables related to diet characteristics, parity, breed, DIM, number of feed distributions, and number of feed pushing per day, PCA was considered. Correlations between variables were assessed using Spearman and Pearson tests when appropriate. In order to avoid redundancy, it was ensured that all variables had a correlation coefficient less than 0.9 (Tabachnick and Fidell 2001). The adequacy of the sample was measured by the Kaiser Meyer

Olkin test, and the communalities of the variables were assessed. The multivariate normality was assessed by the Bartlett test of sphericity. After the assumption's assessment, NE_L , NDF, the number of feed distributions per day, $peNDF$, and ADF were excluded from PCA analysis. Only the components with eigenvalues greater than 1 were retained in the analysis. PCA was conducted with the PCAmixdata package (Chavent et al. 2014), which allowed for both continuous and categorical variables. In addition, the PCArot function was considered to evaluate the possibility of improving the clarity of the data interpretation (Chavent et al. 2012).

The effect of ETC and breed on MY, milk composition, BCS, and RT was assessed considering ETC and breed as fixed factors, diet and parity as block factors, and DIM as a covariate in a general or generalized linear model when appropriate. A similar model was used to assess the effect of breed on ET. In particular, the breed was considered a fixed factor, diet and parity as block factors, and DIM as a covariate in a general linear model. In all the models considered, multiple testing was conducted using the sequential Bonferroni procedure.

Results

Descriptive statistics of animal traits, composition of diets, performance, feeding behaviour, and feed management are reported in Table 1. Dairy cows had on average 2.5 ± 1.5 lactations, 183 ± 111 DIM. Diets were primarily based on farm-produced forages (corn silage, haylage, hay, and some legumes, and silage accounted for approximately half of the diet (47.57 % as fed), with only 14% of farms not using silage in rations. This was also reflected in the particle size data (5.14 to 7.07 mm, Q_{25} and Q_{75} , respectively), and in DM values (56.02 to 64.25 %, Q_{25} and Q_{75} , respectively). As regards the fibrous component, NDFd ranged from 60.27 to 69.14 %NDF (Q_{25} and Q_{75} , respectively) and $peNDF$ had average values of 40.97 ± 6.71 %DM. The different composition of the diets led to different energy levels, with 1.36 to 1.63 kcal NE_L /kg DM (Q_{25} and Q_{75} , respectively).

The cows had an average production of 30.41 kg of milk per day. Regarding feeding behaviour, the average values of ET and RT were 227.6 and 536.0 min/d, respectively (± 66.1 and $\pm 58,0$ min/d,

respectively). Different feeding management practices distinguished the farms. In particular, feed pushing per day showed high variability, with extreme values ranging from 3.5 to 24.0 (Q₂₅ and Q₇₅, respectively), and feed delivery numbers per day (1.50 ± 1.22 on average) showed considerable variability due to various types of conduction using automatic feeding systems or traditional ones.

Table 1. Descriptive characteristics of 779 dairy cows and rations of the farms involved in the study.

Item	Total			
	Mean	SD	Q ₂₅ ¹	Q ₇₅ ¹
<i>Animal traits</i>				
Parity (n)	2.1	0.9	1	3
DIM (d)	182	111	102	249
<i>Diet characteristics</i>				
Particle size (mm)	6.46	2.73	5.14	7.07
DM (%)	60.48	11.04	56.02	64.25
Ash (%DM)	7.14	0.76	6.85	7.49
CP (%DM)	14.98	1.69	13.81	16.84
EE (%DM)	2.89	0.64	2.39	3.34
NFC (%DM)	35.40	4.85	32.59	37.90
NDF (%DM)	39.58	6.16	34.13	43.96
ADF (%DM)	23.05	4.46	18.51	26.25
ADL (%DM)	4.18	1.10	3.18	5.26
NDFd (%NDF)	64.15	6.59	60.27	69.14
NE _L (Mcal/kgDM)	1.48	0.17	1.36	1.63
peNDF (%DM)	40.97	6.71	36.42	43.54
<i>Performance</i>				
FPCM (kg/d)	29.82	8.55	23.69	35.60
Milk Yield (kg/d)	30.41	9.11	24.49	36.50
Milk fat (%)	3.87	0.82	3.35	4.34
Milk protein (%)	3.42	0.29	3.21	3.61
Milk lactose (%)	4.80	0.29	4.74	4.97
Milk urea (mg/dL)	23.09	6.02	18.75	26.80
SCC (x 1,000/mL)	214	515	33	142
BCS (points)	3.24	0.61	2.75	3.75
<i>Behaviour</i>				
ET (min/d)	227.6	66.1	179.6	273.4
RT (min/d)	536.0	58.0	501.1	577.4
<i>Feed management</i>				
Feed delivery (n/d)	1.50	1.22	1.00	1.00
Feed pushing (n/d)	11.43	10.01	3.50	24.00
COF (kg/d)	3.84	1.54	2.70	4.80

DIM = days in milk; DM = dry matter; CP = crude protein; EE = ether extract; NFC = nonfiber carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; NDFd = neutral detergent fiber degradability; NE_L = net energy for lactation; peNDF = physical effective NDF; FPCM = fat- and protein - corrected milk (Kok et al., 2016); SCC = somatic cell count; BCS = body condition score; ET = eating time; RT = rumination time; COF = compound feed offered; ¹Q₂₅ = 25th percentiles; Q₇₅ = 75th percentiles.

The PCA of ETC relative to the component map of the numerical variables and of the categorical are shown in Figure 1a and Figure 1b, respectively. The first two components explained 39% of the total variance and the ETC was mainly linked to the first principal component (30% of the variance explained) with a squared loading (SL) of 0.44. The ETC-1 and 2 were well discriminated by ETC-3, 4, and 5 on the first principal component, primarily by diet-related characteristics. In particular, the lowest ETC (1 and 2) were positively associated with EE (SL, 0.67), CP (SL, 0.64), NFC (SL, 0.69) and NDFd (SL, 0.72), and negatively associated with ADL (SL, 0.81). Along the second principal component (9% of the variance explained), the lowest ETC (1 and 2) were positively associated with DIM (SL, 0.13) and negatively associated with feed pushing (SL, 0.18). In order to improve the clarity of the relationship between the variables, the simple correlations are shown in Table 3. In agreement with the PCA results, ETC was positively correlated with FP, particle size, and ADL ($p < .01$) and negatively correlated with parity, CP, EE, NFC, and NDFd ($p < .01$). In contrast, no significant correlation was found between ETC and DIM ($p > .05$). Similar results were found with the same variables, but considering the within breed correlations.

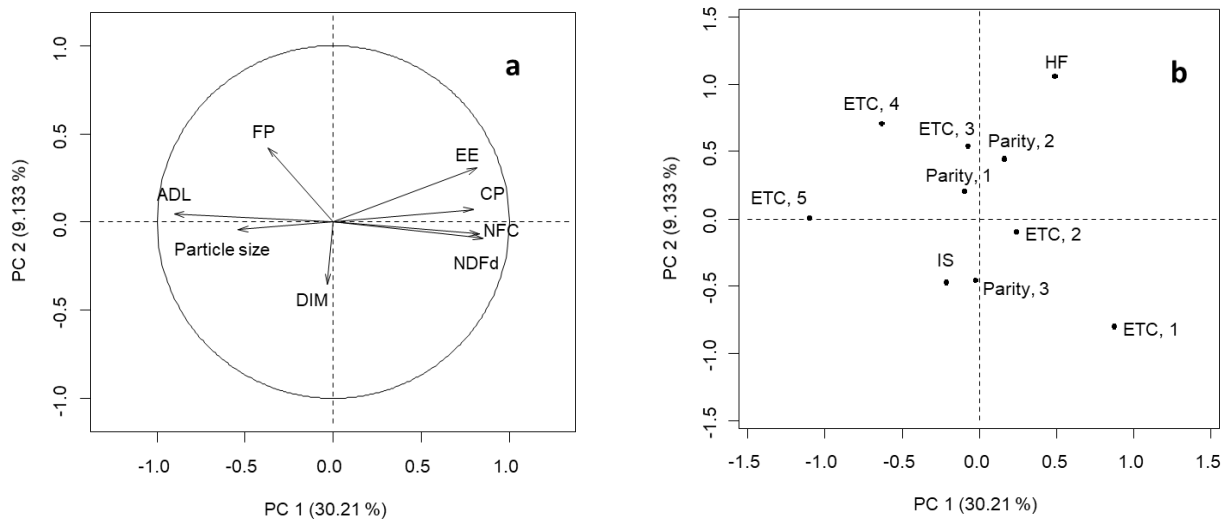


Figure 1. Results of the principal component analysis (PCA) analysis for the variable related to eating time class. a: component map with factor scores of numerical variables; b: component map with factor scores of levels.

- a. FP = number of feed pushing per day; DIM = days in milk; ADL = acid detergent lignin (%DM); EE = ether extract (%DM); CP = crude protein (%DM); NFC = nonfiber carbohydrates (%DM); NDFd = neutral detergent fiber degradability (%NDF).
- b. ETC = eating time class. ETC-1 ≤ 180 min/d; ETC-2 from >180 to ≤ 220 min/d; ETC-3 from >220 to ≤ 260 min/d; ETC-4 from > 260 to ≤ 300 min/d; and ETC-5 > 300 min/d. Parity 1 = primiparous; Parity 2 = multiparous (2 parities); Parity 3 = multiparous (>2 parities); HF = Holstein Friesian; IS = Italian Simmental.

Table 2 depicts the results of performance and feeding trait differences among the five ETC. The distribution of cows from the two breeds in different ETC classes revealed a prevalence of IS cows but the frequency of the two breeds was the same across the different ETCs. In fact, the ratio HF/IS cows had a limited range of variation (between 23:77 in ETC-5 to 39:61 in ETC-3), and the statistical analysis (chi-square test, $p > .05$, not in table) did not show significant differences in breed ratio across the ET classes. Cows within the highest ETC were the most productive animals compared to those of the shortest ETC (31.91 kg/d vs. 27.04 kg/d of milk, respectively; $p < .01$). There were no significant differences in milk composition and FPCM increased according to the ET class with a significant linear trend ($p < .01$). The only difference was given by urea, which was significantly different from ETC-1 with the highest value compared to other ETC ($p < .01$). Looking at the concentration of SCC in milk, their presence decreases linearly from less to more productive animals (from 224 x 1,000/mL to 118 x 1,000/mL; $p < .05$). Regarding BCS, animals in the lowest ETC had

a significantly higher value compared to the highest ETC (3.24 vs. 3.05, respectively; $p < .05$). On the contrary, animals within the ETC-1 had the lowest RT (520.9 min/d; $p < .05$).

Considering the differences between the two breeds (Table 2), HF had significantly higher milk production than IS, both in absolute terms (30.60 vs. 28.46 kg/d; $p < .01$) and as FPCM (29.56 vs. 28.21 kg/d; $p < .01$) and there were no significant differences in milk composition between the two breeds. The only component that had a significant difference was urea, with half a point in favor of HF (23.17 vs. 22.66 mg/dL, respectively; $p < .05$). Otherwise, SCC was significantly and strongly higher for HF compared to IS (223 vs. 157 x 1,000/mL, respectively; $p < .05$). Concerning BCS, significantly higher values were recorded in IS than in HF (3.47 vs. 2.81 points; $p < .05$). Regarding feeding behaviour, RT was significantly different between the two breeds, with HF ruminating almost 20 minutes more than IS ($p < .01$). Finally, the ET differed between the two breeds, with higher values for HF than IS (254.7 ± 5.2 min/d for HF and 227.9 ± 2.2 min/d for IS, $p < .01$, data not shown in the Table).

Table 2. Estimated marginal means of milk yield and composition, body condition score and ruminating time as affected by eating time class and breed.

Item	Eating Time Class (ETC) ¹					Breed		SEM	Breed, <i>p</i> -value	ETC, <i>p</i> -value	Contrasts, ETC <i>p</i> -values	
	1	2	3	4	5	HF	IS				Linear	Quadratic
HF:IS ratio	27:73	29:71	39:61	36:64	23:77	-	-	-	-	-	-	-
Parity (n)	2.3	2.2	2.0	1.9	1.8	-	-	-	-	-	-	-
DIM (n)	199	170	185	183	170	-	-	-	-	-	-	-
Milk yield (kg/d)	27.04 ^E	28.72 ^D	29.89 ^C	30.23 ^B	31.91 ^A	30.60	28.46	0.058	< .01	< .01	< .01	.01
FPCM (kg)	26.71 ^e	27.97 ^d	29.28 ^c	29.54 ^b	31.08 ^a	29.56	28.21	0.059	< .01	< .01	< .01	.13
<i>Milk composition</i>												
Fat (%)	3.87	3.84	3.82	3.87	3.77	3.77	3.90	0.213	.80	1.00	1.00	1.00
Protein (%)	3.45	3.44	3.40	3.39	3.41	3.37	3.47	0.196	.83	1.00	1.00	1.00
Lactose (%)	4.80	4.79	4.80	4.80	4.79	4.80	4.79	0.078	.96	1.00	1.00	1.00
Urea (mg/dL)	23.58 ^A	22.76 ^B	22.95 ^B	22.63 ^B	22.65 ^B	23.17	22.66	0.107	.03	< .01	< .01	< .01
SCC (x 1,000/mL)	224 ^a	255 ^a	189 ^a	179 ^a	118 ^b	222.5	157.1	11.36	.02	< .01	< .01	.13
BCS (points)	3.24 ^a	3.19 ^{ab}	3.10 ^{ab}	3.13 ^{ab}	3.05 ^b	2.81	3.47	0.024	< .01	.02	< .01	.72
RT (min/d)	520.9 ^E	531.3 ^C	532.3 ^B	528.7 ^D	536.1 ^A	540.1	519.8	0.06	< .01	< .01	< .01	< .01

¹ETC = eating time class. ETC-1 ≤ 180 min/d (n = 197); 220 min/d ≤ ETC-2 > 180 min/d (n = 179); 260 min/d ≤ ETC-3 > 220 min/d (n = 174); 300 min/d ≤ ETC-4 > 260 min/d (n = 111); ETC-5 > 300 min/d (n = 118).

DIM = days in milk; FPCM= fat- and protein - corrected milk (Kok et al., 2016); SCC = somatic cell count; BCS = body condition score; RT= rumination time; HF = Holstein Friesian; IS= Italian Simmental; ^{A, B, C, D, E} means of ETC in the same row with different superscript are significantly different (*p* < .01); ^{a, b, c, d, e} means of ETC in the same row with different superscript are significantly different (*p* < .05).

Table 3. Correlation coefficient (r_s) between ETC and variables included in principal component analysis (PCA).

	Parity	Breed	DIM	FP	Particle size	CP	EE	ADL	NFC	NDFd
ETC _t	-.18**	-.02	-.06	.30**	.44**	-.48**	-.46**	.54**	-.62**	-.59**
ETC _{HF}	-.30**	-	.01	.32**	.52**	-.48**	-.45**	.51**	-.52**	-.52**
ETC _{tIS}	-.13**	-	-.08	.27**	.42**	-.54**	-.58**	.57**	-.65**	-.67**

DIM = days in milk; FP = number of feed pushing per day; CP = crude protein (%DM); EE = ether extract (%DM); ADL = acid detergent lignin (%DM); NFC = nonfiber carbohydrates (%DM); NDFd = neutral detergent fiber degradability (%NDF); ETC = eating time class; Parity 1 = primiparous; Parity 2 = multiparous (2 parities); Parity 3 = multiparous (>2 parities); breed: Holstein Friesian (HF), 1; Italian Simmental (IS),

** : $p < .01$.

Discussion

Changes in diet composition and milk production traits are explored in relation to variations in ET of dairy cows on commercial farms, accounting for the contributions of the two breeds considered in the study.

Eating time and diet composition

The relationship between diet composition and DMI in dairy cows has been extensively studied over the years and dietary NDF has been widely recognized as one of the dietary components most likely to influence DMI (Mertens 1997; Allen 2000). Instead, much less information is available on the effect of diet on feeding time. Beauchemin (2018), in an extensive review of cow feeding activity, summarizes that the main drivers of chewing time are the physical and chemical characteristics of the diet (particle size, moisture, and fibre contents). In particular, the author states that ET and forage NDF intake have an overall positive relationship but with considerable dispersion due to several other factors influencing the feeding behaviour of lactating dairy cows.

This indirectly occurred also in this investigation: the lowest ET classes were associated in the PCA analysis positively with NFC and negatively with ADL, and these latter dietary components were highly correlated with NDF ($r = -0.96$ and 0.83 , respectively). NDFd, on the other hand, had a negative correlation with ETC, as did dietary chemical characteristics that often rise with compound

feed consumption (such as NFC, EE, and CP). Several studies have demonstrated the positive impact of fiber digestibility (NDFd) on the performance of dairy cows (Dado and Allen 1994; Oba and Allen 1999), mainly due to a high disappearance and passage rate in the rumen, while there are no studies on its effect on ET. The negative relationship between ETC and NDFd found in our experiment suggests a low necessity for cows to intensively masticate a more degradable fiber. Conversely, high levels of poorly degradable fibre decrease rumen transit time, accelerate rumen filling (Stergiadis et al. 2015), and could increase ET.

Beauchemin (2018), in the previously cited review, states that increasing silage particle size augments ET, but this relationship appears to be highly variable across studies. Particle size affects daily DMI, dietary digestibility, and MY not only in maize silage-based diets but also in other forage-based diets (Haselmann et al. 2019). In general, excessively long particle size increases ET because cows require longer mastication to swallow the feed bolus (Grant and Ferraretto 2018). According to this, Kononoff et al. (2003) found an increased daily ET of 36 min/d as silage particle size increased, as well ET was reduced by 43 min/d for cows fed the finer chopped silage (Fernandez and Michalet-Doreau 2002). Particle size also has an effect on RT, with a considerable drop when the particle size is very tiny (< 5 mm) (Nasrollahi et al. 2015). The results of our study have confirmed that the increase in particle size was correlated with ETC ($r = 0.44$; $p < .01$; Table 3). In the farms involved in this study, we found different dietary strategies (both silage- and hay-based diets), and particle size was related to dietary fibre content expressed as NDF ($r = 0.64$; $p < .01$; data not reported in Table). Jiang et al. (2017) observed a 1.8 h/d greater ET when dietary forage content increased from 40 to 70%, but RT only increased by 35 min/d.

In general, eating activity increases throughout the day when there is frequent mobilization of feed in the bunk (feed delivery or feed pushing). The delivery of fresh feed has been demonstrated to be a strong stimulus to initiate feeding activity, so frequent distribution throughout the day exhibits more desirable feeding patterns to support production and rumen health (Hart et al. 2014). DeVries et al. (2005) tested one, two, and four deliveries of feed per day and found an increase in the daily feeding

time with increasing frequency of feed provision (+10 and +14 min/d, respectively for two and four deliveries). It is generally believed that dividing the total daily meal into several portions over 24 hours allows ruminal homeostasis, which has advantages in terms of MY and animal welfare. Instead, Benchaar and Hassanat (2020) argue that changing the daily frequency of feed delivery from one to two to four times does not lead to an increase in DMI, milk production, or even in the digestibility of the nutrients. In our study, the frequency of feed pushing contributed only a small part, but highly significant, to ET ($r = 0.30$; $p < .01$; Table 3).

Eating time and milk production

As the ETC decreases, there appears to be a gradual increase in the DIM. Indeed, DIM was weakly related to ETC-1 and 2, along with the second principal component (Figure 1 a, b), probably due to the reduction of milk production as lactation progresses and, consequently, to the reduction of nutritional requirements. Cows with more than two lactations were associated with lower ETCs. This is also reflected in Table 3, where a low but significant correlation of parity with ETC is reported for both breeds ($r = -0.30$ for HF and $r = -0.13$ for IS, $p < .01$). It is known that multiparous cows spend less time feeding and have a generally higher DMI than primiparous cows (Azizi et al. 2009). This is due to differences in body weight, which, in addition to influencing rumen capacity and meal size, also influence bolus size (Dado and Allen 1994; Aikman et al. 2008).

The positive relationship between DMI and MY is expected and well demonstrated (Dado and Allen 1994; Ben Meir et al. 2019) while more controversial is that with ET. In fact, the same studies report rather weak or absent correlations between milk and ET, although we found a positive relationship (Table 2). In a study comparing two groups with high and low MY (Azizi et al. 2009), no significant difference was found in terms of ET, but the more productive cows had an additional 11% of feeding rate than the other group. However, Dado and Allen (1994) reported a negative correlation of 0.64 between milk production and feeding rate (expressed in min/kg of DM). The aforementioned publications reported experimental data obtained in a controlled environment; our study considered

cows on commercial farms with differences between and within herds. Other factors that may have conditioned our results were the PMR feeding technique (high-yielding dairy cows receive more feed from AMS) and the diverse levels of competition at the feed bunk (competitive situations increase the feeding rate).

The group of cows with an ET of less than 3 h/d was essentially made up of low MY cows, where even the little compound feed amount offered by AMS combined with the PMR exceeded their actual nutritional requirements (both energy and protein). This translates into a higher concentration of urea in the milk (an indicator of protein nutrition) and a better BCS (energy nutrition). Another possible interpretation of this result was related to ruminal activity in animals with different feeding behaviours. During the digestion of feeds in the rumen, proteins are partially degraded by bacteria and protozoa into amino acids, which are then deaminated to ammonia. The liver synthesizes urea from ammonia absorbed in the rumen, which is subsequently released into the blood, where it is subject to clearance in several ways and, to a minimal extent, excretion in milk. Therefore, a longer RT favours bacteria growth and digestion with increased bacterial protein synthesis, which leads to a reduction in urea production and subsequent elimination through milk (Beauchemin 2018). This was little reflected in our results, in which animals with extreme values of RT had only slightly higher values of milk urea concentration (23.58 mg/dL with 520.9 min/d vs. 22.65 mg/dL with 536.1 min/d, $p < .01$).

The SCC result is more difficult to interpret. It is clear that in the high ETC, there were cows with a high MY, and among these, there was a lower concentration of SCC in milk, as observed also by Fogsgaard et al. (2012). In fact, the data proposed in this study confirm that the increase in SCC causes a reduction in the synthesis capacity of the mammary gland. However, there does not appear to be a direct effect of mastitis on feeding behaviour. Few studies were performed to probe a relationship between acute or clinical mastitis and changes in animals' behaviour and assess protocols for early detection of disease. In the study by Fogsgaard et al. (2012), the effect of mastitis on behaviour was investigated, and ET and RT decreased from 24 to 48 hours after infection. This was

probably due to a switch of energy from normal metabolism activities to the immune system to overcome infection. A similar result was found in the study of González et al. (2008), which concluded that feeding time could be a useful early indicator of mastitis if related to other variables such as MY and milk conductivity, which are easily available thanks to the data collected by AMS.

Breed effects on body conditions, udder health, and feeding behaviour traits

The lower BCS of the HF cows was an expected result, given the higher MY of this breed compared to IS (30.6 vs. 28.4 kg/d, $p < .01$; Table 2). In fact, the more intensive selection programs favouring only milk production increase the animal's nutritional needs, which are only partly supplied by feed intake increments. This agrees with two studies (Walsh et al. 2008; Ferris et al. 2014) that both compared a dairy breed (HF) with a dual-purpose breed (Norwegian Red) and found that HF cows had a significantly lower BCS during all lactation ($p < .001$). Another aspect affected by stronger selection for MY in HF is the resilience of the animal, in particular the udder health of dairy cows. Some studies have investigated the different susceptibilities of breeds to the insurgency of mastitis. For example, a study explored potential differences in udder health between breeds on commercial dairy farms and found that HF cows had a higher MY, but udder health was significantly better for dual-purpose cows (Norwegian Red). Furthermore, the proportion of cows that developed mastitis was lower for dual-purpose breeds compared with dairy ones (6% vs. 11.9%, $p < .05$; Begley et al., 2009). Ferris et al. (2014) discovered that HF and Norwegian Red cows had SCC levels of 282 and 176 x 1,000/mL, respectively, which were fairly similar to our data, indicating that the HF breed has a high susceptibility to mastitis.

As regards differences between the feeding behaviours of HF and IS, we found that the two breeds differed in terms of ETC and RT, with a longer time for HF than IS. In the literature, some studies evaluated feeding behaviour in different breeds and found little or no significant differences in terms of total daily ET but differences in terms of eating rate (Aikman et al. 2008; Olson et al. 2019; Romanzin et al. 2022). This suggests the possibility that breeds with increased nutrient requirements

(like highly productive dairy breeds) may compensate, at least partially, for different ingestion quantities by increasing the amount of feed ingested per unit of time. Other studies found substantial variations in feeding behaviour between HF and other breeds (especially Jersey), with the first spending more time eating than the latter (Munksgaard et al. 2020; Gündel et al. 2022). These results agree with those found in our study, where IS cows ate significantly less than HF (about 27 min/d; Table 2) and are associated with the lowest ETC (Figure 1b). This is explained by the higher milk production of HF. Finally, considering RT, Aikman et al. (2008) found that HF animals ruminated longer than Jersey ones (623 vs. 538 min/d; $p < .05$), despite no significant differences in terms of ET per day.

Conclusions

Our results revealed that ETC is well related to aspects concerning diet characteristics, the performances of cows, and almost the health status of the animals. In particular, fibrous and energy content can significantly change the daily duration of the ET, as well as different kinds of ration management. Furthermore, ET and milk performance condition each other, in fact, cows with the highest ETC perform better than others with less ETC. Breed could also define different eating patterns. The study provides preliminary results for future research to better define the role of ET in the causation of the overall feed efficiency and health status of cows. The potential perspective could be using the ET as a useful tool to rank lactating cows within herds for selective and/or management purposes.

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Disclosure statement

The authors declare no real or perceived competing interests.

Ethical statement

All research reported in this study has been conducted in an ethical and responsible manner and is in full compliance with all relevant codes of experimentation and legislation.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CHAPTER IV

Whole lactation feeding behaviour of Simmental cows in commercial dairy farms

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ABSTRACT

Understanding the changes that occur in dairy cows during lactation may be important to better manage the herd and optimise production. Today, thanks to the spread of new technologies in commercial dairy farms, it is possible to obtain numerous individual data about the animal throughout its productive career. In our study, we investigate the relation between feeding behaviour, parity, and performance in Italian Simmental dairy cows during the whole lactation, using daily data collected with sensors available in commercial farms. The aim was to get a greater understanding of the ways in which lactation induces significant deviations in feeding behaviour as a possible practical implication of this new knowledge. Individual data of milk performance, eating time (ET), rumination time (RT), and body weight (BW) about 80 dairy cows during the whole lactation were collected for 12 months. Animals were divided into two groups considering parity (primiparous, PP, and multiparous, MP) and feeding behaviour classes from low to high values (eating time classes, ETC; rumination time classes, RTC). Data was statistically analysed with a factorial design with animals as repeated measurements. As expected, the positive relation between parity and milk production was found ($p < 0.01$), but no differences were found on milk composition or somatic cell count between the two parity groups ($p > 0.05$). PP cows have greater eating activity (264 vs. 249 min/d, $p < 0.01$) but less estimated dry matter intake than MP cows (21.85 vs. 23.43 kg/d, $p < 0.01$), which meet their requirements with an increased eating rate (105 vs. 89 g/min, $p < 0.01$) and a tendentially higher RT (525 vs. 506 min/d, $p < 0.05$). ETC is positively related to milk composition, as animals in the higher ETC are more productive, especially at the two ends of the lactation curve ($p < 0.01$). An interesting relation between ET and RT was found. Milk production is also positively related to RTC, but surprisingly, no effect was found on milk fat ($p > 0.05$). All this information can be combined and integrated to provide valuable support to the farmer in managing the herd.

Keywords: Eating time – Rumination time – Dairy cows – Commercial farms

Highlights

- Primiparous cows increase their feeding activity after lactation peak.
- Eating time and rumination time are positively related with milk production.
- There is a relationship between eating time and rumination time.
- Rumination time is not related to fat composition of milk.

Introduction

Feeding behaviour is an emerging topic that links behaviour and nutritional sciences. Some recent studies have investigated how feeding behaviour (eating and rumination time) affects the performance, milk quality, health status, and production efficiency of dairy cows (Castaneda et al., 2024; Jurkovich et al., 2024). This scientific interest comes from the fact that different feeding behavioural patterns affect daily nutrients' intake and functionality of the rumen, which is the primary driver of nutrients utilisation in ruminants and makes their digestive processes efficient. Abnormal behaviours can cause ruminal pH imbalances, which have a severe impact on production and animal health (Antanaitis et al., 2024). The effect of rumination time has been extensively explored, and it is often used as a proxy for changes in the health and welfare of the animal, as well as being potentially linked to some milk quality traits (fat composition; Beauchemin, 2018). Otherwise, eating time is less used as an indicator, despite some research suggesting that chemical and physical properties of diets have some influence on it (Florit et al., 2023) and that it is related to milk production (Johnston and DeVries, 2018).

In recent years, remarkable progress has been made in developing innovative technologies known as “precision livestock farming” on commercial farms. The main goals for this development are to direct the intensification of livestock production toward greater productive efficiency and to respond to social requests to improve sustainability and animal welfare. These technologies are aimed at monitoring and sensing the health, physiology, and activity of animals, as well as their feeding behaviour. Milking robots and accelerometers are certainly the most common technologies available

in modern commercial dairy farms, with consolidated use over the years. These technologies allow collecting several individual data concerning milk production, body weight, and feeding behaviour throughout the entire lactation period and provide useful insights to the farmer regarding the presence of nutritional, management, or welfare issues.

It is well known that during lactation a complex evolution of nutritional and physiological conditions occurs in the dairy cow, which has to support milk production, begin and maintain a reproductive cycle, and restore the body reserves. Changes in behaviour could provide relevant information about the nutrition, reproduction, health, and overall well-being of dairy cows (Benaissa et al., 2019). In particular, having continuous data on feeding behaviour allows us to link it to production aspects and obtain useful indicators for better herd management in every single lactation phase. The scientific literature contains limited information about the modification of feeding behaviour of cows measured by accelerometers on commercial farms and on the changes that occur during lactation. In addition, most nutritional research in the dairy industry is conducted on the most specialised and widely distributed dairy breeds in the world (Holstein Friesian), while there is a scarcity of experimental data on dual-purpose breeds. These breeds are gaining popularity due to their potential to support sustainable livestock systems based on a balanced production of meat and milk. The objective of this study was to investigate how feeding behaviour changes during the lactation phases and in relation to the parity and production performance of Simmental cows.

Materials and methods

Data collection

Five commercial dairy farms in the northeast of Italy (Friuli Venezia Giulia region) with a total of 350 Italian Simmental dairy cows were considered for this study. Within these animals, we considered cows that had complete data for the whole lactation ($n = 80$), which were monitored over 12 months (between November 2022 and October 2023). The inclusion criteria for the farms were as follows: all the dairy cows were housed in a cubicle system and milked using an automatic milking system

(AMS) with free and voluntary access to milking; all the AMS had a scale to measure the body weight (BW) at every milking event; all the cows were equipped with a specific sensor capable of detecting feeding behaviour (SenseHub™ Dairy, Allflex® Livestock Intellingence™, SCR Engineers Ltd., Netanya, Israel). Specifically, the sensor was a 3D accelerometer attached on the left side of the animal's neck. Raw data of feeding behaviour were recorded every minute and summed over 2 hours. The average of these values returns the daily feeding behaviour data (min/d).

The management of partial mixed rations (PMR) and compound feed was similar within farms and over the year in terms of ingredients and chemical composition. The farms were visited monthly, during which PMR and compound feed samples were collected. The particle size was assessed by fractionating a fresh sample of PMR with the Penn State Particle Separator (mesh diameter: 19, 8.8, and 1.18 mm) as reported by Kononoff et al. (2003). The dry PMR (obtained in a hoven at 60°C for 48 h) and compound feeds were assayed in duplicate for DM, crude protein (CP; N x 6.25) (AOAC, 2016; methods 930.15, 976.05, and 942.05, respectively) and for the neutral detergent fibre (NDF) content, measured by an Ankom II fibre analyzer (Ankom Technology Corporation, Fairport, NY) following the procedure of Van Soest et al. (1991) without correction for residual ash, and by applying a pre-treatment of samples with α -amylase. The PMR, provided *ad libitum*, was primarily based on fibrous ingredients (such as whole plant corn or sorghum silage and alfalfa hay) and compound feed consisting mainly of cereal flours, corn and barley, and soybean meal (DM = 55.8 ± 2.2%, NDF = 47.4 ± 4.4% DM, CP = 13.4 ± 0.7% DM; particle size = 7.78 ± 1.56 mm). The pellet compound feed offered in the AMS during milking has a similar chemical composition within the farms (DM = 88.8 ± 0.8 %, NDF = 24.6 ± 2.3 % DM; CP = 20.0 ± 1.5 % DM), and the quantity administered individually to cows was based on expected individual milk yield.

During the monthly visits, individual data has also been collected. Data including performance data (days in milk (DIM), lactation number, milk yield (MY), milk composition (fat, protein, and lactose), and somatic cell count (SCC), as well as feeding behaviour such as eating time (ET) and rumination time (RT), and BW were extracted from the AMS computer (Horizon management software, Lely

Industries, Maassluis, the Netherlands) for the 30 days preceding each visit. On the day of the visit, all cows were assessed for body condition score (BCS) by a trained expert, using the method described by Edmonson et al. (1989), with a 5-point scale and scores recorded in increments of 0.25 points.

Data calculations

Fat- and protein-corrected milk (FPCM) was calculated using the following equation (Kok et al., 2016):

$$\begin{aligned}
 \text{FPCM (kg/d)} &= \text{milk yield (kg/d)} \times [0.337 + 0.116 \times \text{fat content (\%)} \\
 &+ 0.06 \times \text{protein content (\%)}]
 \end{aligned}$$

Estimated dry matter intake (eDMI) was calculated using the equation derived from the NRC 2021 (equation 2-1) (see Supplementary Material):

$$\begin{aligned}
 \text{eDMI (kg/d)} &= [3.7 + (\text{Parity} \times 5.7) + 0.305 \times \text{Milke (Mcal/d)} + 0.022 \times \text{BW (kg)} \\
 &+ (-0.689 - 1.87 \times \text{Parity}) \times \text{BCS}] \times [1 - (0.212 \\
 &+ \text{Parity} \times 0.136) \times e^{(-0.053 \times \text{DIM})}]
 \end{aligned}$$

where parity is a factor ranging from 0 (primiparous cows) to 1 (multiparous cows), BCS ranging from 1 to 5 points, and the daily energy milk yield (Milke, Mcal/d) was calculated using the equation 3-14a from NRC 2021 modified to obtain daily data:

$$\begin{aligned}
 \text{Milke (Mcal/d)} &= (9.29 \times \text{kg fat/kg milk} + 5.5 \times \text{kg crude protein/kg milk} \\
 &+ 3.95 \times \text{kg lactose/kg milk}) \times \text{milk yield (kg/d)}
 \end{aligned}$$

The eating rate (ER, g DM/min) was calculated by dividing eDMI by ET.

To determine individual changes in BW, weekly means of BW were computed using daily measures for each animal. Daily change in BW (Δ BW) was calculated by the difference between the last and

the first measure of a week, divided by the number of days between measurements. Then ΔBW has been reported as a percentage of BW.

We calculated the energy requirements for lactation (NEL_t) following the NRC 2021 equations, considering net energy for lactation (NEL) as a basic unit. In particular, NEL maintenance requirements were calculated using Equation 3-13 as follows:

$$NEL_{maint} (Mcal/d) = 0.10 \times BW (kg)^{0.75}$$

The NEL concentration in milk is equivalent to the sum of the heats of combustion of individual milk components and was calculated using the same equation previously described, modified to obtain daily data (Milke; Equation 3-14a). The sum of NEL_{maint} and Milke is the total daily energy requirements of cows.

Statistical analysis

All the statistical analysis were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The normality of data distribution was checked using the UNIVARIATE procedure. Outlier removal for feeding behaviour traits was performed as described in detail in Florit et al. (2023).

Each lactation phase (n = 10) was defined every 30 DIM starting from the calving day (DIM = 0).

To analyse the association between parity group and phase of lactation, animals were divided into two groups, primiparous (PP; n = 25) and multiparous (MP; n = 55).

For the analysis of the association between feeding behaviour and phase of lactation, cows were distributed in three classes of ET length (ETC) and RT length (RTC) according to Codl et al. (2023).

Daily data including ET and RT were divided based on their arithmetic mean and standard deviation:

Low class (L-ETC and L-RTC) included values lower than $\bar{x} - 1/2$ SD; Medium class included values between $\bar{x} - 1/2$ SD and $\bar{x} + 1/2$ SD (M-ETC and M-RTC); High class included values higher than $\bar{x} + 1/2$ SD (H-ETC and H-RTC).

The data about feeding behaviour (ET and RT), feed intake, energy requirements, milk performance and animal characteristics were analysed according to a factorial design using the MIXED procedure considering animal as repeated measurement. The following model was used:

$$Y_{ijkl} = \mu + \alpha_i + (\beta_j)_{i+} + \gamma_k + \delta_l + \gamma\delta_{kl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the experimental data, μ is the overall mean, α_i is the random effect of the farm (block, $i=1,5$), β_j is the random effect of cows within farm ($j_i=1,80$), γ_k is the effect of lactation phases ($k=1,10$), δ_l is the fixed effect of parity ($l=1,2$), and ε_{ijkl} is the random error. The LSMEANS were adjusted with the Tukey-Kramer for multiple comparisons.

The analysis of the association between ETC and RTC and phase of lactation were performed with the above-described model where the parity factor was substituted with the fixed effect of ETC ($l=1,3$) and RTC ($l=1,3$).

The statistical significance was declared at $p \leq 0.01$ and $p \leq 0.05$.

Results

Table 1 and **Table 2** show the effect of parity and lactation phase on milk performance, BW, eDMI, and feeding behaviour (ET, RT, and ER) of dairy cows. Within both parity classes, MY increased significantly from the first to the subsequent lactation phase and then was stable till 121-150 (or 61-90 in MP) DIM before declining, reaching a low point after 241 DIM. A similar trend was found with FPCM. The MY of PP was lower ($p \leq 0.01$) than MP over the first six months of lactation. Lactation phase significantly affected milk composition, SCC, and BW variations ($p \leq 0.01$). Milk traits (fat, protein, fat: protein ratio, and SCC) showed a usual pattern, with a decrease from the first to the subsequent lactation phases, followed by low values and a regrowth at the conclusion of lactation. BW increased during lactation, and there was a significant difference due to parity (681 vs 761 kg for PP and MP, respectively; $p \leq 0.01$). During the beginning of lactation, there was an intense mobilisation of BW, while thereafter BW incremented.

For both parity classes, the lowest ET was detected at the start of lactation, followed by an increase and rather consistent values (from 61 to 210 for PP and 31 to 210 for MP), with a tendency to drop in the latter lactation stages, where the PP cows showed values higher than MP (between 211 and 270 DIM). RT had the lowest value during the first 30 DIM, then remained consistent until 91-120 DIM and grew somewhat in subsequent lactation phases. PP cows had a slightly shorter RT than MP cows (506 vs 525 min, $p \leq 0.05$). Within both parity classes, eDMI increased from the first to the second lactation phase, then stabilised in PP cows but declined in MP cows. Also, the NEL needs increased from the first to the subsequent lactation periods, remained steady from 31 to 150 DIM, and subsequently decreased. PP consumed less DM and had lower NEL needs than MP cows ($p \leq 0.01$) for the first six months of lactation.

Table 1. The effect of parity and lactation phases on milk performance, BW and BW changes.

Item	Parity	DIM (d)										Avg.	RMSE	p-value		
		0-30	31-60	61-90	91-120	121-150	151-180	181-210	211-240	241-270	271-305			Parity	DIM	Parity*DIM
MY (kg/d)	PP	22.87 ^{CY}	29.18 ^{AY}	29.32 ^{AY}	28.28 ^{ABY}	27.19 ^{ABY}	25.79 ^{BCY}	25.38 ^{BC}	24.19 ^{BC}	21.61 ^{CD}	17.43 ^D	25.12	3.711	< 0.01	< 0.01	< 0.01
	MP	32.67 ^{CX}	38.05 ^{AX}	36.29 ^{ABX}	33.98 ^{BCX}	32.09 ^{CX}	29.82 ^{DX}	27.40 ^D	24.45 ^E	20.67 ^F	18.27 ^F	29.37				
	Avg.	27.77	33.62	32.80	31.13	29.64	27.80	26.39	24.32	21.14	17.85	-				
FPCM (kg/d)	PP	22.94 ^{BY}	27.92 ^{AY}	28.55 ^{AY}	28.14 ^{AY}	27.60 ^{AY}	26.69 ^{AB}	26.52 ^{AB}	25.22 ^{AB}	22.79 ^{BC}	18.83 ^C	25.52	4.068	< 0.01	< 0.01	< 0.01
	MP	32.19 ^{BX}	35.92 ^{AX}	34.90 ^{ABX}	33.58 ^{ABX}	32.29 ^{BX}	30.36 ^{BC}	28.36 ^{CD}	25.57 ^D	21.82 ^E	19.49 ^E	29.45				
	Avg.	27.56	31.92	31.72	30.86	29.94	28.52	27.44	25.39	22.30	19.16	-				
Milk fat (%)	PP	4.24	3.62	3.70	3.86	4.04	4.23	4.29	4.26	4.36	4.64	4.12	0.331	0.85	< 0.01	0.54
	MP	4.13	3.67	3.74	3.88	3.99	4.07	4.21	4.31	4.40	4.60	4.10				
	Avg.	4.19 ^{BCD}	3.65 ^G	3.72 ^{FG}	3.87 ^{EF}	4.01 ^{DE}	4.15 ^{CD}	4.25 ^{BC}	4.28 ^{BC}	4.38 ^B	4.62 ^A	-				
Milk protein (%)	PP	3.94	3.48	3.50	3.54	3.58	3.59	3.60	3.62	3.64	3.58	3.61	0.176	0.28	< 0.01	0.53
	MP	3.82	3.48	3.48	3.48	3.51	3.54	3.56	3.58	3.65	3.61	3.57				
	Avg.	3.88 ^A	3.48 ^D	3.49 ^{CD}	3.51 ^{CD}	3.54 ^{BCD}	3.57 ^{BCD}	3.58 ^{BCD}	3.60 ^{BC}	3.65 ^B	3.60 ^{BC}	-				
SCC ($\times 10^3$ /mL)	PP	225	64	63	82	87	151	134	85	109	186	119	189.0	0.14	< 0.01	0.21
	MP	218	125	99	142	159	126	119	182	255	208	163				
	Avg.	221 ^A	95 ^B	81 ^B	112 ^{AB}	123 ^{AB}	139 ^{AB}	126 ^{AB}	134 ^{AB}	182 ^{AB}	197 ^{AB}	-				
Fat: Protein	PP	1.08	1.05	1.06	1.09	1.13	1.18	1.20	1.18	1.21	1.30	1.15	0.103	0.92	< 0.01	0.82
	MP	1.10	1.06	1.07	1.12	1.14	1.15	1.18	1.21	1.22	1.29	1.15				
	Avg.	1.09 ^{EF}	1.05 ^F	1.07 ^F	1.11 ^{DEF}	1.14 ^{CDE}	1.17 ^{BCD}	1.19 ^{BC}	1.20 ^{BC}	1.22 ^B	1.29 ^A	-				
BW (kg)	PP	660	649	661	671	674	677	688	698	712	721	681 ^Y	31.7	< 0.01	< 0.01	0.89
	MP	739	727	751	756	759	759	765	774	785	793	761 ^X				
	Avg.	700 ^{DE}	688 ^E	706 ^{DE}	714 ^{CD}	716 ^{BCD}	718 ^{BCD}	727 ^{BC}	736 ^{AB}	749 ^A	757 ^A	-				
Δ BW (%BW)	PP	-0.18	0.02	0.04	0.04	-0.01	0.03	0.04	0.02	0.06	0.00	0.01	0.204	0.86	< 0.01	0.69
	MP	-0.13	0.00	0.07	0.02	-0.05	0.04	0.03	0.05	-0.02	0.05	0.01				
	Avg.	-0.16 ^B	0.01 ^A	0.05 ^A	0.03 ^A	-0.02 ^A	0.03 ^A	0.03 ^A	0.04 ^A	0.02 ^A	0.02 ^A	-				

Parity: PP = primiparous; MP = multiparous; DIM = days in milk; RMSE = residual mean square error; MY = milk yield; FPCM = fat- protein corrected milk; SCC = somatic cell count; BW = body weight; Δ BW = daily BW variation in percentage of animal BW.

Within each lactation phase different letters show statistical differences between parity groups at a $p < 0.01$ (X, Y) and at a $p < 0.05$ (x, y).

Within each parity group different letters show statistical differences between lactation phases at a $p < 0.01$ (A, G) and at a $p < 0.05$ (a, g).

Table 2. The effect of parity and lactation phases on feeding behaviour parameters, feed intake, and dietary energy requirements.

Item	Parity	DIM (d)										Avg.	RMSE	p -value		
		0-30	31-60	61-90	91-120	121-150	151-180	181-210	211-240	241-270	271-305			Parity	DIM	Parity*DIM
ET (min/d)	PP	192 ^D	240 ^C	271 ^A	283 ^{AB}	291 ^A	279 ^A	284 ^A	280 ^{ABX}	271 ^{AX}	254 ^{BC}	264	29.8	0.09	< 0.01	< 0.01
	MP	220 ^C	250 ^{AB}	255 ^A	261 ^A	266 ^A	259 ^A	257 ^A	246 ^{AY}	236 ^{BCY}	236 ^{BC}	249				
	Avg.	206	245	263	272	278	269	271	263	254	245	-				
RT (min/d)	PP	471	491	502	500	509	514	524	524	517	508	506 ^y	30.9	0.02	< 0.01	0.07
	MP	510	526	517	514	516	530	538	539	530	526	525 ^x				
	Avg.	491 ^C	508 ^{BC}	509 ^{BC}	507 ^{BC}	512 ^{AB}	522 ^{AB}	531 ^A	532 ^A	524 ^{AB}	517 ^{AB}	-				
ER (g DM/min)	PP	111 ^A	97 ^{ABCD}	86 ^{BCDY}	84 ^{BCDY}	82 ^{DY}	86 ^{BCDY}	82 ^{CDY}	82 ^{CDY}	85 ^{BCDY}	91 ^{BCD}	89	16.3	< 0.01	< 0.01	< 0.01
	MP	105	106	108 ^X	106 ^X	102 ^X	103 ^X	103 ^X	105 ^X	109 ^X	102	105				
	Avg.	108	102	97	95	92	95	93	93	97	97	-				
eDMI (kg/d)	PP	18.90 ^{BY}	21.50 ^{AY}	22.25 ^{AY}	22.52 ^{AY}	22.45 ^{AY}	22.27 ^{AY}	22.54 ^A	22.44 ^A	22.17 ^A	21.49 ^A	21.85	1.150	< 0.01	< 0.01	< 0.01
	MP	20.55 ^{FX}	24.28 ^{BX}	25.20 ^{AX}	24.65 ^{ABX}	24.38 ^{ABCX}	23.87 ^{BCDX}	23.64 ^C	23.03 ^{DE}	22.54 ^E	22.12 ^E	23.43				
	Avg.	19.72	22.89	23.73	23.59	23.42	23.07	23.09	22.73	22.36	21.80	-				
NELt (Mcal/d)	PP	30.98 ^{BY}	33.95 ^{AY}	34.31 ^{AY}	34.05 ^{AY}	33.70 ^{AY}	33.94 ^{AB}	32.96 ^{AB}	31.90 ^{AB}	30.16 ^{BC}	27.26 ^C	32.22	2.852	< 0.01	< 0.01	< 0.01
	MP	38.22 ^{ABCX}	40.40 ^{AX}	39.32 ^{ABX}	37.92 ^{BX}	36.98 ^{CDX}	35.59 ^{DE}	34.05 ^{EF}	31.95 ^F	29.12 ^G	27.65 ^G	35.12				
	Avg.	34.60	37.17	36.81	35.99	35.34	34.27	33.50	31.93	29.64	27.45	-				

Parity: PP = primiparous; MP = multiparous; DIM = days in milk; RMSE=residual mean square error; ET = eating time; RT = rumination time; ER = eating rate; eDMI = estimated dry matter intake; NELt = total daily net energy requirements (maintenance and lactation).

Within each lactation phase different letters show statistical differences between parity groups at a $p < 0.01$ (X, Y) and at a $p < 0.05$ (x, y).

Within each parity group different letters show statistical differences between lactation phases at a $p < 0.01$ (A, G) and at a $p < 0.05$ (a, g).

Considering that the trends and differences along the lactation phases were already discussed previously by description of results in **Tables 1** and **2**, in the subsequent presentation of results, we focused only on the most interesting effects of ETC and RTC (**Figure 1** and **Figure 2**). MY increased significantly ($p \leq 0.01$) accordingly with ETC (**Figure 1**) both at the start (0-30 DIM), and at the end of lactation (241-305 DIM). Cows who ate more time during the day also ruminated more (504, 519, and 530 RT, $p \leq 0.01$ for L-ETC, M-ETC, and H-ETC, respectively; **Figure 1**). Cows in the L-ETC exhibited the highest ER value compared to medium, and high ETC throughout the lactation cycle (**Figure 1**). For eDMI and NEL requirements, the sole statistical difference across ETC occurred in the final phase of lactation, whereas both variables increased from the first to second lactation phase, peaked between 60 and 120 DIM, and subsequently fell (data not reported).

MY and FPCM increased significantly ($p \leq 0.05$, **Figure 2**) with RTC only in the second lactation phase (31-60 DIM). Cows who ruminated more time throughout the day had longer ET (260 and 255 vs 244 min, $p \leq 0.01$ for H-RTC and M-RTC vs. L-RTC; **Figure 2**). Cows in the L-RTC had the greatest ER value compared to M-RTC and H-RTC throughout the lactation cycle (105 vs 98 min, $p \leq 0.01$, data not shown in table). Within each RTC, eDMI increased from the first to the second lactation phase, then declined. Only at lactation phases corresponding to 31-90 DIM, the M-RTC and H-RTC had higher eDMI than L-RTC cows (data not shown in table).

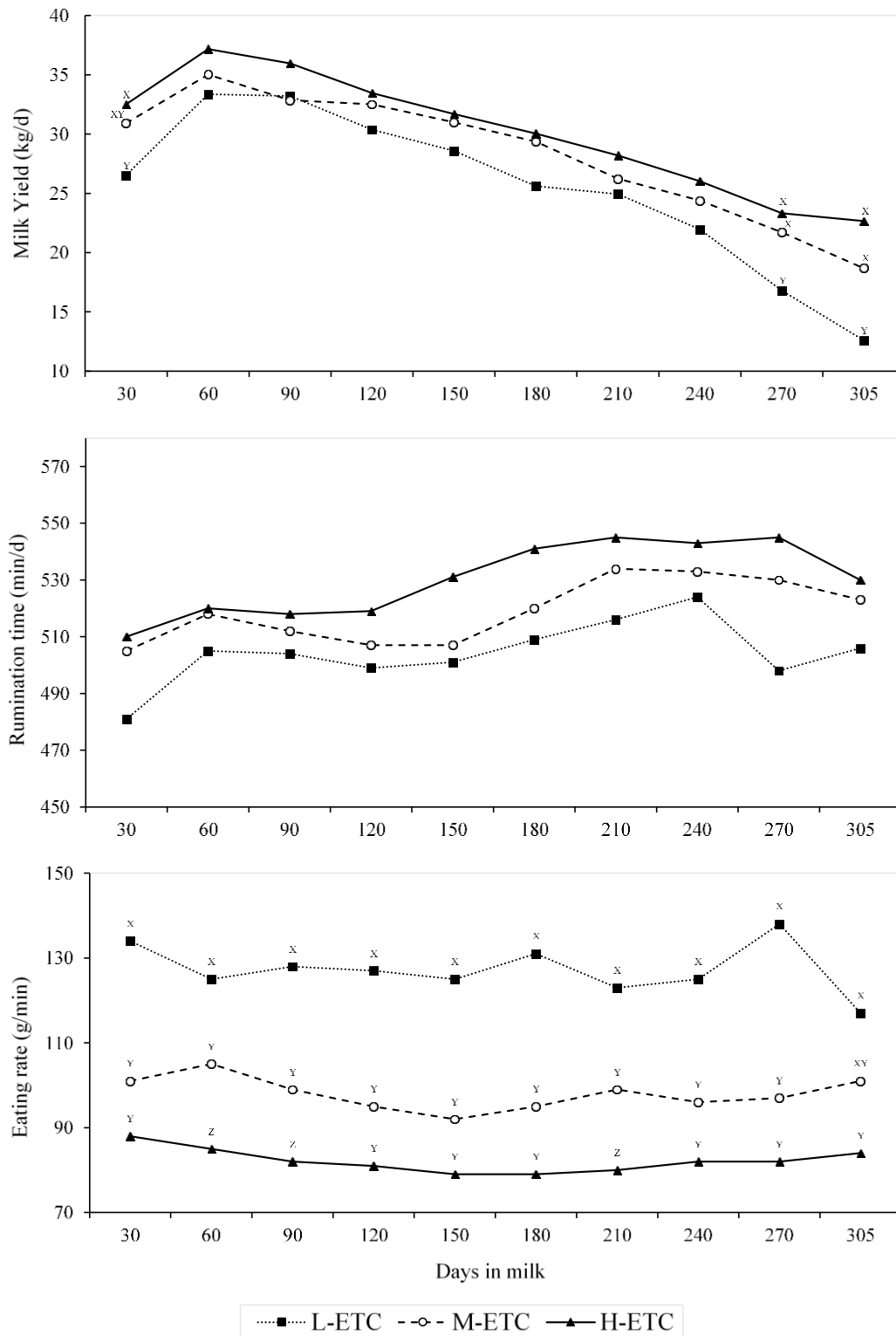


Fig. 1. Milk yield, rumination time and eating rate of cows in different eating time classes (ETC: L-ETC = animals with values of eating time $< \bar{x} - \frac{1}{2} SD$; M-ETC = animals with values of eating time between $\bar{x} - \frac{1}{2} SD$ to $\bar{x} + \frac{1}{2} SD$; H-ETC = animals with values of eating time $> \bar{x} + \frac{1}{2} SD$) during the whole-lactation. ETC bearing different capital letters differ at $p \leq 0.01$ and lowercase letters differ at $p \leq 0.05$.

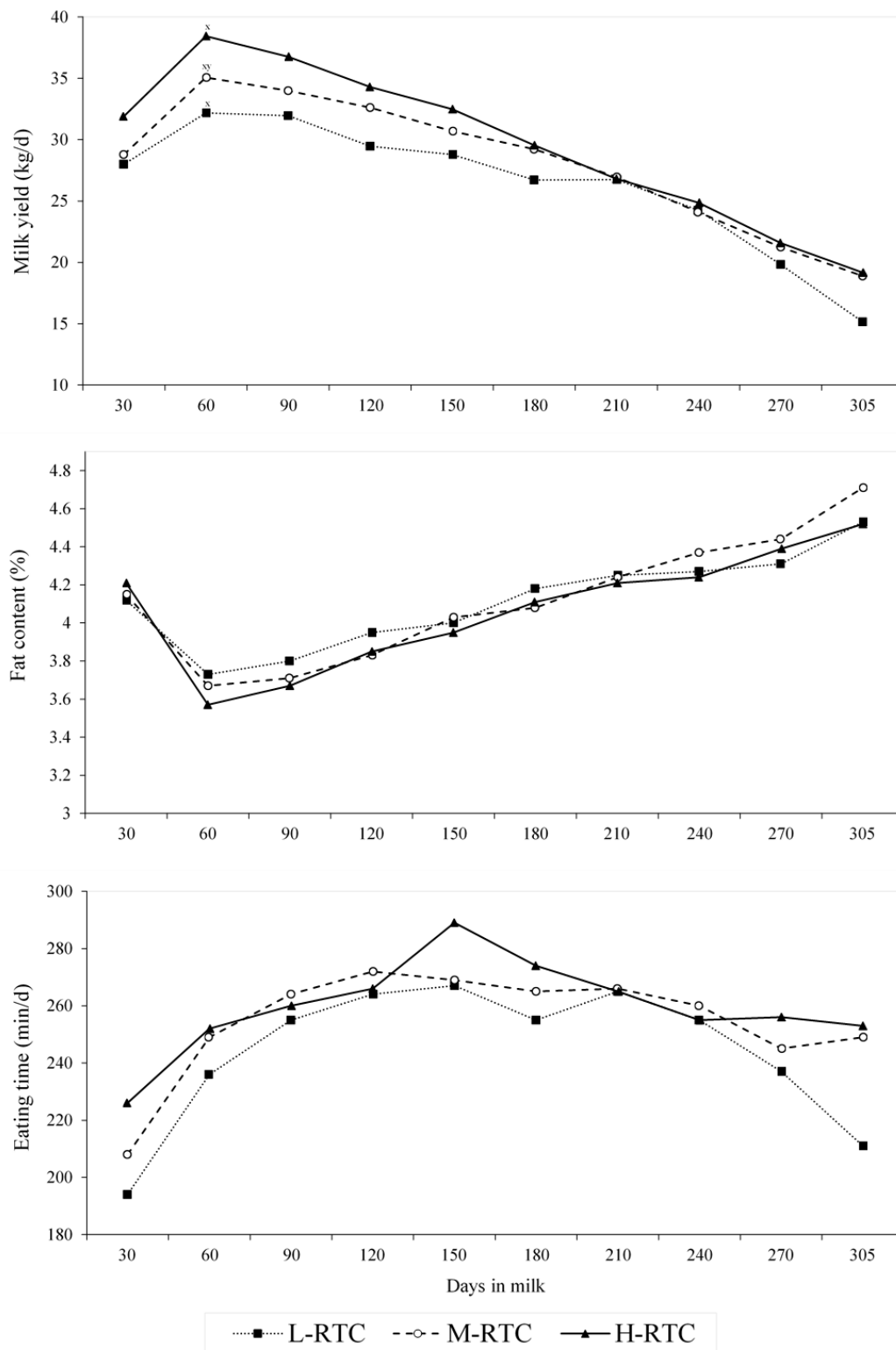


Fig. 2. Milk yield, fat content in milk and eating time of cows in different ruminant time classes (RTC: L-RTC = animals with values of rumination time $< \bar{x} - \frac{1}{2} SD$; M-RTC = animals with values of rumination time between $\bar{x} - \frac{1}{2} SD$ to $\bar{x} + \frac{1}{2} SD$; H-RTC = animals with values of rumination time $> \bar{x} + \frac{1}{2} SD$) during the whole-lactation. RTC bearing different capital letters differ at $p \leq 0.01$ and lowercase letters differ at $p \leq 0.05$.

Discussion

The interest in studying the mechanisms related to feeding behaviour in dairy cows has increased significantly, especially thanks to the spread of sensors in commercial farms useful for measuring individual data every day. Nonetheless, studies on feeding behaviour are mainly focused on specific stages of lactation, particularly those most critical for the animal (transition period or early lactation; Sadiq et al., 2024), where it can be a useful indicator of health and wellness status. Otherwise, they rarely consider differences between different phases in the whole lactation, as was done in the present survey.

Parity and performance traits

The milk yield of dairy cows in this survey is estimated to be 75-90t for the whole lactation, which is comparable to the average Italian Simmental milk yield (AIA, 2023) and 25% lower than the most widespread specialised breed in Italy (Holstein Friesian) (AIA, 2023). However, our study shows that Simmental cows perform better in terms of fat and protein content in milk (4.11% and 3.59%, respectively) than Holstein Friesian (3.85% fat and 3.35% protein; AIA, 2023). Despite these differences in milk yield, the trend of milk production and the other related traits (such as BW, SCC, and milk components) follow the typical shape found in specialised breeds.

The DMI was estimated using daily collected individual data (see Supplementary Material) and is consistent with the known research on Simmental cows (Stauder et al., 2020). The initial increase of feed intake (during the first 90 DIM) is related to the growth of milk production, which leads the animal to be in a phase of negative energy balance due to a consistent growth of nutritional needs (Dahl, 2020). This explains our findings where high milk production and energy requirements are associated with a decrease of BW (especially in the first times after calving) (**Figure 3**; see Supplementary Material).

The available literature is rich of studies regarding the positive relationship between parity and increased milk production (Brown et al., 2022; Munksgaard et al., 2020). MP cows tend to have better

performance and improved physiological adaptations to lactation requirements compared to PP cows, as also indicated by our results (**Table 1**). As expected, there is an effect of the lactation phase on SCC, as it is known that the number of cells gradually increases, especially in the last times before the dry-off period (Sharma et al., 2011), and the higher values in the first phase of lactation might be the result of the mammary structure's requirement to rebuild its productive functionality following the dry period, which causes a notable loss of epithelial cells in a small amount of milk (Schalm et al., 1971). On the other hand, an effect of parity on SCC is also expected. Previous studies support the knowledge that parity is one of the main risk factors for mastitis, as with age the physiological and anatomical defense mechanisms can deteriorate (Jamali et al., 2018). In this study, MP cows have numerically higher values of SCC, but do not reach the significance given the known variability of this trait (**Table 1**).

Feeding behaviour traits and parity

Voluntary feed intake of dairy cows is regulated by several factors, including the stage of lactation, the size of the animal (which in turn depends on breed and age), the social interactions (hierarchy and competition), and the management and composition of the ration (in terms of number of distributions, particle length, and fibre content) (Grant and Albright, 2000). In addition, feeding behaviour plays a role within these mechanisms, but the knowledge regarding the relationship between DMI and ET is still conflicting (De Mol et al., 2016; Pahl et al., 2016; Azizi et al., 2009). Despite the lower milk production and nutritional requirements of PP cows, they numerically ate longer, but less quantity, than the MP cows (**Table 2**), in agreement with the findings of Codl et al. (2020), Stauder et al. (2020), and Azizi et al. (2009). This may be related to their reduced intake capacity (in terms of digestive volume), or, as supposed by Jaeger et al. (2019), MP cows have adapted their behavioural pattern with experience and managing to eat more by reducing their total ET (4.5% less per day). Another possible explanation is related to the social organisation of the herd. Typically, in commercial farms, cows are raised in mixed groups (PP and MP in the same barn), and the effect of hierarchy may lead

PP cows to eat for a shorter time because they have to compete with older, bigger, and more dominant cows. Probably, in our study, the *ad libitum* feed management and the free milking with AMS mitigate this effect, resulting in behavioural patterns similar to those seen in PP and MP cows raised apart (Grant and Albright, 2001).

The significantly higher ER (in 7 lactation stages) (**Table 2**) in MP cows could be explained by the fact that older cows have a greater feed prehension thanks to their larger size (therefore size of the mouth), which leads to a more feed intake in less time (Reyes et al., 2024; Brown et al., 2022; Azizi et al., 2009). ER is established to be moderately to strongly related to feed efficiency in beef cattle (Romanzin et al., 2022; Montanholi et al., 2010) and dairy cattle (Brown et al., 2022; Green et al., 2013), as animals with low ER have longer chewing activity and slower intake, with a consequential better mastication, rumen fermentation, and nutrient digestibility (Aikman et al., 2008). While the tendentially higher RT (**Table 2**) is needed to process a larger bolus (Dado and Allen, 1994; Adenuga et al., 1991) and is related to a better utilisation of feed by ruminal microbiota, maintaining the rumen homeostasis, and favouring the passage of feed through the gastrointestinal tract (Kaufman et al., 2018).

Feeding behaviour traits and performance

Finding the links between dairy cow feeding behaviour and milk production is critical to supporting proposals for ways that enhance milk composition and yield; however, there are few studies on this topic. The relationship between ETC and milk performance throughout lactation (**Figure 1**) supports the previous findings of Johnston and DeVries (2018), where milk production increased for about 1.74 kg/d for every increased hour of time spent eating, and Jaeger et al. (2019), where daily feeding activity increases from 15% to 25% from low MY class (< 15 kg) to high MY class (> 30 kg). Interesting is the positive association found between ETC and RT (**Figure 1**). Previous results were conflicting, as some authors found a weak or negative correlation between ET and RT ($r = -0.14$,

Jaeger et al., 2019; $r = -0.64$, Dado and Allen, 1994), while others found a positive, albeit weak, relation ($r = +0.27$, White et al., 2017).

The RT is currently considered an important parameter for defining the health and welfare of cattle, as well as for assessing the management of the farm (Castaneda et al., 2024). Chemical composition (fibre) and physical characteristics (long particle size) of the ration are important drivers of different rumination patterns (Beauchemin, 2018). The association between RT and milk production has been studied extensively (Souza et al., 2022; Soriani et al., 2013), and as we expected, the positive association between RTC and MY was found (**Figure 2**). Considering that a normal rumination activity contributes to a favourable rumen pH, digestion of fibre, and regulation of biohydrogenation processes (Souza et al., 2022), an effect on milk composition, especially fat content, was expected but not found (**Figure 2**). This is in line with the assumptions of recent studies, which underline how an increase in rumination activity is weakly linked to an increase in milk fat (Souza et al., 2022; Andreen et al., 2020).

Conclusions

In this study, relationships were found between feeding behaviour and the performance level of animals. Also, a positive relationship between the two main feeding behaviours measured, ET and RT, is to be underlined and deserves further investigation. These results can lead to the conclusion that, thanks to the help of the different technologies present in commercial farms, it is possible to collect individual data useful for studying and describing different aspects of the lactation curve and the factors involved in its progress. This, combined with other knowledge, can assist the farmers in managing problems within the herd, also with particular attention to the individual animal, from the perspective of precision livestock farming.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at ...

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Supplementary material

A fundamental aspect in this work is the relationship between measured feeding behaviour traits and intake of animals during the entire lactation. The DMI of cows was predicted by an equation provided in NRC 2021 and based on animal characteristics and performance (de Souza et al., 2019), because we wanted to highlight the variations due to animal factors (e.g stage of lactation, milk yield, BW, etc.). The other equation by NRC 2021 is based on ration characteristics (Allen et al., 2019) and is more useful in situations of differences between rations and feeding systems. The selected equation was created using a Holstein cow dataset and has low bias, high accuracy, and precision. However, as commented in NRC 2021, the equation predicts "... DMI reasonably well for Jersey and Holsten crossbred cows", and we used it in Simmental cows because this breed could be close to productive, and conformation traits of Holstein crossbred (e.g. milk yield). In our knowledge, this is the first application of the equation in Simmental cows. **Figure 3** provides a visual representation of the expected DMI and the equation's principal predictors (such as BW and Milke) during the whole lactation, using the Simmental cow dataset of our study.

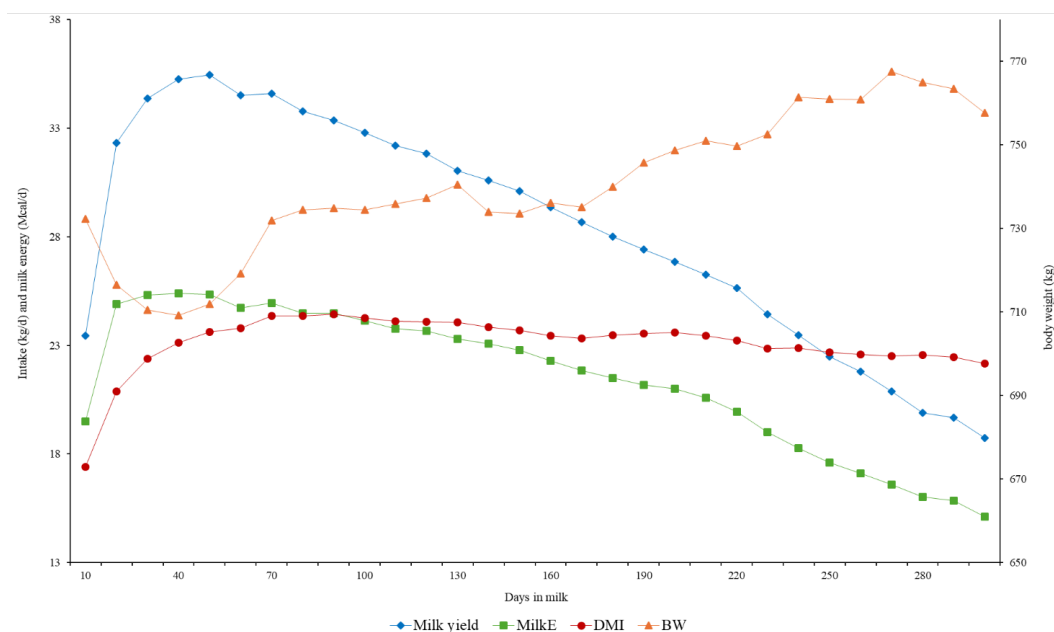


Fig. 3. Lactation curve of the Simmental dairy cows in the dataset. The red line with circles shows the estimated DMI by NRC 2021, the blue line with diamonds shows the lactation curve (milk yield), the green line with squares shows the observed milk energy (Milke) and the orange line with triangles shows the measured BW

APPENDIX

Sorghum silage and cover crop silage in diets for late-lactation cows: Effects on feed intake, digestibility, feeding behaviour, and milk yield

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ABSTRACT

Climate change is imposing new challenges on dairy cow nutrition. Sorghum has received interest due to its greater water use efficiency and high tolerance to drought conditions. The use of cover crops has grown in popularity as a means of boosting residual soil nitrogen and preventing soil erosion during periods of bare soil. The aim of the study was to assess the feed intake, apparent total tract digestibility, feeding behaviour, performance, and milk quality of dairy cows in late-lactation fed diets differing in silage type (Diet 1: sorghum silage; Diet 2: cover crop silage). Eight Holstein Friesian dairy cows in the last stage of lactation fed experimental diets in a cross over design trial. Diet 1 had higher apparent digestibility of dietary protein ($P < 0.05$), lower rumination chews ($P < 0.05$), and tendentially lower eating chews ($P < 0.10$) than Diet 2. However, no effect was found on dry matter intake, milk yield, or milk composition ($P > 0.05$). Cover crop silage can be used as an alternative to sorghum silage in the diets of dairy cows in late-lactation.

Key words: alternative silage, apparent digestibility, milk production, rumination time

Introduction

Climate change has greatly increased the difficulties in the supply of raw materials for the livestock sector. To address this issue, in many areas farmers are changing cultivation and ensiling processes and introducing alternative crops (McCary et al., 2020). In this context, sorghum has received interest, and a large number of studies have been performed to confirm its water use efficiency and high tolerance to drought conditions due to its physiology (Bhattarai et al., 2019; Safian et al., 2022). Sorghum silage is mostly used in cattle with reduced energy- and higher fibre requirements in the diet. Considering milk production and quality, it is well known that sorghum silage can be widely used in late lactation dairy cows if the diets are adequately integrated with starch sources (Bhattarai et al., 2019).

An alternative to sorghum silage is cover crops silage, consisting for instance of small grains and clovers. The use of this alternative silage in dairy cow rations has grown in popularity as a way to offset planting costs, increase the annual forage yield, prevent soil erosion in the absence of crops, provide residual nitrogen in soil, and control weeds and insects (Harper et al., 2017). Maxin et al. (2020), studying seven different species of cover crops, showed that these plants are suitable for ruminant feeding and, despite the moderate energy content, have a good amount of useable protein at the ruminal level, reducing the production of methane and ammonia.

In this study, we investigated diets differing in silage type on late-lactation dairy cows. Our hypothesis is based on the knowledge that cows in this phase have relatively low nutritional requirements that could be met by alternative crops. This leads to a reduction in feed costs and allows responding to new demands resulting from climate change without altering the production and welfare of dairy cows. Therefore, the aim of the study was to compare cover crop silage with sorghum silage in late-lactation cows, considering apparent digestibility, feeding behaviour, milk production, and milk quality.

Material and methods

Animals, housing, dietary treatments, and experimental design

The study was conducted in agreement with EU Directive 2010/63, in accordance with Italian legislation (DL n. 26, 4 March 2014), and observing the rules of the University of Udine. All animal treatments were approved by the ethical committee of the University of Udine (prot. n. 4/17).

Eight multiparous Holstein Friesian dairy cows in the last stage of lactation (days in milk > 200 d) were considered in the study. Cows were housed individually in a tie-stall barn (A. Servadei experimental farm, Pagnacco, Udine, Italy), fed and milked twice a day.

The dietary treatments consisted of two diets that differed in the type of silage. Diet 1 contained a sorghum silage (*Sorghum bicolor* L. Moench). Sorghum was harvested at the soft dough grain stage and chopped. The chopped sorghum was ensiled in a rectangular trench silo with a slight slope to facilitate the drainage of moisture. Diet 2 contained a cover crop silage composed of a mixture of cover crops, which included triticale (*Triticosecale* Wittmack), oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), and vetch (*Vicia sativa* L.). This mixed crop was ensiled in polyethylene film-wrapped round bales without the addition of inoculants.

The trial was carried out with a crossover experimental design. After an adaptation period of 14 days in the barn, four cows were fed Diet 1 for 20 days. After a washing-out period of 31 days, the same animals were then fed Diet 2 for an additional 20 days. The other dietary group of four cows received the same treatments, but in the opposite order. During the adaptation and washing-out periods, dairy cows were offered hay ad libitum, in order to meet or exceed the fibre requirements suggested by the NRC (2001), and 9.2 kg DM of compound feed. This was composed of corn (426 g/kg), soybean meal (215 g/kg), wheat bran (157 g/kg), sunflower meal (81 g/kg), wheat middling (78 g/kg), and minerals and vitamins (44 g/kg). Diet 1 offered during the experimental period consisted of 6 kg DM of sorghum silage, grass hay ad libitum, and 9.2 kg DM of a compound feed as previously described. Diet 2 consisted of 6 kg DM of cover crop silage, grass hay ad libitum and 9.2 kg DM of the same

compound feed. The ingredients and chemical composition of the feed offered to the dairy cows are reported in Table 1. In particular, the silages differed in physical characteristics (particle size and DM %) but had a similar chemical composition (in terms of fibre and protein content). The two silages had a pH level of 4.5 for cover crop silage and 4.0 for sorghum silage, respectively. The absence of mycotoxins has been confirmed, and the organoleptic quality parameters (smell, texture, color) fell within the desired range for the crops considered.

Table 1. Chemical and physical characteristics of feed offered to dairy cows

Item	Hay	Sorghum silage	Cover crop silage	Compound feed
Dry matter (%)	90.4	27.9	67.1	89.7
Chemical composition (% DM)				
Crude Protein	6.7	6.5	6.9	24.5
Ether Extract	1.6	2.3	1.8	6.5
Starch		16.7	1.6	24.7
Crude Fibre	39.2	37.7	38.6	10.0
Neutral detergent fibre	70.3	58.4	63.0	24.6
Acid detergent fibre	41.4	36.5	36.7	12.9
Acid detergent lignin	9.4	7.0	6.7	3.0
Ash	8.8	6.4	5.8	7.5
Nonfibre carbohydrates	12.6	26.4	22.5	36.9
Net energy for lactation (Mcal/kgDM)	0.95	1.24	1.21	1.87
Particle size fraction (% retained as-fed basis)				
>19 mm	95.8	2.7	87.8	
>8.0 mm	2.5	64.6	9.1	
>1.18 mm	1.4	31.3	2.8	
Bottom pan	0.2	1.3	0.2	

At the beginning of the first experimental period, dairy cows were assigned to two balanced dietary groups, according to milk yields (16.6 ± 1.30 kg/d [means \pm SD]; $P > 0.05$), days in milk (247 ± 35 d; $P > 0.05$), and body weight (650 ± 60.3 kg; $P > 0.05$). The animals involved in the trial were non-pregnant, had a mean age of 5.8 ± 2.05 years, and had a somatic cell count less than 200,000 cells/mL.

Samples and data collection

Samples of ingredients were collected weekly and analysed for chemical composition. Before morning milking, the daily individual dry matter intake (DMI) of hay, silages, and compound feed was determined by weighing the amounts of offered and refused feed, and the chemical composition was assessed.

During the last six days of each experimental period, the apparent total tract digestibility (ATTD) was assessed, as reported in Ponce et al. (2013). Briefly, samples of ingredients were collected daily, and the DMI of each ingredient was obtained as previously described. Fecal samples were collected twice a day from the floor immediately after defecation for each cow at 08:00 and 16:00 and homogenized by animal. At the end of the collection period, fecal samples and diets ingredients were analysed for chemical composition. Diet chemical composition per animal was obtained considering the chemical composition and DMI of ingredients.

Individual daily milk yield was recorded during the last three days of each experimental period, and individual milk samples were collected from each cow during morning milking. The milk yield was expressed as fat-corrected milk at 4% fat (FCM; Tyrrell and Reid, 1965).

In the two experimental periods, dairy cows were equipped with a noseband pressure sensor (RumiWatch system, ITIN-HOCH GmbH) validated to assess feeding behaviour by Ruuska et al. (2016). The raw data obtained was processed according to the procedure described by Romanzin et al. (2018). The following variables of feeding behaviour were considered: rumination and eating time (min/d), number of rumination and eating chews (n/d), number of rumination boluses (n/d), and rumination intensity (n bites/bolus). The values obtained in the last three days of each experimental period were averaged per animal and considered for statistical analysis.

Chemical analysis

Feed, feed residuals, and fecal samples were pre-dried at 60 °C for 48 h. Feed samples and fecal samples were then milled through a 1-mm screen (Pulverisette; Fritsch). Analysis of residual DM

was performed by heating at 105 °C for 3 h (AOAC, 2016). Ash was measured by incineration in muffle at 550 °C for 2 h (AOAC, 2016). Acid insoluble ash (AIA) was measured according to Van Keulen and Young (1977).

The N content was determined using the Kjeldahl method (AOAC, 2016), and the crude protein (CP) content was calculated as $N \times 6.25$. The neutral detergent fibre (NDF) was determined using a fibre analyzer (Ankom II Fibre Analyzer; Ankom Technology) following the Van Soest et al. (1991) technique, without correcting for residual ash, and applying a pre-treatment with α -amylase to the samples. Only for feed samples, ether extract (EE) was determined using the Soxhlet method (AOAC, 2016). The energetic value of feeds, expressed as net energy for lactation (NEL), was estimated following the INRA standard (INRA, 2010).

Organic matter (OM) ATTD was calculated as follows:

$$100 - \left(100 \times \left(\frac{\% \text{ AIA in feed}}{\% \text{ AIA in feces}} \right) \times \left(\frac{\% \text{ OM in feces}}{\% \text{ OM in feed}} \right) \right)$$

The same was done for CP and NDF.

Milk samples were analysed for chemical composition (fat, protein, and lactose) according to ISO 9622:2013 using a MilkoScan FT6000 (FOSS Electric).

Statistical analysis

Data were analysed using R software, v. 4.1.2 (R Core Team). The total sample size was determined using the PowerTOST package (Labes et al., 2024) considering a power of the statistical test of at least 80%. Shapiro-Wilk test was considered to check the normal distribution of the data, which were Tukey-transformed for parametric testing when appropriate. The effect of dietary treatment (Diet 1 vs. Diet 2) on variables was assessed as a cross-over design. In particular, dietary treatment, period (P1 vs. P2), and the sequence with which the animals were fed with the experimental diet were considered as fixed factors, while dairy cow was treated as a random factor. Differences between

dietary treatments at $P < 0.05$ were considered statistically significant, while differences at $P < 0.10$ were considered as a tendency towards statistical significance.

Results

The effects of silage type on feeds, nutrient intake, and ATTD are reported in Table 2. No changes were observed in total DMI ($P = 0.83$), and Diet 1 and Diet 2 intakes were also comparable ($P = 0.30$). Hay intake also did not differ between the treatments. Overall, the Diet 2 group ingested 0.7 kg DM more forage than the Diet 1 group, but the difference was not significant ($P = 0.13$). Only the compound feed intake differed between the treatments (+0.7 kg for Diet 1, $P < 0.01$). This difference was partly reflected in the intake of CP and starch, which were higher in the Diet 1 than in the Diet 2 (+1 p.p. and +4 p.p., respectively, $P < 0.01$), and in the higher NDF intake observed in the Diet 2 ($P < 0.01$). The ATTD of OM and NDF did not differ between the dietary groups ($P > 0.05$). Diet 1 group had a higher ATTD for CP (+4.1 p.p.; $P = 0.02$) than Diet 2.

Table 2. Effects of silage type on feeds and nutrient intake and digestibility of dairy cows

Item	Dietary group		RSD	P-value
	Diet 1	Diet 2		
Intake				
Compound feed (kg DM)	8.9	8.2	0.45	<0.01
Forage (kg DM)	6.8	7.5	0.85	0.13
Silage (kg DM)	3.7	4.1	0.71	0.30
Hay (kg DM)	3.1	3.4	0.42	0.19
Total (kg DM)	15.8	15.7	0.87	0.83
Crude protein (%)	16.9	15.9	0.66	<0.01
Starch (%)	17.5	13.5	0.53	<0.01
Neutral detergent fibre (%)	41.8	44.6	1.37	<0.01
Apparent total tract digestibility (%)				
Organic matter	68.3	67.7	3.15	0.74
Crude protein	70.8	66.7	3.57	0.02
Neutral detergent fibre	49.2	51.6	4.02	0.24

Diet 1 - sorghum silage diet; Diet 2 - cover crop silage diet; RSD - residual standard deviation

Average milk production was approximately 12.0 kg/d and became very similar when corrected for energy (12.4 kg/d) between the two diets ($P > 0.05$; Table 3). The use of cows in late-lactation, in addition to having led to a limited production of milk, was also reflected in the higher content of fat and protein in milk. However, no differences were observed between treatments regarding milk components (protein, fat, and lactose) or somatic cell content ($P > 0.05$).

Table 3. Effect of silage type on milk yield and milk composition of dairy cows

	Dietary group		RSD	<i>P</i> -value
	Diet 1	Diet 2		
Milk yield, kg/d	11.8	12.2	2.24	0.71
Fat-corrected milk, kg/d	12.4	12.4	1.89	0.99
Milk composition (%)				
Protein	3.48	3.43	0.300	0.75
Fat	4.33	4.19	0.542	0.60
Lactose	4.37	4.48	0.146	0.17
Somatic cell count, cells/mL	229474	199992	47724	0.22

Diet 1 - sorghum silage diet; Diet 2 - cover crop silage diet; RSD - residual standard deviation

The feeding behaviour of dairy cows is shown in Table 4. The Diet 2 treatment always reached numerically higher values in both eating (eating time and eating chews) and rumination (rumination time, rumination chews, number of boluses, and rumination intensity) variables. However, these differences were statistically significant only for the number of rumination chews (+4842 chews/d, $P = 0.01$), while a tendency towards significance was found for the number of eating chews (+2369 chews/d, $P < 0.10$). Instead, although they differed by approximately 1 hour per day, the rumination times of the dietary groups did not differ ($P = 0.12$).

Table 4. Effect of silage type on feeding behaviour of dairy cows

	Dietary group		RSD	<i>P</i> value
	Diet 1	Diet 2		
Rumination time, min/d	462	521	75.3	0.12
Eating time, min/d	235	258	50.9	0.37
Rumination chews, n/d	24,299	29,141	3,875	0.01
Eating chews, n/d	11,993	14,362	3,671	0.10
Boluses, n/d	481	545	78.5	0.10
Rumination intensity, n chews/bolus	48.3	49.8	2.92	0.32

Diet 1 - sorghum silage diet; Diet 2 - cover crop silage diet; RSD - residual standard deviation

Discussion

The results in Table 2 show that dietary treatment did not affect total DMI. In fact, the difference in compound feed consumption was compensated by, although not significant, differences in forage consumption levels. It should be noted that the two groups of cows consumed different amounts of compound feed, likely affecting animal performance indicators.

The CP content of the two silages was similar, while there was a difference in starch content (16.7 vs. 1.6% for sorghum silage and cover crop silage, respectively; Table 1). This contributed to determining a different composition of the DMI (CP, NDF, and starch). Therefore, it can be assumed that the variable consumption of compound feed was the only cause of the difference in CP intake (+1 p.p.). On the other hand, the greater difference in starch intake (+4 p.p.) can be attributed, in addition to the compound feed, also to the different compositions of the two silages. In this sense, it is surprising that the cows that received the silage lowest in starch (Diet 2) were also those that consumed the least compound feed.

Differences in NDF intake could be linked to the greater forage intake (although not significant, $P = 0.13$) in Diet 2 compared to Diet 1, and to varying levels of NDF in both silages. The different composition of DMI, in addition to the different particle sizes between the two silages, are likely the main factors that led to differences in CP digestibility. However, this different composition of the

ingesta did not determine changes in overall digestibility. In contrast, a study comparing three diets based respectively on corn silage, whole plant grain sorghum silage, and forage sorghum silage, integrated with increasing levels of corn meal, found a higher total-tract digestibility for OM and NDF in the forage sorghum diet compared to the other two and no difference in digestibility for CP (Colombini et al., 2012). Sorghum has a protective coating that covers the seeds, reducing the degradability of the starch. Herrera-Saldana et al. (1990) state that starch degradability rates in sorghum are lower than in oats, wheat, barley, and corn. In the present study, as previously mentioned, cows fed Diet 1 ingested higher amounts of starch, but their main source of starch was compound feed (about 2.2 kg compared to 0.6 kg from sorghum). This may have reduced the effect of the lower starch degradability, partly explaining the lack of difference in milk yield between treatments.

In the literature, numerous studies compare the yield and composition of milk from traditional forages with sorghum or cover crop silage. Amer et al. (2012), substituting alfalfa with sorghum silage in diets of early to mid-lactation dairy cows, observed a reduction in milk yield and an increase in milk fat, which led to a similar FCM yield. The authors explained that these results could be due to differences in NDF and CP intake by animals, which were higher and lower, respectively, in sorghum silage compared with an alfalfa-based diet. In general, an increase in dietary fibre intake is accompanied by a reduction in available energy. In a study on the partial replacement of corn silage with wheat or triticale silages, Harper et al. (2017) reported no differences in milk yield and composition in animals fed wheat or triticale silage. Interestingly, the two experimental groups showed similar DM, CP, and NDF intakes. Li et al. (2020), substituting corn silage with four different inclusion levels of sweet sorghum silage, observed that milk yield did not differ, and it was comparable to our study (ranging from 11.4 to 12.6 kg/d). In this case, the lack of differences in milk production and composition may be due to the low nutritional requirements of late-lactation cows. In the present study, we could also expect that the different forage to concentrate ratio would lead to a change in milk fat content, but this did not occur. In short, it could be hypothesised that the differences in CP, starch, and NDF intake observed in the dietary groups were not sufficient to influence milk

composition and yield. Schalla et al. (2012) reported that the apparent CP digestibility was negatively related to the milk protein. The authors state that the milk CP percentage was associated with microbial CP production and that the latter may cause a decrease in the apparent CP digestibility. In our study, although CP digestibility was higher in Diet 1, no differences were found in milk CP content. This could be attributed to the other factors (total DMI and ATTD of the OM) that did not change between our dietary groups but, according to Schalla et al. (2012), could affect milk yield.

In this study, feeding behaviour was evaluated with a noseband pressure sensor capable of recording and interpreting the chewing movements, distinguishing between eating and rumination. Overall, the results obtained are comparable with other studies that have used the same instrument in similar conditions (tie-stall; Leiber et al., 2016). However, as expected, they differ from those obtained on grazing cows, especially regarding feeding time (Romanzin et al., 2018). The Diet 2 reached numerically higher values in both eating and rumination parameters than the Diet 1. However, the different silages influenced the number of rumination chews, and a tendency towards significance was found in the number of eating chews. Feeding behaviour may be influenced by the physical and chemical properties of the diet, which include particle size, fragility, DM, NDF content, and lignin concentration. In a recent study, Florit et al. (2023) found that lignin and particle size are among the main factors capable of increasing eating time on commercial dairy farms. Considering physical properties, in particular particle size, it is known that excessively long particles increase the necessary chewing to swallow a bolus of feed (Grant and Ferraretto, 2018). In the present study, cows fed Diet 1 received sorghum silage composed of rather short particles and consumed more compound feed. While in the Diet 2 group, the cover crop silage was composed of 87.8% long particles (> 19 mm; Table 1). Finely processed feed or, in general, diets with a high percentage of short particles lead to a reduction in eating and rumination time (Beauchemin, 1991). Considering chemical properties, increasing NDF intake and lignin in silages are associated with an increase in rumination and eating chews. Beauchemin (1991) defines a high correlation between NDF intake and rumination activity ($r = 0.94$). In the study of Rinne et al. (2002), four different maturity stages of grass ensiled were

compared, resulting in an increase in NDF intake associated with an increase in the time of mastication per day, according to our results. These authors found the same trend for CP digestibility, which decreased with an increasing chewing period. This was partially confirmed by Tafaj et al. (2005), which explained the higher chewing needed in fibrous diets to compensate for a low microbial fibre breakdown and to avoid the most available surface for microbial activity (the contribution of chewing activity to the particle reduction was about 70-80% and that of the microbial degradation was 15-25%; McLeod and Minson, 1988). Probably, the increase in chewing activity in fibrous and/or long fibre diets (Diet 2) was not sufficient to maintain a level of protein digestibility similar to that observed in Diet 1.

Conclusions

The inclusion of cover crop silage can provide an alternative dietary source for cows in the late stage of lactation, which is characterised by lower energy requirements and milk yields. Cover crop silage-based diets tend to result in reduced apparent digestibility of dietary protein and a higher number of feeding chews without altering milk performance, milk quality, or feed intake. However, the actual impact of these findings is limited by the high consumption of compound feed (about 50% of the diet) that may have masked the effect of the different silage types offered to both groups of cows. Therefore, further studies could deepen the understanding of the potential role of alternative silages by including lower levels of compound feed in diets offered to lactating dairy cows.

Conflict of interest

The Authors declare no conflict of interest for this article.

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CHAPTER V - DISCUSSION

The discussion section follows the aims of the papers presented in the thesis, focusing in particular on:

1. The complex relationship between feeding behaviour and feed efficiency
2. The insights gleaned from sensor data in commercial dairy cattle farms, exploring the associations between feeding behaviour and parity, lactation stage, performance, health status, ration composition, and feed management strategies.

1.1 Feeding behaviour and feed efficiency

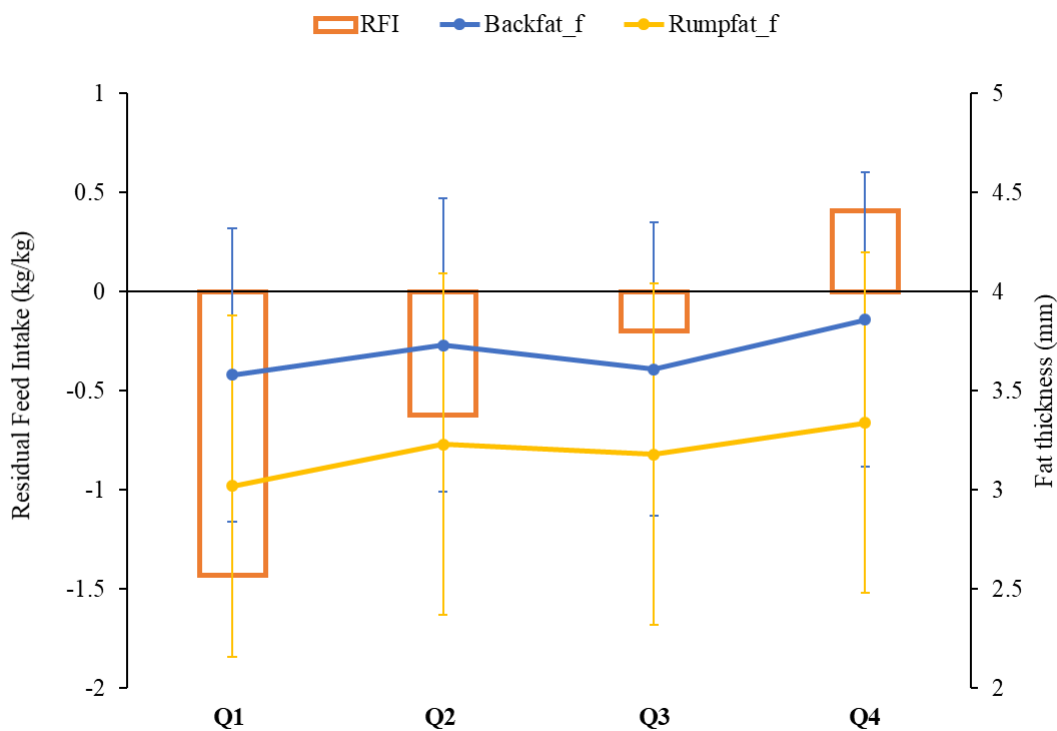
As already mentioned, feed efficiency is a complex, multifactorial trait that remains partially understood. There is a growing interest among farmers and researchers in breeding cattle with improved feed utilization. The aim is to genetically enhance cattle populations, recognizing the significant impact of feed efficiency on animal well-being, environmental sustainability, and economic viability. Individual feed intake is closely tied to feeding behaviour, which results from a complex combination of nervous signals triggered by external stimuli.

The exploration for the link between feeding behaviour and feed efficiency has been conducted in various studies, particularly with considering beef cattle kept on pasture or housed in experimental farms but results are controversial. Several studies found that efficient animals tend to have lower feeding rates (Brown et al., 2022; Romanzin et al., 2021; Parsons et al., 2020; Connor et al., 2013). Research on eating habits varies, with some efficient animals having shorter ET (Xi et al., 2016; Fitzsimons et al., 2014; Green et al., 2013) and others showing no correlation (Brown et al., 2022; Holló et al., 2022; Romanzin et al., 2021; Menezes et al., 2020). These discrepancies can be partly explained by different animal categories, measurement techniques, and raising systems (pasture, tie-stall, or free-stall barns).

In the **Paper 1** we investigated the relationship between feeding behaviour and feed efficiency in dual-purpose breed beef cattle. While we found no correlation between FT and RFI, we identified a notable positive relationship between feeding rate (FR) and feed efficiency. More efficient breeds exhibited a lower FR, consuming up to 15 grams per minute less than the less efficient breed. Additionally, we observed a significant correlation between FR and RFI, albeit not strong ($r = 0.24$). We must clarify that the system utilised for monitoring behaviour consented to the collection of data regarding FT, which is the time the animal spent within the trough (calculated as the difference between the entrance and the exit). This data is not the same as ET, which is the time the animal spent eating. Despite this, the FT reflected the behavioural patterns of the animals, as the so-called missing events (with an ingested amount less than 100 grams) were eliminated as outliers from raw data. Regarding meal frequency, we found that the most efficient breeds had completely different patterns in terms of meal number per day (7 more for the most efficient breed). In general, frequent and smaller meals can positively impact feed digestion, rumen pH stability, milk production, fibre digestibility, and overall production efficiency, while reducing health issues and large fluctuations in rumen pH (Hart et al., 2014). Our findings suggest that the influence of feeding behaviour may not be evident in daily variations, but rather in within-meal behaviour. Given the promising results obtained with FR, and considering its moderate heritability (0.44-0.56 in steers; Kelly et al., 2021; Chen et al., 2014), we assumed that it could be considered as a potential selection criterion for improving feed efficiency in breeding programs. The possibility of incorporating feeding behaviour into the efficiency assessment has also been considered in other studies. Fischer et al. (2018) found that among all the biological traits that contributed to almost 60% of the phenotypic variability of the REI, the main ones involved were activity (26.5%) and feeding behaviour (21.3%). In a recent communication, Lemal et al. (2024) evaluate the possible use of adding behaviour patterns to select cattle for heat tolerance, finding that behaviour data (ET and RT) helps to explain a high part of the variation in the reaction for heat stress, suggesting the possible use of this trait in genetic evaluation for heat tolerance, and also helps the farmer to detect heat-stressed animals in farms.

All our relationships between feeding behaviour and feed efficiency in dual-purpose bulls were made using a common RFI, based on performances measured as gross live weight gain. In reality, a different tendency of animals to accumulate in gain fat or protein, which have different energy contents (5.6 vs 9.2 kcal/g, respectively) and links with water (78 and 13%, respectively), could cause relevant difference in energy of live weight gains. As a result, the RFI could be corrected for degree of fattening. To take this into account a parallel study was conducted to investigate the association between RFI and body fat by measurements of fat thickness in two points (Backfat and Rumpfat). In **Graph 1**, the examined bulls were divided into quartiles considering the RFI value.

Graph 1. Ultrasound measurements of dorsal fat thickness in growing Simmental bulls grouped into quartiles using RFI (n = 222)



Quartiles: Q1 = \leq Q25; Q2 = $>$ Q25 and \leq Q50; Q3 = $>$ Q50 and \leq Q75; Q4 = $>$ Q75.

RFI = Residual Feed Intake (kg/kg); Backfat_f = Ultrasound measurement between the 12th and 13th ribs (mm); Rumpfat_f = Ultrasound measurement between the biceps femoris muscle and the gluteus medius muscle (mm).

Table 3. Regression of the RFI model and the RFI_{fat} model considering ultrasound measurements of dorsal fat thickness.

	RFI (kg/d)	RFI _{fat}			
		Backfat _f	Backfat _Δ	Rumpfat _f	Rumpfat _Δ
r^2	0,433	0,448	0,457	0,437	0,462
b	-	0,19 ± 0,09*	0,20 ± 0,07**	0,09 ± 0,08	0,21 ± 0,07**

RFI = Residual Feed Intake; Backfat_f = Ultrasound measurement between the 12th and 13th; Rumpfat_f = Ultrasound measurement between the biceps femoris muscle and the gluteus medius muscle; Backfat_Δ, Rumpfat_Δ = Increase in fat thickness between the beginning and the end of the experimental trial.

* $p \leq 0.05$; ** $p \leq 0.01$

Our analysis revealed that more efficient bulls exhibited lower fat thickness and slower fat growth rates. By including these fat measurements in the RFI model, we achieved a significant improvement in model fit, explaining an additional 3% of the variation in feed intake (**Table 3**). This finding aligns with previous research highlighting the value of incorporating body fat data in feed efficiency assessment. Basarab et al. (2011) found an improvement of up to 3.8% ($P < 0.05$) in predicting the variation in DMI with the inclusion of Backfat data, while Holló et al. (2022) also included the Rumpfat data in the RFI calculation model, achieving an improvement in the prediction of the variation (R^2 increased from 0.98 to 0.99 and standard error of estimate decreased from 0.06 to 0.03). Based on the current results, the influence of fattening appears to be present but not significant, demonstrating that the RFI based on gross live weight growth is accurate. It is possible that fattening variances in other breeds are more relevant than in dual-purpose cattle, and this phenomenon warrants additional research.

It is important to acknowledge the distinction between trials conducted under experimental conditions or in commercial raising systems. Experimental trials, conducted in controlled environments, allow for the precise investigation of specific factors while minimizing external influences. This approach has been instrumental for research, as it has allowed for the deep understanding of animal factors and processes. However, commercial farms present a more complex scenario, where animals are subject to a multitude of environmental, social, and managerial factors that can lead to different results. Moreover, as has already been widely emphasized, the applicability

of tests conducted in experimental farms to commercial farms is very difficult due to high costs and specific infrastructure requirements hinder their application with the normal management of a farm. Therefore, there is a need for alternative approaches that can bridge the gap between research and commercial reality. Precision livestock farming, with its advanced technologies, offers a promising solution by enabling the collection and analysis of real-time data on individual animals.

1.2 Feeding behaviour in commercial farms

Given the inherent challenges of assessing feed efficiency in commercial dairy farms and recognizing the critical role of this metric in genetic selection, we sought to identify additional factors that could contribute to a more comprehensive understanding of feed efficiency. Building upon previous research highlighting the link between feeding behaviour and efficiency, the primary objective of the second part of the thesis was to investigate the factors that could have an influence on feeding behaviour. To overcome the limitations of traditional data collection methods, we took advantage on modern precision livestock farming technologies installed on commercial farms. These technologies enabled the collection of a large amount of individual animal data, including detailed information on feeding behaviour. To achieve this, we first approach a general screening in regional commercial dairy farms encompassing two breeds (Italian Simmental and Holstein Friesian), dietary variations, and levels of automation in feed management. This approach allowed us to identify inter-animal variability as well as differences associated with varying dietary characteristics. Subsequently, we adopted a more focused approach, concentrating on a smaller number of farms with consistent management practices, including similar diet quality and overall management of the farm (same breed, automatic milking system, same sensor), allowing us to delve deeper into intra-animal variability throughout the entire lactation period.

Identifying the relationships between cow feeding behaviour and productivity is crucial for implementing strategies that optimize milk composition and yield. The interesting link that has been

found in **Paper 2** regarding ET and milk production can help us distinguish animals that are efficient from a production standpoint. Johnston and DeVries (2018) reported similar findings, showing that increasing daily ET by one hour led to a 1.74 kg/day increase in MY. Krpáľková et al. (2021) investigated the relationship between feed efficiency (FCR), feeding behaviour, and milk performance. They found that more efficient cows exhibited active feeding behaviours (eating and rumination) while maintaining lower FR and high milk productions, suggesting that incorporating feeding behaviour into DMI model predictions can enhance accuracy of the results. Additionally, ET data has taken on greater importance in modern dairy farming, as it is often used in sensor algorithms with RT to identify health issues like acidosis, ketosis, and ruminal stasis, rather than solely focusing on reproductive status. Therefore, in **Paper 2**, we wanted to verify whether the feeding behaviour was associated with the incidence of mastitis, a major health and economic challenge in dairy farming. In fact, this disease costing approximately 198 EUR per case (Berry et al., 2004) leads to significant milk loss (averaging 375 kg per lactation; Seegers et al., 2002) and increased culling rates. The observed relationship between longer ET and lower somatic cell counts is difficult to explain. The most likely explanation is that the animals that spent the most time eating were also the most productive, so the link with udder health is quickly established. However, it seems unlikely that somatic cell count directly influences feeding behaviour. This is particularly true for the cases of subclinical mastitis, where prevention is crucial due to their spread (15-40 times more frequent than clinical form; Cobirka et al., 2020), duration, and difficulty detection. Unfortunately, following our results, we think that feeding behaviour is not a reliable indicator for predicting or preventing mastitis.

Research on the impact of housing, nutrition, and management practices on dairy cow feeding behaviour is continually growing. **Paper 2** also highlighted the importance of ration characteristics and feed management strategies for the profitability of the farm. Cattle producers aim to optimize diets to meet animal needs, minimize feed waste, and maintain production levels. The link between feeding behaviour, ration composition, and feeding management practices suggests that feeding behaviour data could serve as a valuable short-term indicator for farmers to adjust diets or

management strategies to better align with animal needs, without compromising productivity or welfare. We observed a negative correlation between ET and the energy and protein content of the diet, but particularly with higher fibre digestibility, while a positive relation was found between ET and lignin content. These results highlight that animals with more digestible fibre (and lower lignin content) require less time to chew and form the feed bolus. This finding could be valuable for farmers, as it demonstrates that feeding a ration with higher fibre digestibility can positively impact animal performance. Additionally, this information can be useful for nutritionists, as fibre digestibility analyses are often time-consuming and not routinely included in ration control analyses. By associating feeding behaviour with fibre digestibility, we can gain valuable insights into ration formulation and management and make the appropriate adjustments to the ration.

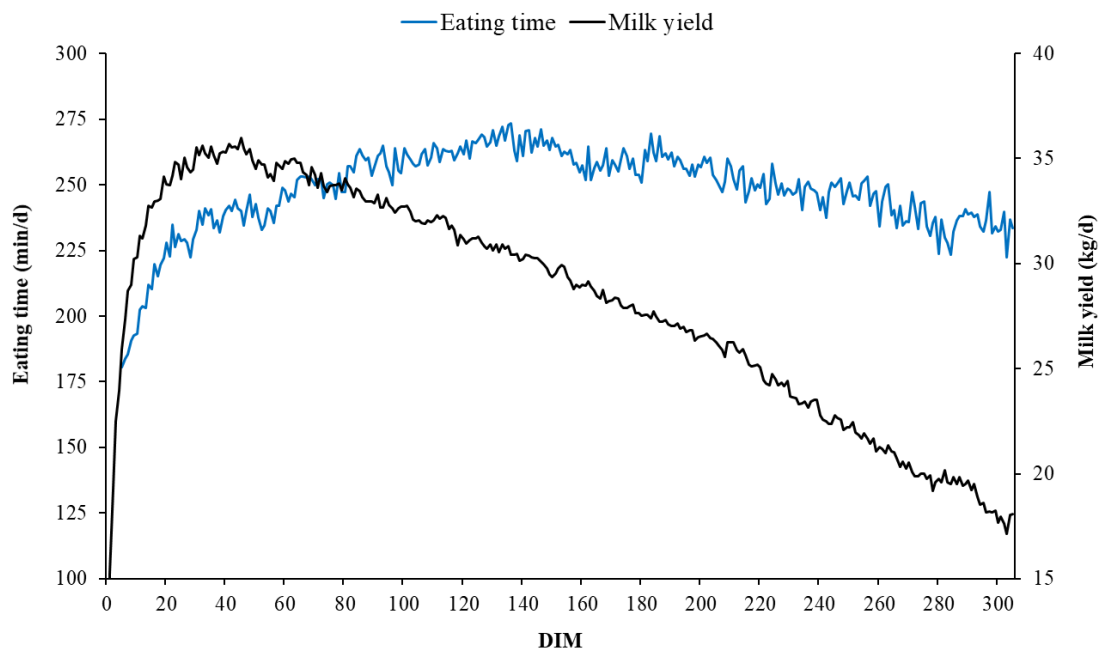
Besides the chemical composition, the particle size of the ration is also important to promote proper ruminal functionality. As expected, very long feed particles were associated with a longer time required by the animal to form the bolus (**Paper 2**). These results have also been confirmed in the **Appendix paper**, where a feeding trial comparing two alternative silages was conducted. The results indicate that the diet based on cover crop silage, which was higher in particle resulted in increased ET and RT, as well as higher number of chews. In this trial also different chemical characteristics of the two diets influenced different behavioural patterns.

Regarding daily ration management, we observed a link between times of feed delivery and feed pushing and eating behaviour. The administration of feed stimulates feeding activity (DeVries et al., 2003). In general, it has been observed in several studies that dividing the total daily intake into multiple meals is beneficial for ruminal health and for reducing potential competitive situations within the group.

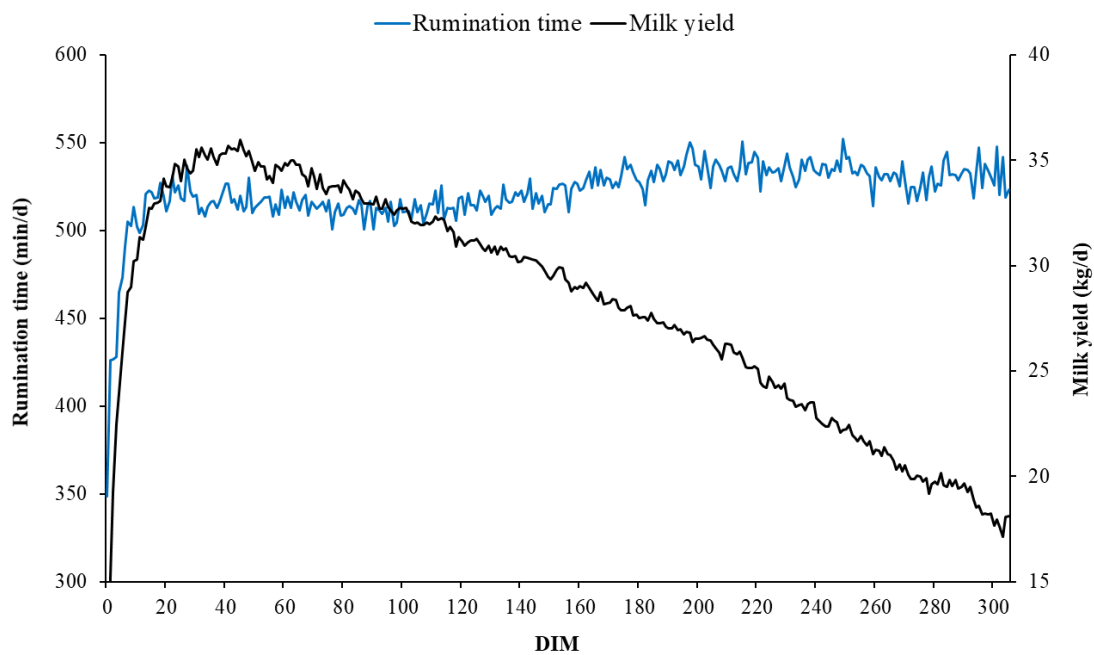
In **Paper 3**, we focused on evaluating how feeding behaviour was modified during the animals' lactation period. The concept that lactation involves physiological, hormonal, and dietary changes at every stage is definitive, and this is also reflected in the feeding behaviour of animals. Despite this aspect being well-established, studies in the literature often focus on specific phases of

lactation, the most critical ones (such as the transition period or the peak of lactation), failing to characterize the animals throughout the entire production period. The trends of the lactation curve for both ET and RT reported in **Graphs 2a** and **2b** are similar with that found by Atashi et al. (2024). The peak of ET occurs after the production peak, when the animals increase their intake to obtain the necessary nutrients for production, and then maintain a more or less constant pattern until the dry period. The RT, on the other hand, reaches its maximum value before the lactation peak, already within the first 10 days after calving, and then remains constant for the subsequent phases and until dry-off.

Graph 2a. Lactation curves for daily milk yield and averaged eating time (min/d) for the animals involved in Paper 3.

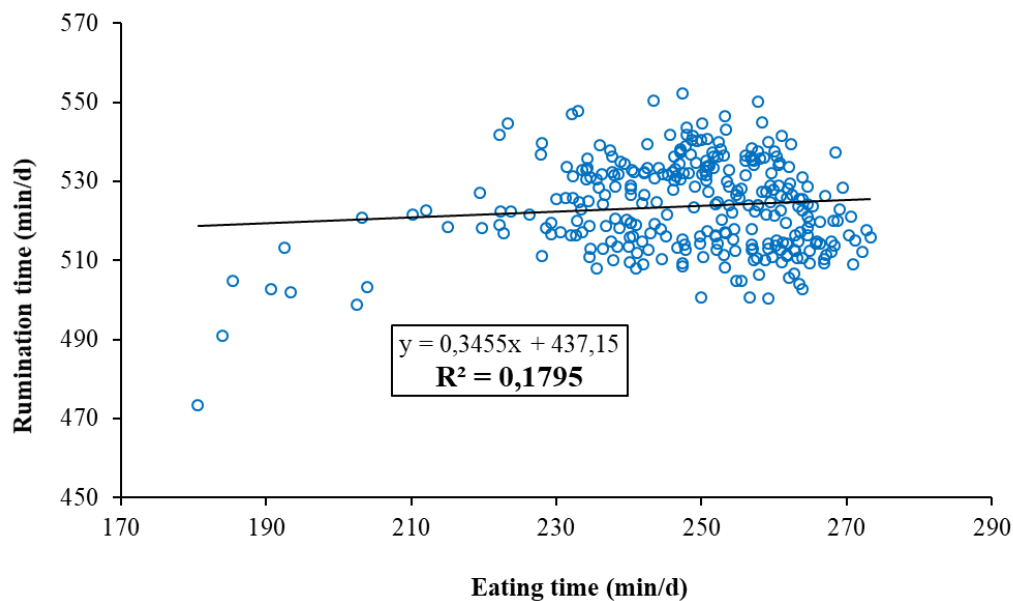


Graph 2b. Lactation curves for daily milk yield and averaged rumination time (min/d) for the animals involved in Paper 3.



Expecting a relationship between animal age, productive capacity, and size, we identified an interesting trend in feeding behaviour across different parity groups (primiparous and multiparous). Younger cows, despite having lower intake and energy requirements, exhibited longer daily ET. In contrast, older cows, to meet their higher intake needs, fed more quickly and spent more time ruminating, likely to compensate for the reduced chewing time during feeding. Another explanation for the higher ER in multiparous cows is their larger body size, which may lead to a greater quantity of feed ingested per bite (Reyes et al., 2024). We have once again confirmed the results of **Paper 2** regarding the relationship between ET and milk production, also adding a positive relationship between RT and milk production throughout the whole lactation period. Interesting is the positive, albeit weak, correlation found between ET and RT (**Graph 3**), that confirms the previous findings of White et al. (2017).

Graph 3. Correlation between eating time and rumination time about data of Paper 3.

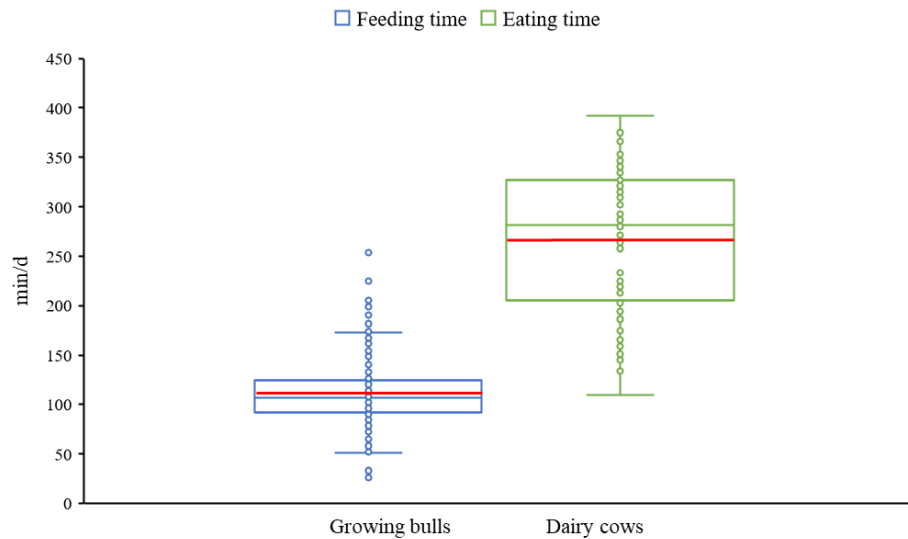


A last comment might be made regarding the comparison of data on feeding behaviour between bulls and dairy cows. **Graphs 4a** and **4b** displays the average feeding behaviour measured individually in growing bulls (**Paper 1**) and lactating cows (**Paper 3**). This latter metric on cows was chosen at 140 DIM because it is a lactation phase in which feeding time peaks and subsequently becomes very steady. As can be observed, feeding duration is around 111 min/d in bulls and 260 min/d in lactating cows, but with the same degree of variability (average 26%CV). Of course, the difference is mainly explained by different DMI between the two categories, but also by the different diet composition (less fibre content in beef cattle) and the measuring systems (automatic feeding system vs. accelerometers). Furthermore, the average FR is comparable across the two animal categories (94 g DM/min for growing bulls and 100 g DM/min for dairy cows) but not significantly different.

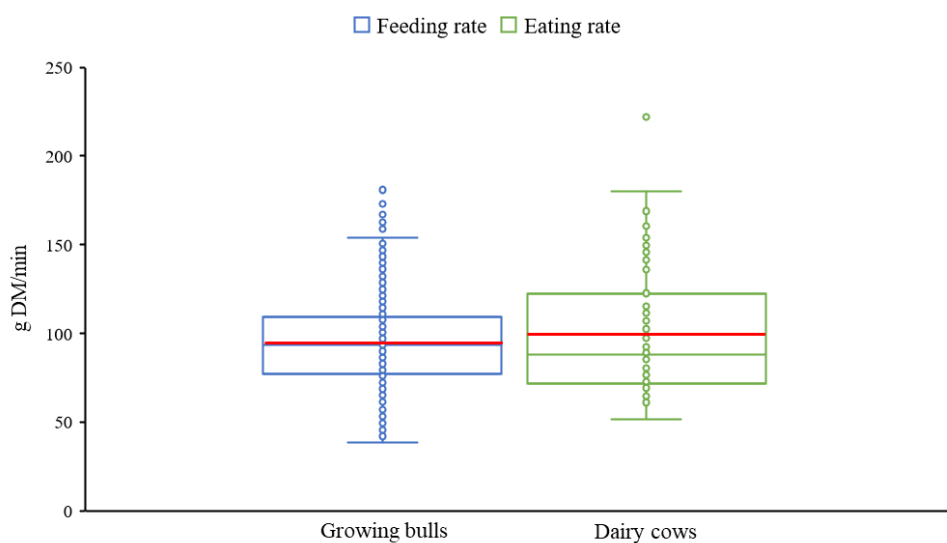
This information may be valuable in terms of genetic approaches to character improvement because it shares a gross phenotypic similarity of the feeding behaviour trait between growing bulls and cows. This serves as a very preliminary precondition for selecting feeding behaviour traits based

on bulls, with the aim of genetically improving the trait across the entire population, including lactating animals.

Graph 4a. Boxplot with average feeding time of growing bulls of Paper 1 (blue) and eating time of dairy cows of Paper 3 (green). Red line represents the mean value.



Graph 4b. Boxplot with average feeding rate of growing bulls of Paper 1 (blue) and eating rate of dairy cows of Paper 3 (green). Red line represents the mean value.



CHAPTER VI - CONCLUSION AND IMPLICATIONS

The findings of this thesis highlight the relationship between feeding behaviour and feed efficiency in cattle. This opens interesting possibilities to address the well-known issue of feed intake measurement in commercial farms.

The results obtained suggest further investigation into the factors related to feeding behaviour, to increase knowledge and implement the current use of feeding behaviour in barns for herd management. Furthermore, having deepened our knowledge by focusing particularly on the Italian Simmental breed, which is the most important dual-purpose breed at the national level, we can enhance the general knowledge on the topic, which at the moment is mainly concentrated on high milk-producing breeds such as the Holstein Friesian.

All of this can serve as a solid foundation for implementing feed intake estimation, which is useful for monitoring animal efficiency. The interest in studying this factor is also growing in the companies of the dairy sector. I had the opportunity to learn during my research experience abroad at a major company in the precision livestock farming industry (Lely International, Maassluis, NL). These companies are increasingly trying to improve the algorithms for predicting or detecting health or welfare issues in animals by integrating various aspects related to the animal, providing increasingly high-performance tools to modern livestock breeders. For this reason, the growth of knowledge in this field continues in our research group, with ongoing investments in research projects and doctoral programs on this topic.

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