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GUIDELINES DEVELOPMENT FOR DIETS FORMULATION FOR ROSSA REGGIANA COW UNDER HEAT STRESS CONDITION

Sviluppo di linee guida per la formulazione di diete per vacche Rosse Reggiane in condizione di stress da caldo

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Abstract

The aim of the present work is to develop feeding guidelines for the Rossa Reggiana cow under heat stress conditions, considering the existing relationships between dietary components and milk constituents. This field trial was conducted in July 2022, involving 9 traditionally fed herds of Rossa Reggiana cows located in the provinces of Reggio Emilia and Parma, in both plain or hilly territory. Two samplings' visits were conducted approximately 2 weeks apart in each farm, for a total of 18 samplings, considering only the lactating group cows. Each sampling involved the measurement of the average feed intake, average digestibility of different nutrients in the ration, average barn milk yield, milk quality and dairy aptitude. Samples of hay, feed, faeces, and bulk milk were collected, and the analyses were conducted at the university laboratory. Chemical analysis data and digestibility values of hay and concentrates were entered into the specific rationing software NDS Professional version (Rumen S.a.s, Reggio Emilia, Italy), to obtain an accurate estimation of the nutritional value of the 18 rations provided during the sampling days. The chemical composition data measured and estimated by the software were then regressed over milk composition, obtained from the laboratory analysis. The same data were also input into the statistical analysis software SPSS v.26 (Armonk, NY, IBM Corp), to obtain and study the correlations between the various parameters. To ascertain the constant presence of heat stress during the sampling days, the Temperature Humidity Index (THI) was calculated using the appropriate formula, and it consistently exceeded the limit level of 72.

By analysing the graphics and correlations obtained between diets parameters and milk production, fat, protein, lactose, urea, and rheological characteristics of milk, several interesting results emerged, some of which being aligned with findings in the scientific literature on dairy cattle. The cows examined had an average milk production of 5,905 kg per lactation, with an average content of 3.40% fat and 3.29% protein. The average diet fed, composed by hay, fresh grass and concentrate, consisted of high levels of forage (55-82 %), high levels of sugars (5-9.3 %), low levels of fat (2.43.5 %), modest levels of starch (7.5-21 %), and protein (11-17 %). All the reconstructed rations were low in concentrate and high in forage, due to the abundance of fresh grass fed. The highly positive correlation ($r = 0.910$; $P < 0.01$) between the measured productions of the single herds and the production estimated by NDS software highlights that the diets reconstruction and estimation were done correctly.

To maximize milk yield, fat, protein, and lactose content, to favour the rheological characteristics of milk, and to not exceed the limit of 30 mg/dl of urea, it was found that the average parameters

in the diets of Rossa Reggiana cows under heat stress should be between 23 and 25 kg of DMI, between 15 % and 22 % of starch, approximately 8% of sugars, between 12 % and 17 % of crude protein, and between 0.80 % and 1 % for Ca. The Lys/Met ratio should be no more than 3:1, as well as the forage to concentrate ratio should be between 1 and 2. Ideal reference values regarding EE were not found because the diets fed had low fat content and little variability.

This study represents a starting point for future works, which may involve the increase of the number of examined herds or the comparison of the results obtained during the summer season with those from other seasons, when heat stress conditions are absent or when fresh grass is not fed.

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Introduction

Parmigiano Reggiano is one of the most significant PDO products of the Italian dairy industry. It is produced exclusively in the provinces of Parma, Reggio Emilia, Modena, Bologna and Mantova, where specific climatic and environmental conditions, together with restricted cow feeding, play a key role (de Roest and Menghi, 2000). For the cheesemaking process, partially skimmed raw milk from the evening is mixed with whole milk in the morning and delivered to the dairy within 7 hours of milking (Malacarne et al., 2006). The production volume and the amount of milk used underscore the importance of this product, as indicated by CLAL data. Parmigiano Reggiano uniquely combines local agriculture, including animal farming and cultivation, with local craftsmanship in cheese production. This approach helps to preserve the landscape as it is recognized (de Roest and Menghi, 2000). The quality of the final product, Parmigiano Reggiano cheese, depends on the quality of the raw material (raw milk), which is enhanced through meticulous management of genetic, managerial, and nutritional aspects on the farm.

Milk is a white and turbid liquid consisting of different structural elements. It is not a uniform liquid, as it is formed by elements that establish relationships of emulsion, dispersion, colloidal solution, and true solution with water, that is the dispersing phase (Corradini, 1995). The milk used to make Parmigiano Reggiano cheese must have specific characteristics and an appropriate content of fat, protein, lactose, and ash. In fact, only raw milk, rennet, and natural milk enzymes can be used to produce Parmigiano Reggiano cheese, so the milk must possess the correct composition at the origin. Milk must have a high protein content, adequate fat and fatty acid content, good rennet properties and specific chemical flavours from local feed. These factors are influenced by genetics, age, lactation stage, season, climate, general management and health conditions, milking, and largely also by feeding (Bertoni et al., 2001).

Nutrition plays a key role in rapidly changing milk composition, although the relationship between diet and milk components is very complex. In particular, the dairy cattle nutrition most affects fat production and composition, but also partly affects protein production and composition. In contrast, lactose concentration may vary weakly in response to diet. There are many factors that must be considered in determining how ingested feed can change milk quantity and composition, but the greatest changes can be made by adjusting the amount of feed, composition, and the pattern in which meals are provided (Sutton, 1989). What most affects Parmigiano Reggiano cheese production is the amount and composition of casein in milk (Formigoni and Biagi 2007), so most studies have focused on this point. It is important to understand what diet is allowed in the Parmigiano Reggiano area, where dried forages, composed mainly of alfalfa and polyphytic cultures of stable meadows, assume great importance, as these are the ones that affect the composition and aroma of raw milk (Formigoni and Mordenti, 2004). The “Disciplinare di Produzione del Formaggio Parmigiano Reggiano” carefully regulates the dairy cows feeding, defining which foods are allowed and which are forbidden.

Heat stress is an important issue of current interest within the dairy industry due to the ongoing climate change, which is making some subtropical and Mediterranean areas much warmer than in the past (Summer et al., 2019). Also affected is the Padana Plain, the cradle of Parmigiano Reggiano cheese production (Coccimiglio et al., 2009). When cattle are subjected to temperatures above 25° C and effective cooling strategies are not implemented, they struggle to maintain body temperature within 38.5° C and begin to suffer from heat stress, decreasing milk production (Joksimović-Todorović, 2011; West, 2003). Milk composition is also affected by heat stress (Summer et al., 2019), as dry matter ingestion decreases and alterations occur in the gastrointestinal system (Kadzere et al., 2002). To cope with this issue, appropriate structural and nutritional strategies need to be implemented during the warmer months (Atrian and Shahryar, 2012). Above all, nutrition plays a pioneering role in this regard and must be adapted to the situation, using diets that are lower in fiber, more digestible and provided during the cooler hours of the day, while also allowing adequate water intake (Fantini, 2018). Heat stress affects high-producing cows the most, as they produce more heat during digestion (Joksimović and Todorović, 2011). In contrast, more rustic, low-producing breeds are more resistant to heat stress (Gantner et al., 2017). Due to greater heat resistance and considering milk production with distinctive characteristics, it would be useful to focus on studying local breeds.

Although today most of the cattle in the area belong to the Holstein or Brown Swiss breeds, to make Parmigiano Reggiano cheese an excellent product with a strong territorial link, it is important to revalue an indigenous breed such as the Rossa Reggiana cattle (de Roest and Menghi, 2000). This breed originally had a triple purpose and was in danger of extinction during the 1980s, but thanks to some interventions to enhance the breed and thanks to milk quality payment systems, today 4450 animals are registered in the Breed Book, mostly found in the province of Reggio Emilia (A.N.A.Bo.Ra.Re., 2023; Beretti et al., 2010). The Rossa Reggiana breed has an average production more than 30 % lower than that of the Frisona breed, but today it is considered as a breed with a purely dairy attitude, whose milk has specific characteristics that make it qualitatively better and more suitable for cheesemaking (Gandini et al., 2007). Although several studies have been conducted for the characterization of the breed from a genetic point of view, to better manage the breed's reproduction and recovery (Bovo et al., 2021), almost no studies have been carried out regarding the nutrition of the Rossa Reggiana breed and the correlation present between nutrition and milk quality. Considering this, the experimental part of this work will focus on a field trial aimed to investigate some aspects related to the feeding of Rossa Reggiana dairy cattle in the provinces of Parma and Reggio Emilia and their correlation with milk composition and quality during summer period, in presence of heat stress.

1. Dairy industry in the Parmigiano Reggiano cheese production area

The milk quality, characteristics and composition are important in the dairy industry, especially for a globally relevant product such as Parmigiano-Reggiano cheese, the most important typical product in Italian agriculture. This cheese can be produced only in in the Emilia-Romagna region and in a small part of Lombardia region, in the provinces of Parma, Reggio Emilia and Modena and parts of the provinces of Bologna (to the left of the Reno river) and Mantova (to the right of the Po river), according to an official decree of 1955. Parmigiano Reggiano has an inescapable link with its area of origin and is a PDO (Product of Designated Origin) product of excellence. To understand the importance of this production, it should be known that the entire production area covers 1.02 million hectares and contains 550,000 hectares of utilized agricultural land, including three main subareas, that are mountain, hill and plain (de Roest and Menghi., 2000).

Parmigiano-Reggiano is an Italian PDO semi-skimmed, hard, cooked and long-matured cheese and is the second national PDO cheese by tons produced. It is made with milk from cows fed mainly on fodder grown in the region of origin and following strict processing procedures. Only raw and unheated milk can be used. The use of additives is severely prohibited, and the diet of dairy cows is strict. Vat milk is the basis of the cheesemaking process and is obtained by mixing the evening milk (partially skimmed by natural creaming) and the next morning's whole milk. Maturation can last more than 24 months (minimum 12 months). Official Parmigiano Reggiano cheese production protocol requires that cows be milked twice a day and that the time between the start of milking and the delivery of milk to the dairy be less than 7 hours (Malacarne et al., 2006).

The distinctive sensory characteristics of PDO cheeses cannot be obtained under different environmental-productive conditions, because some milk characteristics are related to specific animal production systems and cheese ripening is influenced by the interaction between the milk (specific) and the traditional technology applied to the processing process (non-specific). Furthermore, the environment for a good ripening stage can be quite specific and not-reproducible. With reference to milk, the typicality factors are species and/or breed, pedo-climatic conditions, animal management system and especially feeding. Other factors that influence cheese quality are milk treatments, milk processing and ripening procedures. However, the secret to the success of PDO cheeses is the combination of modern technology and tradition, with the aim of adapting the product to market demand without losing specificity, originality and authenticity (Bertoni et al. 2001).

The supply chain of this famous PDO cheese is composed of farmers, dairies and wholesaler-agers. It is governed by a third-party institution, the "Consorzio del Formaggio Parmigiano Reggiano" (CFPR), which is responsible for establishing common rules for all members of the chain and for exercising control and promotion of the product on the market (Giacomini et al., 2010).

1.1 Parmigiano Reggiano cheese production and market data

The total number of dairy cows in the Parmigiano Reggiano area is 336,000, equivalent to 15% of the entire Italian dairy cattle herd. In the 1950s, many Dutch Friesian cattle were imported, which, starting in the 1970s, began to completely replace local breeds. Currently, Italian Friesian cows represent 85% of the cows in Parmigiano-Reggiano farms. About 60% of the semen used in Italian Friesian cow herds comes from Holstein Friesian bulls. There is also a substantial number of Italian Brown Swiss cows (15%). Finally, two native breeds, Rossa Reggiana and Bianca Modenese, are relatively insignificant compared to the total herd size. However, because of EU restrictions on milk production (milk quotas) and the introduction of a milk quality payment system for Parmigiano-Reggiano, there is new interest in cattle breeds that produce high-quality milk (Malacarne et al., 2006).

According to CLAL data and to Parmigiano-Reggiano cheese supply regulation plan 2023-2025, drawn up by the "Consorzio del formaggio Parmigiano Reggiano", 4.002.318 Parmigiano-Reggiano wheels were produced in 2022 in 295 dairies spread throughout the territory, for a total of 160.097 tons of cheese. In recent years, the number of dairies has decreased (- 43% from 1993 to 2008), but the total quantity of processed milk has increased, leading to an increase in the average size of the dairies (Giacomini et al., 2010). While in 2015 the average size of cheese factories was 9,300 wheels/year, in 2020 it reached 12,300 wheels/year, so the average size of productive dairies in just 5 years has increased by 32%. The dairy farms in the Parmigiano-Reggiano district are 2543, divided in 915 mountain dairy farms (36%) and 1628 plain dairy farms (64%). The price of Parmigiano-Reggiano cheese changes from 10 euros per kg in a price crisis period to 15 euros per kg when supply is insufficient to cover the demand. Over 75% of Italian milk production is destined for cheesemaking and Parmigiano Reggiano production absorbs about 18% of the volume of milk produced in Italy, the equivalent of 2.025,9 tons of milk in 2020. Most farmers Parmigiano Reggiano area use their milk for the Parmigiano Reggiano cheese production and only 0.42% of milk is for other uses. The demand for Parmigiano-Reggiano cheese in 2020 was 58% Italian and 42% foreign, so the export of this product plays a key role in the Italian dairy industry. Parmigiano-Reggiano and Grana-Padana cheeses represent 74.3% of the total Italian PDO cheese production and show an annual average revenue from their export equal to 734 e/ton (Lovarelli et al., 2019).

1.2 Parmigiano Reggiano production system

The Parmigiano Reggiano production system is an eloquent example of rural development because of its great impact on rural employment and the maintenance of labor-intensive artisanal production techniques on which the final quality of the cheese depends. It is an excellent example of a European PDO product, that has been able to secure a large and successful market. The production of Parmigiano Reggiano owes its success to compliance with a specific production disciplinary, that directs the production processes and compulsorily affects production factors and managerial choices of farmers and producers. This differentiates the production and processing of Parmigiano Reggiano milk from the milk produced and processed in industrial dairies. The success of this system depends mainly on the fact that consumers appreciate it and are willing to pay more for a high-quality product. The milk price of Parmigiano Reggiano is always higher than that of industrial milk, except in years when there is an overproduction of cheese and prices fall. The production cost of milk for Parmigiano Reggiano is about 20 % higher than that of industrial milk. Silage is banned on Parmigiano Reggiano farms, so farmers rely on concentrates for a larger share of the cows' ration. Hay harvesting requires machinery and manpower with higher labour intensity per unit of food energy than silage production. On the other hand, on industrial dairy farms, the variable cost of growing corn is higher than that of alfalfa. To compete in the international milk market, industrial farms are generally larger than Parmigiano-Reggiano farms, and Parmigiano Reggiano farms employ more labour units than industrial farms. However, it is necessary to try to reduce costs to avoid developing too large a cost-price difference between the real PDO product and industrial substitutes. In fact, the main threat to the production system of Parmigiano Reggiano is the risk that its characteristics will become indistinguishable from those of its main market competitors, such as Grana Padano and other Grana-type cheeses, undermining the degree of typicality and exclusivity of the product. To avoid this, it is possible to produce new niche products, partly overlapping and partly distinct from the Parmigiano Reggiano markets. One successful example is Parmigiano Reggiano made with milk from cows of the Rossa Reggiana breed, an ancient and traditional breed, which is priced about 50% higher than regular

Parmigiano Reggiano. The use of biological milk in Parmigiano Reggiano production is another attempt to capture a niche market (de Roest and Menghi, 2000).

To obtain a quality final product, it is necessary for cows to produce milk, the raw material, of high quality in terms of composition. In fact, a specific composition of milk is required by dairy industries to produce Parmigiano Reggiano cheese, otherwise a valid product is not obtained. To do this several requirements must be observed and certain aspects that directly affect milk production and quality, including feeding, can be modified.

2. Importance of raw milk composition on Parmigiano Reggiano cheese

The relationships between dairy farm practices, raw milk composition and properties and the quality of the resulting cheese are complex. The quality and characteristics of cheese depend largely on the composition and properties of the raw milk, which are related to many farm factors. In PDO cheese production, knowledge of the raw materials is of paramount importance. In fact, the link between the quality of raw milk and the quality of the final product is very strong for PDO cheeses. This underscores the importance of knowledge of raw milk supply. Specific milk production conditions should be considered when milk is intended to produce cheeses with unique characteristics. Scientific identification of these conditions would improve the current understanding of the complex associations between raw milk quality and farm and management factors. Recent decades have witnessed a rapid intensification of the dairy industry, with dairy farms becoming larger and larger. Herd size has increased and there has been an increase in milk production per unit of input, such as feed, labor, or land. In parallel with the ongoing changes in on-farm dairy production, the research community has reported interactive and often confounding effects of multiple farm factors on the composition and properties of raw milk (Priyashantha and Lundh, 2021).

To best understand how raw milk can affect the characteristics of Parmigiano-Reggiano cheese, it is necessary to start by analysing the composition of cow's milk, with particular interest in those parameters that can vary the cheesemaking and characteristics of Parmigiano-Reggiano cheese.

2.1 Cow milk definition and composition

The free dictionary simply defines milk as a whitish liquid containing milk protein, fat, lactose, and various vitamins and minerals, produced by the mammary glands of all adult female mammals after giving birth and used as food for their young (Guetouache et al., 2014). The composition of milk varies from species to species, and this chapter will consider the composition of cow's milk, which is useful to produce dairy products, in the specific case of Parmigiano Reggiano cheese.

Milk is formed in the glandular epithelium of the mammary gland. Only a few of its constituents are derived directly from the blood, while the constitution of most components occurs in the mammary gland during secretion. The mammary gland is an organ influenced by a complex neuroendocrine control, which acts on the myoepithelial secretory cells located in the alveoli that form the lobes of mammary gland. They pour their secretion into the various lumens, influenced by the presence of oxytocin, which allows their contraction and the release of milk. The lobes ducts flow into the gland's cistern, which is connected to the nipple sphincter, from which milk ejection occurs. In addition to oxytocin, other hormones are involved in milk production, such as prolactin, somatotropin, and lactogenic placental hormone (Corradini, 1995). Also, exogenous hormones have a dramatic effect on milk yield, but it hasn't yet been established how effectively they can be used to alter milk composition (Sutton, 1989).

The primary osmotically active milk component is lactose. It is produced in the Golgi, secreted in vesicles, and then those vesicles travel to the apical membrane where they release their contents into the alveoli. The amount of lactose in milk remains remarkably constant because lactose is osmotically active, but it cannot exit the Golgi or secretory vesicles. The endoplasmic reticulum is the primary site of synthesis for milk proteins and milk fat triglycerides. The protein enters the Golgi where it is secreted alongside the lactose in the same vesicles. However, the fat droplets travel to the apical membrane and are secreted as milk fat globules into the alveoli. The secretory pathway for lactose and protein is therefore the same, but it is quite different from that for fat (Sutton, 1989).

Milk appears as a uniform, white and turbid liquid. It is not a homogeneous liquid because it consists of different structural elements, which establish with the dispersing phase (water) relationships of emulsion, dispersion, colloidal solution, and true solution. Under the optical microscope, spherical droplets can be seen rotating in a still turbid liquid, called plasma. With the electron microscope, on the other hand, it is possible to distinguish casein micelles and submicelles (spherical protein particles) and fat globules, covered by a membrane. If the fat globules and protein micelles/submicelles are removed, whey is obtained, an opalescent liquid containing globular proteins, lipoprotein particles and, in true solution, the other components of milk, such as carbohydrates (lactose). In relation to water, the fat globules are in an emulsion state and the casein micelles in a pseudocolloidal dispersion, while the whey proteins and lipoprotein particles are in solution and colloidal dispersion respectively (Corradini, 1995).

In general, at the macronutrient level, cow's milk is typically composed of water (85-87%), fat (3,8-5,5%), protein (2,9-3,5%) and carbohydrates (5%). At the micronutrient level, bovine milk contains many bioactive compounds, including vitamins, minerals, biogenic amines, organic acids, nucleotides, oligosaccharides, and immunoglobulins (Foroutan et al.,2019). In the Italian Frisona breed, the most common breed in the Parmigiano Reggiano production area, the fat content of milk is 3,7%, the lactose content is 4,8%, the total protein content is 3,2% and the ash

content is 0,65 percent (Corradini, 1995). Specifically, today Friesian cows bred in Italy subjected to functional controls produce an average of 99 quintals per lactation, with 3,73% fat and 3,33% protein, compared to 71 quintals with 3,51% fat and 3,08% protein in 1991 (Canavesi, 2018).
The technology applied to most PDO cheeses, such as Parmigiano Reggiano cheese, uses only raw milk, rennet, and natural lactic acid bacteria, so the milk must be suitable for processing at origin. The specific milk characteristics that ensure a high success rate for PDO cheeses are a high protein content, good rennet properties, adequate fat content with appropriate fatty acid composition and the presence of chemical flavours from local feed. In addition, appropriate microflora is also of great importance. Factors that contribute to obtain milk suitable for processing into PDO cheese include genetics, age, lactation stage, season, climate, general management and health conditions, milking, and especially feeding. These aspects influence nutrient availability, endocrine response, health status and the presence of microbes and chemicals that enrich or reduce milk-cheese quality. Many of these factors are regulated by producer associations (Bertoni et al., 2001).
Cheese making ability depends on quantitative and qualitative characteristics of milk. The most important variables in milk composition are casein and fat contents (Buchberger and Dovc, 2000). The next subchapters will consider the milk components that most influence the cheese yield and chemical and physical characteristics of Parmigiano Reggiano cheese. More specifically, are considered fats, proteins and carbohydrates contained in milk, but also minerals and somatic cells. These components are also the most easily modified through some external factors.

2.2 Milk lipids

Adequate percentages of fat in milk and correct casein ratios are safety and quality factors for Parmigiano Reggiano cheese production, as the speed and effectiveness of skimming for milk cleaning, cheese yield and ripening characteristics are affected. In particular, the percentage of fat in milk should be 3.5%-3.7% or more. The quali-quantitative composition of fat in milk is also important in conditioning the flavour and taste of milk (Formigoni and Mordenti, 2004). Fat plays a passive role during the coagulation process, as fat globules are entrapped in the para-casein matrix and thus positively affects cheese yield and the recovery of stir-out time and energy in the curd (Stocco et al., 2021).
With more than 400 different fatty acid species, milk and dairy fat is the most complex fat in the human diet. Short, medium, and long-chain fatty acids, odd-chain fatty acids, branched chain fatty

acids, conjugated linoleic acids (CLA), ruminal trans fatty acids (vaccenic acid), n-3 and n-6 fatty acids are all present in dairy fat (Bertoni et al., 2001).

The lipids in bovine milk are mainly present in globules as an oil-in-water emulsion. These fat droplets are formed by the endoplasmic reticulum in the epithelial cells in the alveoli and coated with a surface material of proteins and polar lipids. When secreted, they are enveloped with the plasma membrane of the cell. The milk fat consists mainly of triglycerides, approximately 98%, while other milk lipids are diacylglycerol (about 2% of the lipid fraction), cholesterol (less than 0.5%), phospholipids (about 1%) and free fatty acids (FFA) (about 0.1). In addition, there are trace amounts of ether lipids, hydrocarbons, fat-soluble vitamins, flavour compounds and compounds introduced by the feed. The milk fat globule size (MFG) increases with increasing fat content in the milk, probably because of a limitation in production of milk fat globule membrane (MFGM). The size of the MFG has crucial influence on the stability and technological properties of milk. Milk fat globules are trapped during the formation of curd and therefore milk with higher fat content results in better cheese yield (Månsson, 2008).

2.2.1 Milk fatty acids

The milk fatty acids (FA) are derived almost equally from two sources, the feed and the microbial activity in the rumen of the cow. Many factors are associated with the variations in the amount and fatty acid composition of bovine milk lipids. They could be of animal origin, associated with genetics (breed and selection), lactation stage, mastitis, and ruminal fermentation, or they could be feed-related factors, associated to dietary fats, fiber and energy intake, as well as seasonal and regional effects. The saturated fatty acids present in milk account for approximately 70% by weight (palmitic acid 30%, myristic acid 11%, stearic acid 12%, butyric acid 4,4%, caproic acid 2,4%). Approximately 25% of the fatty acids in milk are mono-unsaturated (oleic acid 23,8%) and poly-unsaturated fatty acids, they constitute about 2.3% by weight of the total fatty acids (linoleic acid or omega-6 1,6%, α-linolenic acid or omega-3 0,7%). Approximately 2.7% of the fatty acids in milk are trans fatty acids with one or more trans-double bonds (vaccenic acid or VA, 2,7%). Milk fat contains even conjugated linoleic acid (CLA), with many different isomers, including rumenic acid (RA) (Månsson, 2008).

In the dairy cattle case, the FA profile is also seen as an important factor in the technological quality of raw milk. Therefore, the FA profile has the potential to contribute significantly to the production of higher value-added dairy products. As such, FAs also have an economic importance.

Current developments in analytical methods and their increasing efficiency make it possible to study milk FA profiles not only for scientific purposes but also in terms of practical technological applications. It is crucial to study the factors that affect FA variability in milk, such as population genetics, farming practices, and specialized animal nutrition. It is equally important to study the health and technological impacts of FAs (Hanus et al., 2018).

2.3 Milk protein

The nitrogen components importance in milk for transformation into Parmigiano Reggiano cheese has always been largely recognized. This is because the casein fractions quality and quantity affect the cheese yield, but also numerous milk dairy properties, such as its response to rennet and the characteristics of the curd (Formigoni and Mordenti, 2004). Caseins have an active influence on the coagulation pattern, they increase the firming speed and firmness of curds and the cheese capacity, with a high cheese yield of processed milk (Stocco et al., 2021).
Proteins are the most valued milk components. Milk proteins belong to two main families. The first is composed of caseins (CN, as αs1, αs2, β and κ), which constitute approximately 80% of true protein. The other 20% comprises soluble or whey proteins, essentially composed of β-lactoglobulin (β-lg), α-lactalbumin, serum albumin, phosphoglycoproteins, transferrin, lactoferrin, plasmin/plasminogen, lactoperoxidase and immunoglobulins. Most of these proteins have several genetic variants. Milk soluble proteins have high nutritional value, but only caseins are of concern to cheesemakers, because cheese yielding properties depend mainly on milk casein content. All the casein proteins, as well as α-lactalbumin, β-lactoglobulin, lactoferrin, and lactoperoxidase, are produced in the mammary gland, whereas the immunoglobulins, bovine serum albumen and plasmin/plasminogen derive from the circulation. Another nitrogen fraction includes non-protein N, composed by predominately urea and peptides with trace amounts of ammonia, creatinine and hippuric acid.
It is important to determine the range of the casein proportion in milk true protein and the factors causing it to vary in countries where large amounts of milk are processed to make cheese, especially since some cheesemakers claim to have observed that the increase in true protein content does not increase cheese yield proportionally. In practice, it can be estimated that cheese yield increases by approximately 7% when the casein/protein ratio increases by 10 g/kg (Coulon et al., 1998; Walker et al., 2004).

2.3.1 Lactoproteins genotypes and characteristics

The casein quantity (CN) and, specifically, the coagulating properties of milk proteins have a significant impact on the ability to make cheese. Casein content and significant variations in the coagulating behaviour of various lactoprotein genotypes are both important determinants of the coagulating properties themselves. This is supported by the fact that there are more variations in the ability of different lactoprotein genotypes to produce cheese than would be predicted only by their quantitative differences. Specifically, quantitative-qualitative variations in casein have a major impact on the industrial performance of dairy processing and, at the same time, are capable of significantly affecting some of the rheological characteristics of the curd, with direct repercussions on the structure of the paste and the quality of the cheese.

Caseins are found dispersed in milk in the form of micelles, held together by ionic bonds with calcium. The colloidal milk fraction is structured in micelles and consists mainly of caseins and in a small amount of colloidal calcium phosphate. It constitutes the casein mass entirely. Colloidal calcium phosphate is intimately associated with casein and is essential for maintaining the structure, playing an important role in all stages of the coagulation process. The structure of the micellar system of milk is maintained by concentration, breakdown and genetic type of caseins, colloidal calcium phosphate and calcium caseinate, in close dynamic relationship with the soluble phase of milk. Even small variations can exert an important influence on the aggregation state of the micellar system.

The most common alleles of the chromosomal loci of αs1-casein are B and C, while the most common variant of αs2-casein is A. β-casein includes A1, A2 and B as common alleles, and κ-casein comprises the A and B variants. The major variants of β-lactoglobulin (LG) are A and B, and that of α-lactalbumin is B.

The polymorphism of αs1, β and k caseins and β-lactoglobulin constitutes an important aspect in determining milk quality. Qualitative variations, related to the nature of the genetic mutation itself, and quantitative variations, due to the different synthesis capacity of the individual alleles controlling the production of these proteins, may be more or less important, both from a technological point of view and from the point of view of industrial processing performance. Variations in caseins exert a non-secondary influence about the presamic coagulation characteristics of milk, especially with reference to those of k-casein and β-casein.

The ratio between the casein amount and total amount of proteins in milk multiplied by 100 is defined as casein number. This is an important parameter for the cheese-yielding capacity of milk.

Numerous studies have been published on the association between β-LG genotypes and casein number and all publications have confirmed that milk containing β-LG BB has approximately 3 % (absolute) higher casein number than milk containing β-LG AA. In fact, β-lactoglobulin A type cows provide milk that is more supplied with whey protein, while β-lactoglobulin B type cows produce milk that is on average richer in casein (100 g casein per quintal), with significant effects on dairy processing yield. Nearly all investigations in Holstein Friesian and related breeds of the association between κ-CN genetic variants and milk composition demonstrated that κ-CN BB milk has a higher content of protein and casein than κ-CN AA milk.

Rennet clotting time (RCT), curd firming time (K20) and curd firmness (CF or A30-A60) represent the coagulating properties of milk. Milk containing k-casein B manifests greater reactivity with rennet and better aptitude for clot formation. In fact, from most of the published data it may be concluded that milk containing κ-CN BB has significantly shorter RCT and higher CF than κ-CN AA milk. Also, β-CN B milk tends to coagulate in a shorter time than β-CN A milk, with important effects on the speed of clot formation. The genetic type of k-CN is known to exert a significant influence on micelle size. Specifically, k-CN B involves a higher proportion of k-CN, which in turn results in smaller micelles. Systems marked by higher proportions of smaller micelles tend to clot in less time and provide clots that can firm faster and have greater strength. In addition, it has been observed that milk containing κ-CN BB showed 8.5 % higher cheese yield than κ-CN AA milk when producing Parmigiano-Reggiano cheese.

In summary, κ-CN B and β-LG B are the most advantageous variants in terms of the ability to produce cheese. Although all lactoprotein genotypes may be more or less correlated with some parameters that affect cheese production, it can be inferred that the most interesting genotypes for the production of Parmigiano-Reggiano cheese are κ-CN BB and β-LG BB (Buchberger and Dovč, 2000; Mariani, 2011).

2.4 Milk lactose

The most abundant and important carbohydrate in milk is lactose, a disaccharide consisting of two monosaccharides, glucose and galactose. The content of lactose in milk is of interest in milk for fresh consumption, because it gives it a more pleasant flavour, whereas this parameter does not appear to be of fundamental importance for dairy processes (Formigoni and Mordenti, 2004). Lactose is the main carbohydrate in mammals' milk. It allows the osmotic equilibrium between blood and alveolar lumen in the mammary gland. It represents the major bovine milk solid and its synthesis and concentration in milk are influenced mainly by udder health and the cow's energy balance and metabolism. There are several biological and physiological aspects related to this milk compound. Additionally, lactose is a crucial food component and provides to the energy value of milk.

Lactose is synthesized in the udder from blood glucose absorbed by the basal membrane of mammary epithelial cells. Around 20% of the circulating blood glucose of a dairy cow is converted into lactose during lactation. It is the main osmotic regulator between the blood and alveolar lumen, and it rules the amount of absorbed water in the alveoli and the volume of produced milk. As soon as lactose is synthesized by the Golgi, it is packed into secretory vesicles. This disaccharide cannot pass through the vesicle membrane, so it determines a strong osmotic pressure and water is required to get into the secretory vesicles and re-establish equilibrium (Costa et al., 2019).

In the early hours after the cheese making process lactose disappears. The fermentation of lactose into lactic acid and the subsequent acidification of the curd is one of the most important processes in the production technology of Parmigiano Reggiano. So, Parmigiano Reggiano can be defined as a 'lactose-free' product (Summer et al., 2017).

2.5 Milk minerals

Minerals represent a small proportion of milk composition (about 0.7%). They are differently involved in milk processing, during milk coagulation and the other phases of the cheese-making process. The mineral fraction contains cations (calcium, magnesium, sodium and potassium) and anions (inorganic phosphate, citrate and chloride). These ions play an important role in the structure and stability of casein micelles. Depending on the type of ion, they are diffusible (sodium, potassium and chloride) or partially associated with casein molecules (calcium, magnesium, phosphate and citrate), to form large colloidal particles (caseins micelles). This composition is considered as relatively constant, but slight variations can be observed in some cases. For example, calcium and phosphate are higher in milks rich in proteins, but also the time of the lactation period varies the concentration of minerals, especially around parturition (calcium concentration in colostrum is much higher). In addition, mineral composition is also modified during mastitis (concentrations of sodium and chloride ions are strongly increased) and the content of some minerals in milk is highly correlated with other milk components, particularly casein.

For the differentiation of milk to produce high-quality natural products, such as PDO cheeses, is important to consider the natural variations in milk minerals, their interactions with each other and also their relationships with coagulation and the cheese-making process, especially when product specifications and restrictions prohibit milk treatments and the addition of minerals before and during cheese processing. It's important to know which minerals are involved during the cheesemaking processes and in which way (Gaucheron, 2005; Stocco et al., 2021).

2.5.1 Principal minerals involved in dairy process

Today is known that there are relationships between coagulation ability, mineral contents, and cheese-making traits of cow's milk. Calcium is one of the most important minerals in milk. It is present in ionic form in the aqueous phase, while in the micellar phase Ca is bound to phosphoseryl residues of casein molecules and inorganic phosphate. Its presence is essential to the protein particles structure and to their technological functionality. High Ca content promotes water retention in the curd, so has a positive effect on both the coagulation pattern and cheese-making traits. It is the mineral with the greatest effect on milk quality and technological properties.

Phosphorus is present in milk as organic (bound to casein) and inorganic phosphates (ions). Phosphorus positively affected the cheese-making traits, increasing cheese yield in terms of curd solids and all the nutrient recovery traits. Although, a very high P content in milk is associated with less fat recovered in the curd. It has the second largest effect on milk quality and technological properties after Ca.

Sodium is present in milk mainly in the aqueous phase, where it is free or weakly associated with ions of the opposite charge. Na contributes to the ionic strength of milk, together with K and Cl. A higher milk Na concentration than normal is often indicative of an inflammatory process affecting the mammary gland, because Na is in osmolar equilibrium between milk and blood. This is associated with increased solubilization of casein and proteolytic activity in milk. While protein recovery is negatively correlated with high concentrations of this mineral, probably reflecting the correlation with subclinical mastitis, coagulation is only marginally affected by changes in the Na content of milk.

Potassium is a monovalent ion. Together with Na and Cl, it contributes a quarter of the osmolality of bovine milk. The mammary cell's secretion mechanisms mainly control the concentration of potassium in milk, which is closely correlated with the balance of glucose and electrolyte

metabolism. K appears to play a more passive role in the coagulation and cheese-making processes. It is related to the salt equilibrium in milk overall and to milk composition.
The technological importance of Mg in milk is largely eclipsed by Ca, which plays an essential role in the structure and stability of casein micelles. However, these 2 minerals act cooperatively during coagulation. High Mg content tends to slow coagulation and reduce cheese yield traits (Stocco et al., 2021)

2.6 Milk somatic cells

The term SC refers to cells derived from the organism, and which are normally present at low levels in milk. These are mostly cells of the secretory tissue of the udder (epithelial cells), the presence of which is a physiological phenomenon, due to desquamation and epithelial regeneration of alveoli and ducts, to which leukocytes (white blood cells) are added to a lesser extent. Mastitis, caused by any intramammary infection, leads to an increase in leukocytes and consequently somatic cells in milk, with a decrease in the quality of milk produced.
Somatic cells are quantified as the number of cells per ml of milk. Cows with more than 200,000 SC/ml of milk are likely to be infected in at least one quarter. The increase in cell count is directly related to the severity of infection and the number of infected quarters in a cow. Selection of dairy animals for higher milk production and removal of milk by mechanical milking impose unnatural stress on the cow's udder, with increased probability of mammary gland infection and increased SC. Milk somatic cell count (SCC) is used as a marker to monitor the prevalence of mastitis in dairy herds, as an indicator of raw milk quality for processors, and also as a more general indicator of the hygienic conditions of milk production on farms. Somatic cell values above 200,000 units/ml represent a detriment to the dairy farmer due to lower milk production from infected quarters, lower milk yield, and increased sanitary thickness (Alhussien and Dang, 2018). Current legislation (Presidential Decree 54/1997) states that the maximum limit of somatic cells in bulk milk is 400,000 cells/ml (Cevolani, 2014).
Somatic cells in milk are influenced by the productivity of the cow, her health (presence of mastitis), lactation stage and the animal's breed. Any change in environmental conditions, poor management practices and stressful conditions significantly increase the amount of SC in milk. Better hygiene and proper feeding help reduce SC in milk. Milk with low SC means better dairy products with a longer shelf life (Alhussien and Dang, 2018). There is normally an increase in SC in the immediate postpartum period, for a situation that, within limits, can be considered

physiological, due to a normal increase in immune defences. It is also possible to find an increase in late lactation, especially in particularly protracted lactations, due to obvious stress on the udder apparatus. Finally, SCCs are positively correlated with the number of lactations. High temperatures with high humidity rates promote the occurrence of mastitis, both because the cow is in a physically unfavourable condition and because high temperature and humidity promote bacterial development, especially if hygienic conditions are not adequate. The deterioration of the milking equipment multiplies the critical points, which are vacuum variations, irregular pulsations, wear and tear of teats and tubes, and poor cleaning. In milking technique, critical points are represented by pre- and post-dipping, elimination of first jets, management of groups of animals with mastitis. Abrupt changes in the type of herd management should be considered stressful events for the cow and may result in increased SCC. These can include changes in groups, with altered social balances, drastic dietary changes or poorly stored or mouldy feed. Nutrition plays a marginal but not insignificant role in the occurrence of mastitis. From the nutritional point of view, what can be done is to try to administer balanced diets, reduce the negative energy balance during early lactation, prevent and control dysmetabolism, pay attention to food quality (mycotoxins), and administer trace elements and vitamins with immune stimulating (Cu, Se, Vit. A and E) and epithelial-protective (Zn and biotin) action at dosages above requirements.

SC content is inversely proportional to milk production. For values above 200,000 cells/ml, there is already a production decrease of about 5%, to even more than 25% with cells above 1,500,000/ml. A cell content above 200,000/ml is already to be considered an alert situation. Therefore, the benefit of keeping somatic cells in milk as low as possible not only relates to the constraint of current regulations, but also has a direct significance on farm productivity. It is important to intervene on the cause and not on the effect, preventing the onset of mastitis, with constant monitoring of SCCs in bulk milk and in each individual cow, performing bacteriological examination and antibiogram before treatment (Cevolani, 2014).

The high somatic cell content of milk has a significant effect on milk yield, protein and lactose, while the effect on fat composition is not as significant. In the milk of infected cows, a total increase in protein is observed, while there is a decrease in the percentage of lactose. Lactose is responsible for 50% of the osmotic pressure in milk, and a decrease in its level causes a drastic reduction in milk production and an increased displacement of ions from the blood to the milk to maintain osmotic balance. Total protein in milk increases due to an increase in whey protein, while caseins tend to decrease. A decrease in β-, α- and κ-casein fractions can be observed, and this affects the cheesemaking process. These changes may be due to the regulation of lactation-related genes in response to infection and induced hydrolysis of milk proteins. In addition, the increase in protein is due to alteration of the integrity of the mammary epithelium by microbial toxins and opening of tight junctions as a result of damage to the mammary epithelium, with influx of blood-derived proteins into the milk. In milk, there is also an increase in the caseinolytic enzyme plasmin, which is derived from plasminogen, which originates from the blood and probably spills into milk due to disruption of the epithelium. Plasmin cleaves β-casein into casein fragments and smaller polypeptides that diffuse into the milk. This leads to poor curdling, lower cheese yield, and a bitter taste in dairy products, which has a negative effect on cheese production. The decrease in fat concentration in cows with mastitis and high somatic cell content may be due to the reduced synthetic and secretory capacity of the mammary gland. An increase in free fatty acids is observed due to disruption of the milk fat globule membrane by the enzyme lipase produced by leukocytes or plasmin through lipoprotein hydrolysis. Sodium and chloride, which are present in high amounts in blood, also increase beyond the normal concentration in milk (Alhussien and Dang, 2018).

3. Nutritional effects on the modulation of milk composition

To the dairy farmer, manufacturer, and consumer the composition and functional properties of cow's milk are of considerable importance. Basically, three are the options for changing the composition and/or functional properties of milk: cow genetics, cow nutrition and management and dairy manufacturing technologies (Walker et al., 2004). Therefore, the two main strategies a farmer can use to change the composition of milk are nutrition and breeding. Traditional breeding methods produce slow compositional changes, even if new methods of genetic manipulation might enable more rapid development. Nutrition, on the other hand, often results in rapid changes, making it a more appropriate way to address the quickly evolving market requirements of the present. In response to some dietary manipulations, such as severe underfeeding, milk composition can change. Despite this, nutrition does not seem to have been considered until relatively recently as a useful or reliable way to manipulate milk composition.

Although the relationship between feed components and milk composition is complex, feeding offers a way to quickly change milk composition. The nutrients that serve as either direct or indirect precursors to the main milk solids are supplied by feed. Milk fat concentration is the area where the greatest changes can be made, over a wide range of about 3 percentage units. Changes in milk protein concentration, on the other hand, can potentially be much smaller, just over one-fifth percentage units. The effects of supplements and lipid intake are best documented, while milk protein and fat concentrations are only slightly affected by dietary protein. It is known that adding fat to the diet may cause milk fat concentration to decrease and increasing dietary protein while maintaining a constant energy intake typically has little to no impact on milk protein concentration. In response to diet, very small changes in lactose concentration occasionally occur, but they are unpredictable and have no practical application. The amount of roughage, forage/concentrate ratio, carbohydrate composition, lipids, intake, and frequency of meals are the most important dietary factors. Specifically, the biggest changes can be made by adjusting the feed's quantity, composition, and meal pattern (Sutton, 1989).

Because more than 60% of milk produced in Italy is transformed into cheese, milk economical strongly depends on cheese yield. Key factors affecting cheese yield include the content of casein and fat in the milk. Particularly for Parmigiano Reggiano and Grana Padano cheeses, one gram of casein results in the production of three grams of aged cheese. Since casein and, to a lesser extent, fat determine the economic value of milk, increasing the production of these two components is an essential goal for dairy farmers. Even though casein and milk fat are mainly influenced by genetic factors, proper management and feeding strategies can increase their concentration in milk (Formigoni and Biagi, 2007).

The next subchapters will consider the dairy cattle ration, the nutritional parameters of dairy cattle feeds and the effects of feeding on the main components of milk, evaluating the major dietary factors that have been shown to affect milk composition, specifically to produce Parmigiano Reggiano cheese.

3.1 Dairy cattle ration in the Parmigiano Reggiano cheese production area

The dairy cows diet normally consists of a mixture of fresh forages, mainly leaf blades, hay forages (leaf blades and stems in spring, seeds and stems if in the form of forage maize) and concentrates (plant seeds). Different seasons and regions of the world change significantly the diet composition of dairy cattle (Elgersma et al. 2006). According to the "Disciplinare di Produzione del Formaggio Parmigiano Reggiano", the rationing of dairy cows in the Parmigiano Reggiano production area is based on the use of forages from at least 75% from the Parmigiano Reggiano production area and at least 50% from farmland. At least 50% of the dry matter in the daily ration must be provided by forages, preferably dried (hay). The basic ration, consisting of forages, must be conveniently supplemented with concentrates, that can balance the intake of the various nutrients in the diet, and the dry matter of the feeds must not exceed that contributed by forages (ratio of forages/concentrates not less than 1). On the other hand, in the diet of cattle in the Parmigiano Reggiano area the use of silage is prohibited, as well as foods that can transmit abnormal aromas and flavours to milk and alter its technological characteristics, foods that are sources of contamination and foods in bad state of preservation. Fats and the use of many products and by-products widely used in other production chains are also prohibited. Finally, limits are placed on the use of all raw materials, especially protein foods (Formigoni and Mordenti, 2004).

According to a study by Dado and Allen (1995) on primiparous and multiparous cows, the average dry matter intake (DMI) of a cow is 22.8 kg, for an average production of 33.1 kg of milk. According to Formigoni and Mordenti (2004) with unifeed it is possible to reach 25-26 kg of DMI, with an average milk production of 33-40 kg/day. In the Parmigiano Reggiano area among forages alfalfa prevails, which can be used between 7 and 14 kg/head/day. It constitutes the main nitrogen source and comes from multiple cuts. However, if alfalfa is used as the sole source of forage, the balance of the ration cannot be maintained, because alfalfa has rapid fermentation in the rumen, with excessive increase in transit rate, alteration of ruminal and intestinal function and excess degradable nitrogen. The solution is to use grass forages between 2 and 6 kg/head/day. Instead of grasses, discrete amounts of wheat straw, although it is poorly digestible, or wheat hay can be used. When alfalfa is present in high amounts in the ration, the need to use different protein sources becomes minimal (Formigoni et al., 2010). According to the "Disciplinare di Produzione del Formaggio Parmigiano Reggiano", fresh fodder obtained from natural meadows, stable polyphytic meadows, alfalfa and clover grass meadows may be fed to dairy cows. Grasses of ryegrass, rye, oats, barley, wheat, granturchin, fallow sorghum, panicum, mazzolina grass (Dactilis), fescue, timothy (Phleum), sulla, and sainfoin, administered singly or in combination with each other, may be used, as well as grasses of pea, vetch, and field beans, provided they are combined with at least one of the forage essences mentioned above. The hays of the aforementioned fodder essences must be obtained by field drying or by forced ventilation (aero-drying with temperatures below 100°C). Chopped fodder obtained from the whole corn plant with milky-waxy or waxy maturity can also be fed immediately after harvest. Finally, cereal straw may be used, with the exclusion of rice straw. For a maximum dose of 2 kg/head/day, fodder treated with a temperature of 100°C or more can also be provided, except for chopped corn.

For cheeses made from raw milk such as Parmigiano Reggiano, the production of quality forages is the most effective way to improve production performance and enhance milk quality characteristics. Forages represent the main way in which the territory marks the typicality of the final product, conditioning the barn microbial ecosystem. They also play an irreplaceable role in promoting chewing, rumen motor and fermentative efficiency and cow welfare. The availability and use of forages is critical to provide degradable fiber, significantly promote daily feed ingestion and formulate rations with less feed to satisfy the imposing needs of cows producing high amounts of milk. By excluding silage and only using green and hay forages, environmental contamination by spores is drastically limited and the presence of bacteria useful for cheesemaking processes and cheese ripening is enhanced. In addition, rations based on the predominant use of forages, improve the ecosystem of the rumen and intestine, positively condition the microbiological endowment of milk and its cheesemaking characteristics and reduce the number of spores eliminated with faeces.

Covering the impressive nutritional needs of high-producing dairy cows requires the utmost care in the selection of forages to be included in rations, which must be done based on sensory and chemical analyses, which provide an estimate of the nutritional value of forages. Laboratory analyses are needed to estimate those parameters, usually defined as protein and fibrous fraction, united with in vitro digestibility of the forage. Fiber digestibility is inversely correlated to the amount of lignin, defined as the indigestible fraction.

An important effect of the abundant use of forage in the diet is the ability to reduce the proportion of concentrates, with positive effects on ruminal physiology and pH dynamics. However, fiber is not the only important component in the diet, which must also be formulated and optimized for starch and protein shares. Both components are used by bacteria to multiply. In particular, bacterial protein is used directly by the animal, but it requires nitrogen from the diet to be composed. In addition, it is possible to manipulate the protein quota and its amino acid composition that can be used by the animal by including bypass foods in the diet. There are no minimum limits for the proportion of starch in the diet, but it is still important not to exceed its use to avoid the development of diseases such as ruminal acidosis. In any case, starch is the primary energy source for several bacterial species and is related to casein production (Formigoni et al., 2010; Mordenti et al, 2017).

The concentrates used include the employment of cereals, among which corn and barley predominate. Among by-products, the most widely used are pulps and bran. The use of sorghum and wheat properly included in the ration also gives similar results to those obtained with a prevalence of corn (Formigoni and Mordenti, 2004). In accordance with the "Disciplinare di Produzione del Formaggio Parmigiano Reggiano", the permitted raw materials for concentrates include cereals (corn, sorghum, barley, oats, wheat, triticale, rye, spelt, millet and panicum), oilseeds (soybean, flax, sunflower), legume seeds (fava bean, field bean and protein pea), fodder (flours of the permitted fodder essences), dried beet pulp and potato protein concentrate. Carob, in an amount not exceeding 3%, and molasses, in an amount not exceeding 3%, may also be used in complementary compound feeds. The use of molasses block feed, including in crushed form, in the maximum daily dose of 1 kg per head is also allowed, but this is not compatible with the use of feed containing molasses. Appropriate "tags" shall be affixed to the feed, listing the individual raw materials in descending order of quantity. Feedstuffs cannot be stored inside the barn. The total amount of crude fat contributed by products and by-products of soybean, flax, sunflower, corn germ and wheat germ shall not exceed 300 grams/head/day. Many raw materials, such as of dried beet pulp when moistened, animal foods, rice and its by-products, extracted meal, seaweed, urea and many others are prohibited in feed.

3.2 Nutritional parameters of dairy cattle feeds

The high-producing dairy cow necessitates a diet that provides the necessary nutrients for high milk production. Water, protein (amino acids), carbohydrates, fats, minerals and vitamins are all nutrients needed by the lactating dairy cow to satisfy the demand of the mammary gland to produce milk and milk components (Erickson and Kalscheur, 2019).

3.2.1 Water

Water is essential to permits the transport of substances in the body and for other reasons, like insulation, temperature regulation and removal of wastes. Cattle obtain water from various sources, including free water (drinking water), water present in feed and water produced through endogenous reactions. Among these sources, free water intake is the primary water source for cattle. As milk production increases, the intake of free water also tends to rise. Additionally, water intake tends to increase when cows are exposed to environments with a temperature humidity index above 68. The range of free water intake typically falls between 92 and 138 L/d (Erickson and Kalscheur, 2019).

3.2.2 Energy

After water, energy constitutes the main nutritional need. Energy deficiency is the main cause of reduced milk production, although it often goes unnoticed. Animals require energy for milk production, growth, weight maintenance and pregnancy support. Assessing the feed energy content or energy requirements of the cow is complex. Energy can be divided into several forms: intake energy (IE), digestible energy (DE), metabolizable energy (ME) and net energy (NE).

IE represents the total energy contained in the diet. It corresponds to the amount of energy in the diet that is taken in by the animal. This value is obtained using a bomb calorimeter, measuring the heat released from the complete combustion of the feed. However, not all the energy contained in the feed is usable by the cow, since some of the feed IE is not digestible and some is lost from the body after absorption. An initial loss to consider concerns energy excreted with faeces. This occurs when the animal cannot fully digest the energy intake. Since many forages are less digestible than grains, much of the energy they contain is lost with faeces. The total energy of the IE minus the energy lost in faeces constitutes the DE. However, the DE does not accurately measure the actual usable energy in the diet, because some of the digestible energy is lost in the form of urine and gases, such as methane. What remains after these losses is ME. Gas energy losses can be significant in ruminants and tend to be greater in fibrous foods than in grains. ME represents the energy available to body tissues, such as muscle, fat and mammary gland, in order to support the body's chemical reactions. This value is expressed in calories (Kcal or Mcal). In addition, even once energy from food reaches the tissues, there are additional losses to consider. When chemical reactions take place in the body, some of the energy is lost as heat. The ME of food, net of heat lost during metabolism, is called NE or retained energy content (RE). NE represents the total amount of energy available to the animal for body functions, growth, pregnancy and lactation.

The energy requirement for maintaining a cow refers to the energy needed to sustain the cow's body and remains essentially constant for adult animals. This requirement must be met before any other production. Since the maintenance requirement is independent of feed intake and is met initially, any units in excess of this requirement increase the energy available for production. When energy intake exceeds maintenance requirements, the extra energy contributes to increased production. This is why higher food intake usually improves productivity. Energy comes from three main food sources: fats, proteins and carbohydrates. Fats have the highest energy value per unit weight of food, followed by protein and carbohydrates. The most common ingredients for increasing feed energy density include forages, cereals, fat supplements and by-products. For example, corn silage or small grains constitute energy-dense forages. Energy-dense grains include corn, barley and sorghum. In some circumstances, food by-products, such as beet pulp, citrus pulp, and bakery waste, are used as sources of supplemental energy (Knowlton, 2003).

3.2.3 Proteins

Protein in feedstuffs is typically measured as crude protein (CP), which is calculated by multiplying the nitrogen percentage (%N) in a feed by 6.25. The value of 6.25 is based on the approximation that feed proteins contain about 16% nitrogen. However, crude protein includes not only true protein but also other nitrogen-containing compounds like amino acids, dipeptides, nucleic acids, NH3-N, and nonprotein nitrogen (NPN) compounds. It is important to note that cattle, like all animals, have an amino acid requirement rather than a protein requirement, as true proteins are defined as chains of amino acids. Amino acids are utilized by cattle for various physiological processes such as enzyme production, synthesis of milk proteins, immunoglobulins, muscle development and overall organ and tissue growth. Any surplus amino acids can be employed for gluconeogenesis (production of glucose) and lipogenesis (production of lipids). The synthesis of milk proteins is crucial as it leads to the formation of bioactive proteins found in the whey portion of milk, which serve protective functions for newborns. The production of casein and whey proteins ensures the provision of necessary amino acids for the growth of young cattle. Soybean co-products are commonly used as the primary protein supplement, supplemented with corn feedstuffs (Erickson and Kalscheur, 2019).

Nitrogen content in feed should be distinguished into soluble protein (non-protein nitrogen and nitrate content), insoluble protein (NDF- and ADF- bound nitrogen) and lignin-bound nitrogen, that is indigestible (Formigoni et al., 2010).

3.2.4 Carbohydrates

Carbohydrates, consisting of carbon, hydrogen, and oxygen, are the largest and most essential components for dairy cattle nutrition. They represent up to 70% of the diet for lactating dairy cattle. Carbohydrates can be classified as sugars or chains of simple sugars. In the feeding of dairy cattle as with other ruminants, the two most common carbohydrates used are cellulose, which is composed of β-1,4 linked glucose units, and starch, which contains α-1,4 (amylose), α-1,4 and α-1,6 (amylopectin) glucose units. It is important to note that cellulose is less easily digested compared to starches. The primary distinction, that causes the different digestion capacity, lies in the type of bonds present within the glucose units.

In dairy cattle nutrition, carbohydrates can be categorized into two fractions: structural carbohydrates and related compounds (cellulose, hemicellulose, and lignin), and non-structural carbohydrates (starches and sugars). Structural carbohydrates are often quantified as neutral detergent fiber (NDF), which includes cellulose, hemicellulose, lignin and any nitrogen (N) bound to the fiber. Lignin is not a carbohydrate, but is included in the NDF fraction. NDF is determined by boiling a dried and ground sample in a neutral detergent solution for one hour. It represents the remaining material after digestion. For lactating cows, it is recommended to have NDF concentrations around 27%-28% of the diet's dry matter (DM). Common sources of NDF are hays, silage, pasture, and roughages. On the other hand, acid detergent fiber (ADF) is obtained by subjecting NDF to a one-hour boil in an acidic detergent solution. This process removes hemicellulose, leaving behind cellulose and lignin.

Hemicellulose content can be determined by calculating the difference between the percentage of neutral detergent fiber (NDF) and acid detergent fiber (ADF). The estimation of lignin content can be done through various methods, such as heating in KMnO4 or 72% H2SO4. Klason lignin is obtained by subtracting the ash remaining after heating the residue in a muffle furnace at a high temperature for 3 hours from the residue after acid hydrolysis. This process removes the cellulose and leaves behind the lignin. Thus, ADF minus lignin equals cellulose. Lignin, which increases as plants mature, is not significantly digested and negatively affects digestibility. Hence, it is advisable for dairy producers to harvest forage at an early stage of growth (grass-pre-boot, legume pre-bud) to maximize digestibility and take advantage of higher nutrient availability.

Non-structural carbohydrates (NSC) in the context of dairy cattle nutrition mainly consist of starches (amylose and amylopectin) as well as simple sugars and disaccharides. Starches and sugars are commonly analyzed enzymatically. However, non-fiber carbohydrates (NFC), which are similar in composition, can be estimated by difference using a specific equation. NFC includes pectins, which act as a binding agent in cells. This highly digestible component in the rumen is not measured when starches and sugars are enzymatically analyzed. Consequently, pectin is classified as a dietary fiber and can be found in beet pulp and citrus pulp. Typically, non-structural carbohydrates should account for approximately 35% of the diet's dry matter (DM) in lactating cows, while their proportion is much lower in far-off dry cows or post-weaned growing heifers. (Erickson and Kalscheur, 2019).

3.2.5 Fat

Fat is the most energy-dense nutrient, providing 2.25 times more energy than carbohydrates or protein. Unlike carbohydrates and protein, fat is not extensively fermented in the rumen, resulting in minimal heat production during fermentation. This property of fat makes it valuable for maintaining caloric intake, particularly in cattle experiencing heat stress. Fat can be classified into two types: glycerol and non-glycerol. Non-glycerol fats, such as waxes and sterols, have limited or no nutritional value. On the other hand, glycerol fats, including triglycerides, phospholipids, and glycolipids, are nutritionally beneficial. These fats consist of a glycerol backbone, a carbohydrate or phosphate component, and long carbon chains. Rumen microbes can incorporate fatty acids into their cell membranes and even convert the typical even-numbered carbon fatty acids into odd-numbered carbon chain fatty acids. Unsaturated fats contain double bonds, while saturated fats are fully hydrogenated. Plant fats are predominantly unsaturated, whereas terrestrial animal fats contain varying levels of saturated fat. It is important to note that nearly all feed ingredients, except for water and minerals, contain some amount of fat. However, certain feeds have higher fat content. Oilseeds like soybeans, cottonseed, canola, and flax typically contain around 20% fat. Meanwhile, less commonly used oilseeds such as sunflower and pumpkin can have fat content approaching 45% (Erickson and Kalscheur, 2019).

3.2.6 Minerals

Minerals are essential inorganic compounds classified as metal elements, serving various functions in the body, including structural support, nerve impulse transmission, and maintaining osmotic balance. Some minerals act as catalysts for reactions or are vital for enzyme function, such as glutathione peroxidase. They are categorized into two groups: macrominerals (including Ca, P, Mg, K, Cl, Na and S), which are required in gram quantities, and microminerals, also known as trace minerals, required in mg or µg quantities (Cu, I, Fe, Mn, Mo, Zn, Se). Adequate mineral nutrition is crucial for the successful lactation of dairy cattle (Erickson and Kalscheur, 2019).

3.2.7 Vitamins

Vitamins play a vital role in metabolism and are organic compounds categorized into two groups: water-soluble and lipid-soluble. Water-soluble vitamins, such as niacin and biotin, dissolve in aqueous environments and are typically synthesized in sufficient quantities within the rumen. However, certain water-soluble vitamins can have beneficial effects when supplemented. Lipid-soluble vitamins, namely vitamins A, D, E, and K, are based on lipids. Vitamin K, necessary for blood clotting, is synthesized adequately by rumen microbes. The supplementation levels of vitamin A, D and E vary depending on the diet fed (Erickson and Kalscheur, 2019).

3.3 Nutritional effects on lipids in milk

The volatile fatty acids (VFAs) acetate and butyrate are produced by digestion of fiber in the rumen. The rumen wall needs butyrate for energy and the rumen wall tissue converts a significant amount of it to beta-hydroxybutyrate. About half of the milk fat is synthesized in the udder from acetate and beta hydroxybutyrate, while the other half of the milk fat is transported from the pool of circulating fatty acids in the blood. These may come from mobilization of body fat, metabolized fat in the liver or absorption from the diet (Tyasi et al., 2015).

Most of the research on milk composition has concentrated on fat concentration, because numerous dietary factors influence it. Cattle feeding strongly influences both lipid content and fatty acid composition in a short time (Formigoni and Mordenti, 2004; Sutton, 1989).

To achieve high production of acetic acid and butyric acid in the rumen, which are essential to produce most triglycerides and medium- and short-chain fatty acids in milk, it is important to maintain optimal environmental conditions for the activity of acetic and butyric bacterial groups, to have a higher percentage of fat in milk. There is a positive correlation between ruminal pH and fat in milk. In fact, if the ruminal pH drops, the production of propionic acid increases, at the expense of acetic and butyric acids, with a reduction in milk fat content. Therefore, there is a close link between the fibrous component of the ration (NDF) from forages and the component given by concentrates, which provide mainly not-structural carbohydrates (NSC). The supply to the udder of partially hydrogenated fatty acids from the rumen, due to the presence of unsaturated fatty acids of plant origin in the ration and ruminal fermentations oriented toward starch digestion and not fiber digestion, with a decrease in ruminal pH, lead to a decrease in milk fat. In managing fat content, it is therefore crucial to control unsaturated fatty acids in the diet and to orient ruminal fermentations through certain ration parameters. In maintaining ruminal pH, it is also important to manage the ration's ionic balance (ratio of anions to cations) by using quality forages, such as alfalfa, and buffering substances, such as sodium bicarbonate. Potassium bicarbonate has also proven useful in improving the ionic balance of the ration and increasing the fat content of milk. In addition, live yeasts can be used, because they have been shown to help reduce the concentration of lactic acid in the rumen, consequently increasing ruminal pH and milk fat content (Cevolani et al., 2014).

Other important factors that most influence the quantity and quality of fat in milk are roughage, forage to concentrate ratio, soluble carbohydrates, dietary lipids, dietary proteins, intake, and frequency of feeding.

3.3.1 Roughage and milk lipids

The role of roughage in preserving milk fat concentration has been extensively documented and special attention has been paid to the importance of physical structure over chemically determined "fiber." Forage is generally the main source of roughage and should not be finely chopped to preserve its roughage qualities. To obtain a good level of fat in milk, it is necessary to ensure that the roughage fiber in the ration does not fall below the value of 17% relative to dry matter. In addition, an adequate supply of fibrous carbohydrates of appropriate physical structure, with a minimum of 30-32% NDF, from good quality forages of adequate length, not less than 6/8 cm, is essential to maintain a constant ruminal pH (>6) and allow good production of acetic acid, the main precursor of short-chain fatty acids in milk. Forage length is a crucial indicator of roughage quality. The component of NDF that has real significance on rumination is called e-NDF (effective NDF) and depends on particle size. The correct fiber size preserves the quality of the roughage and maintains the fat concentration in milk. The way rations are fed also plays a crucial role in maintaining ruminal pH, so it is necessary to allow continuous availability of feed, which must be easy for animals to access (Cevolani et al., 2014; Formigoni and Mordenti, 2004; Sutton, 1989).

3.3.2 Forage to concentrate ratio, soluble carbohydrates and milk lipids

The fat concentration in milk generally decreases when the ratio of forage to concentrate in the diet is reduced, but the pattern of response varies greatly. As long as about 50 % of the diet consists of forage, the fat concentration in milk seems to be fairly stable. However, when forage intake is reduced, the fat concentration in milk fluctuates (Sutton, 1989). To have a good fat level in milk, the forage/concentrate ratio should not be less than 50/50. A 60/40 ratio favours a higher level of lipids in milk (Cevolani et al.,2014)

The concentration of carbohydrates in concentrates is probably the most significant of the many factors that cause this variation. It is important to avoid an excess of easily fermentable carbohydrates in the diet, which stimulate increased propionate production. It is possible to modulate the intake of grains with different degrees of fermentability. Starch fermentability is decreasing in wheat, barley, oats, corn, and sorghum (Cevolani et al., 2014). When concentrates are based on rolled or ground barley or flaked corn, the decrease in fat is rapid and often severe, leading in extreme cases to milk fat concentrations below 2.0 %. The size of the milk fat depression caused by ground corn in low roughage diets, however, is smaller than that caused by rolled barley. In conventional diets, oats have been found to result in lower milk fat concentration than barley. This is probably due to oats oil content rather than to any differences in starch digestibility. High quality (highly digestible) fiber found in many byproducts, such as corn gluten feed, sugar beet pulp and citrus pulp, is a readily fermentable carbohydrate alternative to starch. These feeds have high NDF concentrations and low starch concentrations, but they also frequently contain significant amounts of poorly identified ingredients like soluble carbohydrates and pectins. Even though they have little impact on fat concentration when added to diets that maintain normal milk concentration, such by-products significantly reduce the milk fat depression caused by high amounts of starchy concentrates (Formigoni and Mordenti, 2004; Sutton, 1989).

Cereals are commonly included in dairy cattle diets because of their economy in providing the easily digestible energy needed to maintain high levels of milk production. While increased grain intake stimulates milk production, it also reduces the percentage of milk fat and alters the fatty acid composition. Specifically, the proportion of 6- to 16-carbon fatty acids in milk is typically reduced, while the proportion of 18-carbon unsaturated fatty acids increases. There are several theories that have tried to explain the cause of grain-induced depression of milk fat, but the exact reason remains unclear. The two most significant theories propose that acetate and butyrate production in the rumen is insufficient to support milk fat synthesis or that cereal propionate stimulates insulin concentration in the bloodstream, redirecting metabolites away from mammary tissue. However, numerous studies have shown that neither theory is the sole cause.
In more recent years, it has been possible to identify trans fatty acids as the main cause of lipid depression in milk. Studies in several locations have revealed an inverse relationship between trans fatty acids in milk and milk fat content, although not all isomers of trans fatty acids are associated with milk fat depression. It is primarily trans-10 fatty acids that cause milk fat depression rather than trans isomers in general. Cereal feeding increases the production of trans-10 fatty acid isomers by rumen microorganisms (Jenkins and McGuire, 2006).
Replacing cereals with different types of soluble carbohydrates, such as lactose, whey, molasses, and condensed molasses solubles, has been found to prevent or reduce the decrease in milk fat content caused by diets poor in roughage. Sucrose, on the other hand, has not shown a similar impact (Sutton, 1989).

3.3.3 Dietary protein and milk lipids

Usually, dietary protein is believed to have minimal influence on milk fat concentration. Nitrogen fractions of the ration do not exert a major role in changing milk fat percentage, although adequate intakes of certain amino acids, such as methionine, supplementation of methionine hydroxy analogue and soluble nitrogen positively influence milk fat concentration. On the other hand, raising the protein content in the diet from approximately 12 to 14% to about 18% can lead to a reduction in milk fat concentration of up to 0.5% unit. Overall, dietary protein does not seem to provide an effective method for manipulating milk fat concentration (Formigoni and Mordenti, 2004; Sutton, 1989).

3.3.4 Dietary lipids and milk lipids

The primary objective of incorporating fats and oils into dairy cow diets is to enhance energy intake and milk production. However, it has long been acknowledged that such supplements also have specific impacts on the concentration of milk fat and the composition of its fatty acids. The effects of dietary fats on milk fat synthesis are complex and occur within the cow's gut, particularly the rumen, as well as within its body and udder. Lipid supplements can significantly influence the composition of fatty acids in milk, which is crucial for milk processing quality.

In brief, adding lipids to regular diets, typically up to approximately 6 to 8% of the total dry matter, generally increases milk production. However, the response in milk fat concentration varies greatly, with reported increases and decreases of over 1.0% unit depending on the amount, physical form, and fatty acid composition of the supplement. The decrease in milk fat concentration caused by lipid supplements is partly due to their impact on rumen fermentation. Consequently, various methods have been attempted to minimize the effect of fat supplements on the rumen, such as feeding whole or crushed oil seeds, using fats protected from rumen processes (rumen-protected fats) through formaldehyde-treated protein, incorporating selected fatty acids (fat prills) and employing calcium salts of fatty acids. These different techniques have varying degrees of success in either maintaining milk fat concentration or even elevating it above "normal" levels, although occasional reductions have also been observed (Sutton, 1989).

Minimum lipid intakes (about 3% of dry matter) are indispensable for proper feeding of cows. The "Disciplinare di Produzione del Formaggio Parmigiano-Reggiano" prohibits the fattening of rations and limits maximum intake of oil (soybean, flax, sunflower, corn) to 300 g per cow per day. The rule still allows the amount of oils needed to ensure those minimum requirements are met, while avoid the risks of overuse of oils. In fact, these affect ruminal and udder function, depressing milk lipid levels and changing composition. Short-chain fatty acids, such as methyl butyric acid and terpenes, are capable of imparting distinctive aromas to cheeses. It can be argued that the smell, taste and colour of milk and cheese can be influenced by the type of feed fed to cows. Feeding can change the expression of cheese aroma by acting on other mechanisms as well, such as milk lipolysis (Formigoni and Mordenti, 2004). In the diet of dairy cows, it is also important to avoid the use as such of mostly unsaturated vegetable oils or those found in the oilseeds of corn or whole soybeans or certain ingredients, such as soybean, canola, sunflower, and flax cakes. Unsaturated fats, if not fully hydrogenated by ruminal microflora, depress fat synthesis in mammary tissue (Cevolani et al., 2014).

3.3.5 Diets intake, meal frequency and milk lipids

Significantly reducing milk fat concentration can be achieved by increasing the intake of cow diets while keeping the concentrate-to-forage ratios constant. Although meal frequency generally has a minor impact on milk fat concentration with most diets, increasing the number of daily concentrate meals in a fixed ration from two to six has shown to greatly alleviate the decrease in milk fat caused by low roughage and high starch diets. These effects of intake and meal frequency highlight the fact that a precise description of diet composition alone is insufficient for accurately predicting milk fat concentration (Sutton, 1989).

3.4 Nutritional effects on proteins in milk

Rumen microorganisms transform dietary protein into microbial protein, which serves as a primary source of essential amino acids for the cow. The cow's mammary gland absorbs these amino acids and utilizes them to produce milk proteins. To support the synthesis of these proteins, glucose is necessary for supplying energy. Glucose can be generated from VFAs propionate in the liver or directly absorbed from the rumen. However, if glucose is insufficient, the cow needs to break down amino acids and convert them into glucose through a process known as gluconeogenesis. This conversion process can diminish the availability of amino acids for milk protein production. Furthermore, certain proteins like albumin and immunoglobulin are directly transferred from the bloodstream to the milk (Tyasi et al., 2015). In addition to ruminal-derived microbial protein, non-degradable dietary proteins in the rumen and by-pass proteins also provide useful amino acids for protein synthesis in milk. Protein synthesis by the udder is closely related to the amount of essential amino acids taken in from the diet, such as methionine, lysine, phenylalanine, and histidine (Cevolani et al., 2014).

In the past years, the nutritional factors that have been extensively studied for their impact on milk protein content include the forage to concentrate ratio, dietary protein amount and source and dietary fat amount and source. In particular, availability of energy and essential amino acids are the most important factor influencing casein synthesis. It is crucial to differentiate between situations that affect the percentage of protein in milk (protein content) and those that affect the daily production of protein (protein yield). Often, dietary modifications that positively influence overall milk and protein yields can have negative effects on protein content. The target in most cases is to increase protein content while maintaining or increasing milk yields (Formigoni and Biagi, 2007; Jenkins and McGuire, 2006). In fact, in order to enhance the value of farm milk, it is increasingly important to produce milk that is rich in cheesemaking protein, allowing high yields to the dairy, even though milk protein is less influenced from a nutritional and/or managerial point of view, since much of the synthesis of milk protein in the mammary gland depends on the genetic component of cows (Cevolani et al., 2014).

3.4.1 Forage to concentrate ratio and milk proteins

The quantity and quality of nitrogenous substances in milk are influenced by cattle feed, although to a lesser extent than fat. It has been shown that the use of rations consisting of forages characterized by lower fiber content (NDF) and higher amounts of non-fiber carbohydrates (NSC), in the presence of correct forage ratios, results in a marked improvement in casein content of milk. Covering ruminal bacterial requirements with feeds with higher fiber digestibility results in an increase in bacterial protein synthesis and ingestion capacity. This is followed by an increase in casein content of milk, which is also observed with dietary intake of amino acids in ruminoprotected form, but not consistently (Formigoni and Mordenti, 2004).

In general, reducing the percentage of forage in a cow's diet leads to an increase in protein content and milk production. Significant increases of 0.4 percentage units or more in the protein content of milk can be achieved by reducing the percentage of forage to 10 % or less of the dry matter (DM) of the diet. However, there are practical limitations due to the need for a minimum forage concentration (usually at least 40 %) in dairy diets to avoid digestive and metabolic disorders. It remains uncertain whether forage directly causes milk protein depression or is an indirect effect of reduced energy intake.

The research conducted in recent years suggest that energy intake plays a greater role in milk protein content, while dietary fiber content has little direct influence. Meta-analysis has related rapidly fermentable dietary carbohydrates to milk protein content. Further investigations focused on the relationship between starch fermentation, propionate production in the rumen, and the effects of insulin on milk protein content and production, found that changes in forage to concentrate ratio can regulate milk protein production. Feeding rapidly fermentable carbohydrates promotes increased propionate and microbial protein production, triggering signals within the cow's body to increase milk and milk protein synthesis (Jenkins and McGuire, 2006).

Overall, poor energy intake depresses protein synthesis in the udder, either due to reduced growth of ruminal microflora or due to a lack of available mammary energy in the form of glucose.

Microbial protein of ruminal origin has an amino acid composition quite similar to that of milk protein, so a proper supply to the gut of microbial protein from the rumen is essential to have a good protein supply in milk. In conclusion, it is necessary to maximize the energy intake in the diet, to facilitate protein synthesis operated by ruminal microorganisms, using carbohydrate sources with different degrees of fermentiscibility (starches, sugars, digestible NDF), to avoid the onset of acidosis. Excesses of by-pass starch (corn and sorghum) do not help maximize ruminal microbial synthesis and, in high-producing cows, very low levels of protein in milk can occur. The addition of barley or flaked corn at 20% of total cereal grains may be useful to improve ruminal microbial protein synthesis and protein title in milk (Cevolani et al., 2014).

3.4.2 Dietary protein amount and source and milk proteins

The effects of the amount and source of protein in the diet on the protein content of milk have been extensively studied, unlike the feed/concentrate ratio. However, it has been found that significant changes in the amount or source of protein lead to only modest alterations in the protein content of milk. According to a study, increasing the protein content of the diet from 15.0% to 19.5%, including a wide range of protein sources, including rumen-protected amino acids, the prevalence of milk protein increased from 2.85% to 3.27%. The increase in milk protein content is about 0.02 percentage units for every 1 percentage unit increase in dietary protein. The limited ability of diet to significantly change the protein content of milk is mainly attributed to the low transfer efficiency (25% to 30%) of dietary protein to milk.

There are studies that explain the limitations of protein transfer from diet to milk. Amino acids are supplied to the mammary tissue in adequate amounts, but poor capture of these amino acids by the mammary gland is a key factor contributing to the low transfer rates. Blood flow through the mammary gland is implicated as a crucial factor affecting this poor capture, which is part of the overall process of coordinating nutrient supply to the mammary gland. Other studies indicate that mammary blood flow and amino acid extraction may adapt, leading to increased milk protein production. This suggests that the mammary gland has the ability to modify substrate uptake from the arterial stream in response to changes in arterial amino acid concentrations, mammary blood flow, and metabolic activity, ultimately enhancing milk protein production (Jenkins and McGuire, 2006).

Therefore, the use of high-protein rations (>16%) does not elevate casein content and may induce a significant increase in milk urea. Urea is not favourable to the dairy aptitude of milk and when it exceeds 30 mg/100 ml in milk indicates the possibility of improving the balance of the ration (Formigoni and Mordenti, 2004). It is important to provide adequate soluble protein (about 30% of total protein) to promote the constant supply of nitrogen to ruminal microorganisms. It is also important to provide non-degradable protein in the rumen or by-pass, not in amounts exceeding 30% of the total. Adequate proportion of the different protein fractions in the diet allows maximum growth of all ruminal bacterial groups, supplying essential amino acids for protein synthesis in milk. It is possible to use ruminoprotected amino acids, maintaining a methionine/lysine ratio of 1:3. However, it is better to focus on improving microbial ruminal protein synthesis through adequate energy and protein supply in the diet rather than adding protected amino acids, as they are more expensive. In rations where animal protein cannot be used, the amino acids histidine and phenylalanine also appear to be limiting. Canola flour can be a good source of these amino acids at low cost (Cevolani et al., 2014).

In conclusion, according to current knowledge, milk protein production can be increased in dairy cows by ensuring that they receive enough metabolizable protein and by providing protein sources with adequate amino acid composition. Strategies to increase milk solids, particularly casein, include improving nutritional evaluation of feeds through advanced laboratory techniques and analytical methods. In addition, a deeper understanding of the nutritional needs of animals and improved animal welfare practices can help reduce nutritional expenditures and improve milk solids production (Formigoni and Biagi, 2007).

3.4.3 Fat amount and source and milk proteins

The amount of casein in milk may drop if too much lipid is used in the ration. As the exploration of fat supplements as an energy source for dairy cows has progressed, it has become apparent that the administration of additional fat often results in a decrease in milk protein content. Consequently, the inclusion of fat in the diet must be limited in markets where milk pricing provides incentives for higher protein content. On average, for every 100 grams of supplemental fat intake, the protein content in milk declined by approximately 0.03 percentage units or about 0.1 to 0.3 percentage units at typical levels of fat feeding. When fat supplementation leads to a reduction in the protein content of milk, the casein fraction suffers the most significant decrease, while the effects on the whey fraction are insubstantial and non-protein nitrogen (NPN) is generally increased. Despite the decline in protein content, fat supplements, when fed appropriately, increased milk yield, resulting in the total daily production of milk protein remaining the same or even increasing.

Over the past years, several important studies have been conducted to understand the mechanism by which fat supplements cause this dilution effect, characterized by a greater increase in milk yield compared to protein yield. One study suggests that elevated levels of fatty acids in the bloodstream, resulting from fat supplementation, decrease the release of somatotropin, which in turn reduces the extraction of amino acids by the mammary gland. Some researchers proposed that fat supplements reduce milk protein concentration by reducing blood flow through the mammary gland, leading to a reduced extraction of blood amino acids. According to their explanation, the increase in milk volume occurs because higher levels of fatty acids inhibit de novo fat synthesis in the mammary gland, resulting in a sparing of acetate for oxidation and in an increased availability of glucose for lactose and milk synthesis (Formigoni and Mordenti, 2004; Jenkins and McGuire, 2006).

3.5 Nutritional effects on urea in milk

The presence of urea in cows' milk is caused by metabolic phenomena in the gastrointestinal tract, resulting in excess ammonia that is not microbially digested in the body. Feed proteins are divided into degradable and non-degradable in the rumen. Those that are degradable are degraded by rumen microorganisms first to amino acids, then to ammonia and branched-chain fatty acids. The bacterial population uses ammonia to grow. The extent to which ammonia is used to synthesize microbial proteins depends largely on the availability of energy generated by carbohydrate fermentation (Gulińsk et al., 2015). Excess ammonia that has been formed ruminally during the processes of degradation of nitrogen compounds in the ration and, to a lesser extent, from ammonia resulting from the processes of amino acid gluconeogenesis, is converted into urea in the liver, released into the bloodstream and then excreted through urine and milk. Therefore, urea is normally present in milk, and the amount found in milk and blood is roughly comparable (Cevolani et al., 2014).

Milk urea varies by season, month, cow group considered, lactation stage and type of sample taken (Roy et al., 2011). Factors that may influence the level of urea in milk are mainly related to the production level of the cows and the feeding plan (Cevolani et al., 2014). In particular, the main reason for high urea levels in milk is related to excess protein content in feed rations and energy and protein imbalance. The increase in the percentage of total protein from 13% to 18%

DM per ration is accompanied by an increase in the level of urea from about 80 mg to over 150 mg in 1 liter of milk. Other factors that influence the level of urea in milk are the frequency of feed intake, the number of milkings and the length of the interval between milkings, the cow's body weight, the volume of water intake, the level of Na and K ration supplementation, and the pH of the rumen (Gulińsk et al., 2015). In addition to dietary protein content, the type of protein and energy from NSC provided in the diet can also influence the level of urea in milk. In practice there may be situations of altered ratio of degradable to non-degradable protein in the ration associated with energy deficiency. If the share of degradable protein available to the ruminal microflora is not matched by an adequate fermentable energy share, unused ammonia is absorbed by the rumen and subsequently converted to urea by the liver, and then eliminated with milk and urine.

Excess urea in milk is a negative index for animal health, fertility, and energy balance. It is also unfavourable from the point of view of milk processing, as urea content is negatively correlated with casein content, leading to lower dairy yield. The optimal range of urea in milk is given by values between 20 and 30 mg/dl. Urea in bulk milk should be monitored frequently for problems related to ration formulation or individual ingredients (soluble protein or ammonia-rich feed). Monitoring allows maximizing production and preventing the occurrence of dysmetabolias and reproductive-related problems. Low urea levels in milk (< 20 mg/dl) may be related to a deficiency of soluble or degradable proteins in the diet. They may also indicate ruminal acidosis. A low urea level in milk leads to lower production, and milk production can be improved by increasing the level of degradable protein in the ration. Excess urea, on the other hand, is more common in areas where alfalfa is a predominant part of the forage, such as in Parmigiano Reggiano cheese production area. Excess urea results in lowered uterine pH and can increase the rate of hypofertility and embryonic mortality. In these cases, it is important to assess protein intake with specific analyses (Cevolani et al., 2014). As seen previously, high dietary protein stimulates milk production, but increased protein has been found to be damaging to the animal's reproductive performance. In addition, protein overeating contributes to environmental pollution and increased feed costs (Roy et al., 2011). To avoid protein overload in dairy cattle, it is also necessary to balance the ruminally degradable protein fraction (2/3 of the total crude protein and the soluble portion must be 50 % of the degradable portion), verify that energy requirements are satisfied (NSC between 38 and 41 % of the dry matter of the ration, correct balance between degradable starch and bypass), and use additives that reduce the share of free ammonia and decrease the liver intake (Cevolani et al., 2014). Recent research have indicated that the addition of natural plant-derived biologically active compounds, such as tannins, saponins, and essential oils, reduces ammonia production and ultimately urea content in milk (Gulińsk et al., 2015).

To summarize, milk urea is a very important parameter, which allows checking potential nutritional errors and preventing potential metabolic disorders. Therefore, given the simplicity of performing it directly in the field, it is an indispensable tool for monitoring the proper management of dairy cattle production efficiency (Cevolani et al., 2014). Milk urea nitrogen concentration (MUN) of dairy cows can serve as an on-farm indicator tool to guide nutritional strategies and help reduce nitrogen (N) emissions to the environment (Spek et al., 2012), as well as reduce feed costs (Gulińsk et al., 2015).

3.6 Nutritional effects on lactose in milk

The lactose content of milk tends to rise according to the animal's energy balance and according to the cereals fed. Corn as the predominant starch contributor increases the lactose content of milk (Formigoni and Mordenti, 2004). However, it is generally considered that lactose concentration cannot be changed by diet, except for severe underfeeding.

There is limited evidence from certain experiments suggesting that decreasing the ratio of forage to concentrate in diets while maintaining constant energy intake could potentially raise milk lactose concentration by up to 0.2% unit. However, other experiments have not shown any significant response. On the other hand, high-fat supplements, whether in free or protected forms, have been reported in some experiments to decrease lactose concentration by up to 0.2% unit, but once again, this response is inconsistent. In specific experiments, the addition of formaldehyde-treated tallow resulted in a reduction of lactose concentration by 0.04% unit per kilogram of supplement during weeks 7 to 13 of lactation. However, during weeks 1 to 6, it caused a non-significant increase of 0.02% unit per kilogram of supplement. Therefore, while it would be incorrect to assume that dietary modifications have no effect on lactose concentration, the observed responses are both small and inconsistent to provide practical usefulness.

There are no extensive studies regarding the variation of lactose in milk in response to changes in diet, as lactose is not subject to legislative regulation and payment-for-quality schemes. In addition, there is no significant correlation between the amount of lactose and Parmigiano Reggiano cheese production (Jenkins and McGuire, 2006; Sutton, 1989).

3.7 Nutritional effects on minerals and vitamins in milk

Feeding cows results in slight variations in the content of some mineral elements, such as Ca, P and Fe, while for others, such as I, Se and Mg, it is possible to significantly change their concentrations in milk through diet. Some research suggests the possibility of naturally and substantially enriching milk with trace elements using fortified forages and specific dietary supplements.

The content of vitamins B, C and K, which are synthesized in sufficient amounts by the bacterial flora of pre-milks, is relatively constant in milk. In contrast, the content of vitamins A, D and E depends largely on the respective content of the diet. Some carotenoids, with provitamin, antioxidant or pigmenting activity, may also transfer in part from the food to the milk and from the milk to the cheese, giving different shades of colour and resistance to oxidizing agents (Formigoni and Mordenti, 2004).

3.8 Dairy cows feeding perspectives in the Parmigiano Reggiano area

To the extent that the price of milk is linked to its components, producers will continue to exploit cow nutrition as a way to modify milk composition for maximum economic return. As greater coverage of the bovine genome becomes publicly available, opportunities to genetically manipulate or develop cow lines that produce milk with a specific composition will be explored, but nutrition will remain an integral part of the expression of this modified genetic potential (Jenkins and McGuire, 2006).

The role of feeding the cows that produce milk for Parmigiano Reggiano must increasingly respond to requirements of strong territorial characterization. This can be achieved by paying particular attention to nutrition and especially to the quality of forages, promoting the use of local ones in increasing quantities to enhance the quality characteristics of milk and improve the health condition of the animals. In fact, only by having excellent and well-preserved forages and providing cows with ideal housing conditions for their welfare can high quality milk production be sustained (Formigoni and Mordenti, 2004). To make Parmigiano Reggiano an excellent product with even stronger territorial connotations, it is possible to breed native and local cattle breeds for its production, such as the Rossa Reggiana cow. This breed allows for a quantitatively lower milk production, but with excellent quality characteristics and greater dairy capacity. Therefore, it is of particular interest to learn about this breed and to determine how through feeding it is possible to optimize the production and quality of its milk.

4. Heat stress and its effects on dairy cattle

The increased frequency with which heat waves affect regions, including Italy, has generated growing interest in studying the impacts that these climatic conditions have on human and animal health. Especially in the context of agriculture and livestock breeding, it is known that environmental conditions characterized by high temperatures and humidity for prolonged periods can cause discomfort to animals, negatively affecting their reproduction, production, and health status (Coccimiglio et al., 2009).

Global climate change is a major recent concern, with the livestock industry being one of the most affected sectors. However, the effects of rising temperatures on livestock vary by geographic location and farming systems. In addition to arid and tropical regions, where heat is already a limiting factor, the most impacted areas will be those with a subtropical-Mediterranean climate, which are exposed to significant heat stress for several months of the year. In addition, intensive production systems in these areas, characterized by a high density of animals raised for production and managed in specialized facilities, present additional challenges (Summer et al., 2019).

Climate is a collection of elements such as temperature, humidity, precipitation, air movement, radiation, atmospheric pressure, and ionization. Climate zones vary globally depending on factors such as latitude, prevailing winds, evaporation, water availability, altitude, proximity to mountains and other parameters (West, 2003). The area between the lower Padana Plain and the northern Adriatic coast is particularly affected by climate warming, showing high maximum temperatures due to low average altitude and high humidity levels from the Adriatic Sea, trapped by the barrier of the Apennines to the south-southwest and the Alps to the north. This area also experiences diurnal convection phenomena, often found in the Alps and throughout the Padana Plain, which can lead to higher summer precipitation than in other parts of Italy due to Atlantic moisture from central Europe (Coccimiglio et al., 2009).

Exposure to unfavourable thermal conditions, caused by high temperatures and humidity combined, exceeds the ability of cattle to dissipate heat, leading to an increase in body temperature beyond physiological limits (38,5°C). This situation, known as heat stress, adversely affects the welfare and performance of dairy cattle. In this state, the conversion of nutrients to energy becomes less efficient, resulting in reduced dry matter intake and increased water consumption, as well as worsened nutrient absorption. This rapidly impacts livestock performance. To assess the combined effect of temperature and moisture and to determine the risk of heat stress in cattle, the temperature-humidity index is used. In the case of dairy cows, climate changes affect the organic and inorganic composition of milk, as well as the efficiency of dairy processes, especially for cheeses made from non-standardized raw milk, such as Parmigiano Reggiano (Summer et al., 2019).

Heat stress affects dairy cattle in various ways, culminating in decreased milk production and overall animal performance. Obvious behavioural signs include shade seeking, refusal to lie down, lack of coordination and mobility, increased respiratory rate and laboured breathing, as well as higher heart rate, profuse salivation, and sweating. In addition, there is crowding around water sources and an increase in fluid intake. Blood circulation to internal organs decreases, the digestion process undergoes alterations, including reduced or absent ruminal activity and a slowing of the passage of food through the digestive system. There is also a decrease in dry matter and feed intake, which impacts milk production and quality. Hormone levels also fluctuate, along with a decline in reproductive performance, a reduction in calf birth weight, and an increase in energy requirements for maintenance. These processes occur progressively and eventually contribute to a decrease in animal production (Atrian and Shahryar, 2012). In situations of high outdoor temperatures, heat emission through conduction, convection and radiation decreases, while heat emission through evaporation by sweating and breathing increases significantly (Joksimović-Todorović, 2011).

Heat stress can occur in early summer or throughout the hot period. In the case of not existent or inefficient animal air conditioning and/or cooling systems it is possible for some or all the cattle to become "sick" from heat stress. This pathological status can be objectively diagnosed by measuring rectal temperature and respiratory rate. An increase of only 0.5°C in body temperature (from 39°C) and a respiratory rate of more than 80 acts per minute is enough to state that the cow is in heat stress. If more than 15 % of the cows have an increase in rectal temperature and respiratory rate beyond the indicated limits, this means that there is a collective risk factor in the barn and the situation needs to be managed. Otherwise, these are individual cows that cannot adapt to heat and therefore need to be managed individually (Fantini, 2018).

4.1 Heat stress genesis and temperature humidity index

The heat stress effect on dairy cows is caused by various external factors, including temperature, relative air humidity, solar radiation, air movement and rainfall. However, quantifying the contribution of each factor to the induction of heat stress is difficult because there is little reliable data available. Most studies on this topic focus mainly on temperature and relative air humidity. The selection of dairy cattle breeds for high performance, focusing on milk production and feed intake, has led to reduced production and reproduction during periods of increased outdoor temperature, because the thermoregulatory capacity of the animals has not been adequately considered. Increased food ingestion results in increased metabolism, requiring efficient thermoregulatory mechanisms to maintain body temperature and physiological balance. The exact determination of when a cow is in heat stress is complex, as its onset is influenced not only by energy balance, but also by the amount of water and sodium, potassium, and chlorine metabolism (Joksimović and Todorović, 2011). Therefore, hot and humid conditions are believed to affect the body temperature of cows. Holstein cows can maintain a stable body temperature as low as 25-26°C, with practices to minimize the increase in body temperature when 25°C is exceeded (West, 2003).

Body temperature is the result of the balance between heat production and heat loss. In high-producing cattle, heat production is higher and the effect of a warm environment is more pronounced. High-production cows reach a higher body temperature than low-production cows, given their faster metabolism and higher heat production. In hot climates or during summer, not only temperature should be considered, but also ambient humidity, which is a critical factor in heat stress. Air humidity can affect the ability of dairy cows to dissipate heat through sweating and respiration. Under high temperatures, humidity makes evaporative cooling less effective. Evaporative cooling occurs when sweat or moisture present on the skin or in the respiratory tract evaporates. This explains why dairy cows experience sweating and increased respiratory rate during heat stress. Wind plays a significant role in heat stress by influencing heat loss through the processes of convection and evaporation. Accurate measurement of heat stress in dairy cows is complex because animal responses to stress involve not only energy balance but also water, sodium, potassium and chlorine metabolism.

The humidity-temperature index (THI) is a parameter used to assess the intensity of heat stress. This index is calculated with the specific formulas using both ambient temperature and relative air humidity. Signs of heat stress become evident in dairy cows when the THI exceeds 72 degrees.

The severity of the heat stress effect is categorized according to THI into four levels, from mild to very severe. For values between 72 and 78 stress is mild, for values between 79 and 88 stress is moderate, for values between 89 and 98 stress is severe, and for values greater than 98 stress is very severe. The most severe THI situations can lead to the death of most of the cows in the herd. Farmers can use thermometers/hygrometers to measure the severity of heat stress, this helps to maintain optimal conditions for cows and prevent the negative impacts of heat stress (Atrian and Shahryar, 2012).

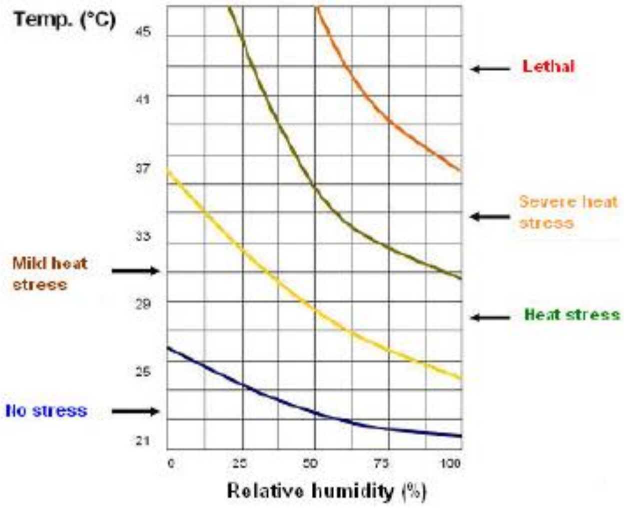


Image 1: different levels of heat stress and its effect on cows (Atrian and Shahryar, 2012).

Temperature	Relative humidity													Stress level
	40	45	50	55	60	65	70	75	80	85	90	95	100	
75	No stress					72	72	73	73	74	74	75	75	Mild
80	73	73	74	74	75	76	76	77	78	78	79	79	80	Moderate
85	76	77	78	78	79	80	81	81	82	83	84	84	85	
90	79	80	81	82	83	84	85	86	86	87	88	89		Severe
95	83	84	85	86	87	88	89	90	91	92	93	94	95	
100	86	87	88	90	91	92	93	94	95	97	98	99		
105	89	91	92	93	95	96	97							

Image 2: THI determined by the use of Relative humidity and Tempertaure (Atrian and Shahryar, 2012).

4.2 Strategies for reducing heat stress

To mitigate and prevent the impact of heat stress in dairy cattle, a complex approach is essential. First, careful genetic selection must be conducted to develop a more heat-tolerant dairy cattle population. In addition, it is essential to improve animal nutrition and optimize the design and environmental control of the structures in which they are housed. It should be emphasized that the process of genetic selection to obtain more heat-adapted cattle is long-term and may be impractical in tight time frames on dairy farms. Therefore, the focus must be on immediate solutions to mitigate the effect of heat stress. Prevention of body temperature rise in hot environments can be achieved through three main approaches. The first is to reduce the temperature of the environment, which can be achieved by cooling buildings or introducing cooling systems for animals, such as the use of ventilators or water foggers. The second approach involves increasing the heat loss of the animals themselves, using techniques such as water sprinkling and the use of ventilators. The third approach aims to optimize energy efficiency from the diet and reduce internal heat production through appropriate feeding strategies (Atrian and Shahryar, 2012).

In the past few years, there has been an increasing adoption of cooling systems for on-farm facilities and for the cows themselves during the summer months, with the aim of improving conditions and reducing losses in milk production. However, despite these measures, heat stress continues to be a key factor negatively affecting health, reproduction, and milk production. Careful and intensive management is critical to mitigate the effect of heat stress on milk efficiency and profitability. Several preventive measures should be taken before the hot seasons arrive, as dairy cows are particularly sensitive to heat stress. These include protecting them from direct sunlight, using ventilators and misting systems for cooling, dispensing high-quality feed with adequate levels of protein, fat, minerals, and vitamins, dividing rations into several meals during the cooler hours of the day, cleaning feed troughs to prevent rations from spoiling, and ensuring continuous access to fresh, clean water. These methods prove effective in managing heat stress.

In summary, three main strategies can be adopted to reduce the negative effect of heat stress in dairy cattle. These strategies include modifying the surrounding environment, providing shade and cooling for the animals themselves, and adopting more heat-tolerant breeds, along with optimizing feeding. Implementation of these combined measures can greatly improve milk production in cows exposed to hot and humid weather conditions (Joksimović-Todorović, 2011).

4.3 Heat stress effects on milk production

Under heat stress situations, the first observable consequence is reduced milk production. High-yielding dairy cows are particularly susceptible to the impact of heat, especially at the beginning of the lactation phase. When the body temperature exceeds 39°C, there is a significant decrease in the amount of milk produced. For example, at an external temperature of 35°C, there is a 33%

reduction in milk production, while at 40°C this reduction reaches 50%. Failure of the organism to adapt to new climatic conditions results in health disorders, reduced nutritional requirements, altered chemical composition of milk and reproductive problems. In the early lactation period, cows have limited resources to cope with heat stress, so the greatest impact on their milk yields occurs in the first 60 days (Joksimović-Todorović, 2011).

During the lactation phase, dairy cows generate a high amount of metabolic heat and accumulate additional heat from radiant energy. This heat accumulation, together with the impaired cooling capacity caused by environmental conditions, contributes to the increased heat load on cows. This increase in body heat leads to a decrease in feed intake and, consequently, a reduction in cow productivity (West, 2003).

Trends in daily milk production are strongly influenced by climatic variations. An increase in temperature and humidity results in a significant decrease in the amount of milk produced daily (in kilograms per day). This decrease can be calculated by means of the formula proposed by Berry et al. (1964), which considers the decline in milk production (in kg/d) as a function of the normal level (NL) of daily milk production (in kilograms per day), recorded at temperatures between 10 and 18°C, and the average daily temperature-humidity index (THI): decline in milk production (kg/d) = 1.075 - 1.736 NL + 0.02474 × NL x THI. Using this formula, it becomes clear that milk production decreases as the THI index increases, especially in the most productive cows (with daily production between 15 and 40 kg). This results in an estimated loss of 0.27 kg of milk for each increase in THI index.

The effect of heat stress on milk fat content is not clearly defined and results are conflicting, while lactose, the predominant component after water, does not appear to be significantly affected by the thermal environment. In the cheesemaking process, the composition of the protein fraction and its seasonal changes play a key role in milk coagulation. Exposure of cows to heat stress can lead to a decrease in protein and casein levels in milk. This decrease seems to be more related to the direct effect of heat stress rather than a decrease in feed intake. In cows exposed to heat stress, there is an increase in casein $\alpha s1$ and a decrease in casein $\alpha s2$, while the other fractions remain unchanged. The effects of heat stress on milk mineral content and distribution are less well understood, while it appears that the number of somatic cells in milk is not affected by heat. The climatic conditions in which cows are kept can affect the pH and titratable acidity of milk, critical factors in cheese quality and yield. Increases in pH and decreases in titratable acidity have been observed when cows are exposed to THI values above 75. These changes have negative consequences on cheese production and its quality. Milk coagulation properties are also affected by the barn climate. These results are related to the close relationship between coagulation properties, casein content, titratable acidity, and mineral content. The effect on milk coagulation time is evident, with a significant increase when the THI index exceeds 75. Overall, milk quality characteristics from cows exposed to heat stress lead to lower cheese yield (amount of cheese obtained from 100 kg of milk) (Summer et al., 2019).

It should be noted that the response of cows to heat stress varies among different breeds. For example, according to Malacarne et al. (2005), the Italian Friesian and Italian Brown breeds show differences in their reaction to the thermal environment. Both experience a decrease in milk production in summer, but this decrease is more pronounced in Italian Friesian cows than in Italian Brown cows. In general, more rustic and low-producing cows resist heat stress better.

Given the trend of climate change, it is essential to adapt animal management systems to the new environmental conditions, to preserve the quantity and quality of dairy products. This requires multiple interventions, which may involve feed planning, selection of resistant animals and adoption of technologies such as cooling systems and automatic feed distribution (Summer et al., 2019).

4.4 Heat stress, physiopathology, and dairy cattle digestive tract

During periods of heat stress, dairy cows reduce their feed intake to limit ruminal heat production and they change the use of some feed components. In dairy cows, especially high-yielding cows, the heat stress effect is accentuated by the release of heat energy during a phase when feed intake is reduced, further exacerbating the typical negative energy balance at the beginning of lactation. This susceptibility is more pronounced in cows with a higher genetic potential for milk production than those with a lower potential (Joksimović-Todorović, 2011), resulting in an increased risk of diseases, such as ketosis and hepatic lipidosis, due to significant weight loss during summer (Fantini, 2018).

High environmental temperatures affect the microorganisms in the rumen, which are responsible for the synthesis of B vitamins, amino acids and fatty acids, crucial elements in ruminant nutrition. Available evidence indicates that rumination activity is reduced during dehydration and heat stress. During these conditions, blood flow to the rumen epithelium decreases, causing a decrease in reticular motility and rumination, while the water content and volume in the rumen increases, promoting the water reserve function of the rumen to compensate for the impact of heat stress on ruminal motility. In addition, the passage of digesta along the gastrointestinal tract of heat-stressed cattle is slower than that of animals under thermally neutral conditions, reflecting reduced feed intake, decreased ruminal activity and lower motility (Kadzere et al., 2002).

During evaporation to dissipate excess heat, a significant loss of electrolytes occurs, especially when the outside temperature exceeds 35°C (Joksimović-Todorović, 2011), resulting in an imbalance of the body's acid-base balance (Atrian and Shahryar, 2012). Consequently, water and macro-mineral requirements in lactating dairy cows are altered during heat stress, as they are closely linked to the demands of maintaining homeostasis and thermoregulation. High-yielding cows tend to increase water consumption, which becomes even more pronounced under heat stress, as these cows have a faster rate of dehydration due to high water turnover. This increase in hydration is beneficial because the high heat capacity of water allows cows to store heat during the day and release it at night.

Fluctuations in the thermal environment can lead to alterations in the activity and function of the digestive system, independent of the change in feed intake. Food intake begins to decrease in lactating cows when the ambient temperature reaches 25-26°C and decreases significantly above 30°C. Under conditions of heat stress, the cooling center of the hypothalamus stimulates the satiety center, which inhibits the appetite center, causing a reduction in feed intake and consequently in milk production. Highly productive animals, such as high-yielding dairy cows, have two to four times higher appetite and metabolic rate than maintenance cows. During heat stress, these cows experience a marked reduction in raw feed intake and rumination activity. The decrease in appetite under heat stress is related to elevated body temperature and may be associated with gut filling. This decrease in raw food intake leads to a decrease in volatile fatty acid (VFA) production and may affect the proportion of acetate and propionate. In addition, rumen pH decreases during heat stress. In contrast, ruminants adapted to high temperatures manage to maintain palatability similar to that under heat-neutral or moderate growth conditions. Dairy cows under heat stress change their feeding pattern, consuming food when temperatures are cooler. The most pronounced reduction in milk production at higher temperatures is the result of lower feed intake (Kadzere et al., 2002).

4.5 Heat stress and dairy cow nutrition

In recent times, there has been a greater understanding of clinical and functional nutrition as a strategy to promote optimal adaptation of cows to high temperatures. Nutritional measures to be taken during the heat stress period involve a significant review of the diets fed to lactating cows and their feeding regimens. Since dairy cows during summer tend to consume food mainly during the night hours, it would be advantageous to maintain lighting in the stables and feed them during afternoon milking. The method considered as the gold standard is to deliver the total daily ration twice a day, concurrently with the morning and afternoon milkings, bringing feed closer to the cows at the feeder at least four times a day (Fantini, 2018). Optimal feed intake occurs early in the morning and late in the evening, as peak feed digestion occurs three to four hours after intake. This avoids the hottest hours of the day (Joksimović-Todorović, 2011). It is recommended to eliminate unappetizing feeds and additives from summer feeding, preferring the use of more easily digestible forages available at the farm (Kadzere et al., 2002).

During summer, water consumption increases by up to 50 %, leading to an average increase of more than 120 litres per day for lactating cows, with a higher concentration (60 %) after milking sessions. Drinking troughs should ideally be located at the rear of the feeding area and at the exit from the milking room or milking robot. If the structure has outdoor paddocks, it is advisable to provide shaded troughs. Decreased feed intake combined with the extensive use of water by cows for body thermoregulation increases the risk of ruminal acidosis, which in turn may contribute to an increased risk of laminitis, a condition often associated with reduced hours of rest in poorly air-conditioned barns during summer (Fantini, 2018).

Several research studies have shown that diets high in concentrates and low in fiber can mitigate heat stress in lactating dairy cows, because the metabolizable energy of concentrate diets is used more efficiently (less internal heat) than diets high in forages (Kadzere et al., 2002). Summer diets tend to contain less starch and more digestible sugars and fiber from concentrates such as bran, soybean hulls, and beet pulps. During summer, protein production from the ruminal microbiota decreases, and this is reflected in reduced protein concentration in milk. The lower dietary intake also affects the amount of protein with low ruminal degradability. The introduction of ruminally protected fat and protein is a valid approach to reduce metabolic heat production and provide an adequate nutritional profile for high-yielding cows during the onset of lactation. During summer, it is advisable to increase the protein portion of the ration, acting on the low ruminal degradability part and including protected amino acids such as methionine and/or lysine, following the recommendations of formulation software.

During heat stress conditions, the management of macro-minerals in the diet changes dramatically, especially when the temperature-humidity index (THI) exceeds 68. Among minerals, sodium bicarbonate is of particular relevance because, in addition to its buffering role in the rumen, it provides sodium needed for the removal of hydrogen ions (H), which might otherwise damage ruminal epithelial cells. High respiratory rate in heat-stressed cows can lead to metabolic acidosis due to high carbon dioxide removal. Some herds in at-risk areas increase the chlorine and sulphur content of diets during the dry period, especially in the run-up to calving, to balance the alkalizing effects of potassium and phosphorus present in large amounts in some lowland hays. Pregnant and non-lactating cows also face challenges during the summer, so it is advisable to reduce or eliminate chlorine and sulphur additions from diets during this period (Fantini, 2018). Thus, adequate nutrient intake must also include a well-balanced mix of dietary minerals, including sodium, potassium, chlorine, and sulphate. These minerals play a crucial role in the body thermoregulation of cows (Kadzere et al., 2002). According to National Research Council (1989) recommendations during heat stress, it is suggested to increase the amount of sodium from 1.2 % to 1.5 %, chlorine from 0.4 % to 0.6 % and magnesium from 0.3 % to 0.35 % in dry matter (West, 1999).

The reduced production of B vitamins due to decreased ruminal fermentations during the summer suggests the possibility of supplementing these vitamins, especially in the form of rumen-protected vitamins, to promote cow health. Niacin, also known as vitamin PP or nicotinic acid, can reduce the emission of fatty acids (NEFA) from adipose tissue and significantly reduce the risk of hepatic lipidosis and metabolic ketosis during summer when administered in the appropriate dosage (at least 6 g of active ingredient per day). Niacin also appears to have vasodilator effects, promoting the dissipation of body heat (Fantini, 2018). In addition, it is necessary to increase the intake of other vitamins, particularly vitamin A (100000 IU/day), vitamin C (50000 IU/day) and vitamin E (500 IU/day), in summer diets (West, 1999).

According to Gantner et al. (2017), less productive and more rustic cattle breeds are less affected by heat stress than high-producing cattle breeds. This was demonstrated by conducting a comparison between the Simmental and Holstein breeds. In the area of Parmigiano Reggiano cheese production, the local breed of excellence is the Rossa Reggiana cattle. Few studies have been carried out on this breed regarding its resistance to heat stress and the nutrition that best suits it in the hot summer period that affects the Padana Plain and the hills of the Appennino Tosco-Emiliano. For this reason, the next chapter aims to rediscover this breed, which has lower production and milk with ideal characteristics to produce Parmigiano Reggiano cheese, while the aim of the experimental part will be to investigate nutrition during the summer period, in presence of heat stress, and to correlate it with the quality and quantity of milk produced.

5. Rossa Reggiana cow breeding excellence, uniqueness and context

Native breeds represent important reservoirs of genetic variability. These breeds are generally well adapted to the production systems in which they developed (Bovo et al., 2021). However, the development of cosmopolitan dairy cattle breeds with high potentials for intensive production has threatened many local dairy cattle breeds associated with modest production. Although the dynamics of loss of local breeds have not been explored in depth in the scientific literature, it is possible that the low number of local remaining animals can be attributed to the low profit potential compared to other breeds in intensive production systems.

In the specific case of Parmigiano Reggiano, the native Rossa Reggiana breed has been replaced and supplanted by more productive breeds, such as Holstein and Bruna Alpina. The 1992 Convention on Biological Diversity recommends maintaining a variety of local breeds, along with a variety of associated breeding systems, as a strategy for preserving genetic variation for future use in the development of livestock production. Therefore, since 1992, some local government agencies and EU agricultural policy have been providing economic compensation to dairy farmers who maintain herds of locally bred cattle. Although this is an effective method to stop the loss of local breeds, it cannot continue forever, so other good reasons must be found for maintaining local breeds with lower production. These include functional traits, such as higher fertility, disease resistance, longevity, and greater rusticity, which have great impact on breeding profitability. An evaluation of the functional traits of local breeds is also necessary to develop sustainable genetic improvement programs (Gandini et al., 2007).

The Reggiana cattle is an autochthonous breed of northern Italy, found especially in the provinces of Reggio Emilia and Parma. In the past it was a "triple-purpose" breed (milk, meat and work) while today it is "dual-purpose" (milk and meat), but specialized mainly in milk production. The first records of the Reggiana cattle breed, also known as the "Vacca Rossa" or "Fromentina," according to agronomist Filippo Re, date back to the early 19th century. However, according to historical sources, the origins of the Reggiana breed are much older and date back to cattle that came to the Italian peninsula with the barbarian invasions in the 6th century. The Langobard people spread westward after the breakup of the Roman Empire and entered Italy in 568, introducing into the Padana plain cattle plundered from the plains of southern Russia and ancient Pannonia of the Roman Empire, present-day Hungary. Many of these cattle, belonging to the Podolian stock, had red coats, a dominant characteristic transmitted from the ancient red cattle of the Russian steppe, still found in Ukraine and central Russia (www.agraria.org;

www.razzareggiana.it; ANABoRaRe). With the Longobards, a livestock conversion began that led to cattle replacing sheep, giving rise to a "dairy revolution". The Benedictine monks in the 12th century supported the dairy revolution with their land reclamation works and the subsequent cultivation of the land. They set the stage for the development of new agricultural activities such as cattle breeding and the production of Grana cheese, giving rise to "Formadio," the progenitor of Parmigiano Reggiano DOP (documented in the Marola parchment of 1159), in the lands of Matilde di Canossa. Triple-purpose Rossa cows found particularly favourable environmental conditions for their development in the territories to the right of the Po' and since the year 1000, they replaced the pre-existing local breeds, maintaining unchallenged supremacy until the first half of the 1900s. The breed was the most widely bred until the mid-20th century in the agricultural and livestock context of Reggio Emilia and Parma, reaching its peak in 1954, with a population of 139,695 head. In 1956 the “Associazione Nazionale Allevatori bovini di razza Reggiana” (ANaBoRaRe), a national cattle breeders' association, was formed and was officially recognized in May 1962 (Lucarini, 2021; Bertolini et al., 2020).
Italy's postwar livestock policy began crossbreeding to replace these cows with cosmopolitan breeds, to achieve greater production through selection and to meet a greater development of the dairy industry, with increasing demands for milk for processing. Deep changes in agriculture and society take place during these years. The main causes of the gradual reduction of the Reggiana breed cattle were the disappearance of sharecropping, changed breeding conditions, delayed payment for milk on quality parameters, the introduction of more productive cosmopolitan breeds and the lack of a concrete genetic improvement program. By the 1970s the Reggiana breed had almost disappeared, but the historical minimum was reached in 1981 with 985 head (corresponding to 0.6 % of the cattle raised in the province of Reggio Emilia). Since 1985, the “Registro Anagrafico delle popolazioni bovine autoctone e gruppi etnici a limitata diffusione” has been established to safeguard cattle breeds threatened with extinction and their genetic heritages (Beretti et al., 2010).
In recent years, thanks to breeders, technicians and public structures, a partial recovery and renewed interest in this breed have been possible and since 1987 it has begun to show a slow recovery in numbers. After years of selective activity by breeders, the Reggiana breed can now be considered exclusively dairy-oriented. The quality of the milk and new valorization strategies, supported by the “Ministero per le Politiche Agricole e Forestali” and the Emilia-Romagna Region, have resulted in a steady demographic recovery, so much so that the number in 2021 is 3032

lactating cows in 102 herds controlled for milk production, with a total of 4450 individuals registered in the herd book (www.razzareggiana.it; ANABoRaRe). This was possible after reconstructing a breed-product identity (production of “Parmigiano Reggiano delle Vacche Rosse” under its own brand name) and its traceability, which allowed the recovery of an economic value that was the basis for the resumption of the breed's numerical development. Today, the Reggiana breed is mainly widespread in the territory of the Reggio Emilia province, distributed in small- to medium-sized herds and is present in the neighbouring provinces of Modena, Mantova and Parma, where it is growing numerically, given the interest aroused by the remunerative potential of its main product (ANABoRaRe). The ANABoRaRE holds the Reggiana Breed Genealogical Book, established in 1996, and oversees the entire production process of Parmigiano Reggiano from the Reggiana Cows. The “Parmigiano-Reggiano delle Vacche Rosse” is placed in the higher market segment of the P.D.O. market. The regulation implies the obligation to feed Reggiana cattle registered in the herd book with fresh green grass, to use fodder more than 90 % from the area and to prohibit the single plate and GMO feed. In fact, unlike Friesians, for the Reggiana the diet is compensated by feeding fresh grasses from the stable meadows in the production area. These are centuries-old meadows that are not processed and contain different types of plants. A diet based on polyphytic grasses makes it possible to obtain milk with rich and varied aromas and taste, with a considerable amount of beneficial compounds, such as β-carotene, an element that gives the most straw colour to the milk and consequently also to the cheese, as well as having positive effects on the animal's health as well. The amount of milk is less and this also has an impact on cheesemaking, with benefits. In addition to local grasses, where possible on farms along the Apennine range, animals are left to pasture. Only after the cheese has been aged for 24 months it becomes "Parmigiano Reggiano delle Vacche Rosse" (Di Berardino 2021; Lucarini, 2021).

Since 2017, the Reggiana breed along with 15 other Italian dual-attitude breeds has been part of the Dualbreeding project. This project, approved by MIPAAF, is aimed at increasing knowledge, enhancing, and safeguarding the genetic typicality of dual-purpose cattle breeds. The main objectives of the project, which integrates for the Reggiana with the existing selection program, are the preservation and characterization of the animal heritage, the maintenance of genetic variability and the identification of possible new indicators related to animal welfare, environmental impact and disease resistance (www.dualbreeding.com).

5.1 Rossa Reggiana breed morphology and characteristics

The Rossa Reggiana cow is characterized by good size, long trunk, solid skeleton, and rather long head. It is a very rustic, fertile, and long-lived animal. The distinguishing feature of the breed is the uniform "fromentino" red coat, from the color of the ripe wheat caryopsis, which can take variations in shade in the inner and lower parts of the limbs, around the eyes, around the snout and on the inside of the tail. From this coloration comes the designation "Vacca Rossa." The skin is of medium thickness, elastic and well lifted, with fine, smooth, and shiny hair. The head is very distinct, characterized by a spacious, slightly concave forehead with a straight fronto-nasal profile, large eyes, and a calm look. The thorax is well developed, the skin is of medium thickness and the limbs are robust. The medium-long horns have a slightly elliptical cross-section, positioned first outward, then slightly forward and then upward. Their colour is yellow with a dark tip or deep red. The limbs are robust and strong, as work in the fields once required, but not coarse, with short, strong fetters, suitable for grazing even in hilly and mountainous areas. The back limbs are often slightly mowed. The hind legs have variable musculature, and although the breed in recent years has been valued primarily for milk, it has a good number of individuals with full thighs and profiles of fair convexity (Lucarini, 2021; www.dualbreeding.com; www.razzareggiana.it).

The udder is the organ that still shows some heterogeneity in breed characteristics, as it is generally developed but not voluminous. Today, the morphological-functional trend is steadily improving, with special emphasis on the mammary apparatus. In general, the udder has normal shape and development, the hindquarters are well developed, the veins are well visible and developed, the skin is fine and rarely very hairy, and the teats are sometimes very long and thick. Subjects of this breed are docile in temperament but nevrile. They present medium size and have a height at withers that varies in adult animals from 145/155 cm for bulls, with an average weight of 9-10 quintals, to 140/145 cm for cows, with an average weight of 6.5-7 quintals (ANABoRaRe; Lucarini, 2021; www.razzareggiana.it). The rusticity of the breed allows, compared to more selected breeds such as the Italian Friesian, to make better use of coarse forages, reduce sanitary expenses and the replacement rate. The Reggiana shows higher reproductive efficiency, with a calving-first insemination interval of 86.50 ± 2.37 days, compared with 97.37 ± 3.04 in the Italian Friesian, a calving-conception interval of 107.48 ± 3.33 days, compared with 126.28 ± 4.28 in the Friesian, and an inter-parturition interval of 387.23 ± 61.97 days, compared with 417.64 ± 78.31 in the Friesian. The Reggiana also has a longer longevity than the Italian Friesian (84.72 months vs. 73.06 months), with 15 months more productive life (58.68 months vs. 43.85 months), a comeback rate of 20.4 % vs. 27.3 %, and one more calving per productive career. On average, more than 5 births per head are achieved, with some exceptions that can reach as many as 10 births. Therefore, the Reggiana breed shows better fertility and longevity, functional traits that condition the profitability of dairy cattle breeding (Pizzi et al., 2003).

In the past, the beginning of the Reggiana breed selection activity had as its main objectives the increase in the number of cattle, thanks to the doses of frozen bovine semen stored in the Association's genetic reserve, and the improvement of dairy aptitude, especially relating to udder morphology and mechanical milking aptitude. Today, the activity is still focused on productive aptitude, which is complemented by new selection goals. In general, within the framework of the Dual Breeding project and with the spread of genomic analysis, the new selection goals for the Reggiana breed are the definitive fixation of the MC1R gene, which is responsible for the "fromentino" red coat colour, improvement of disease resistance, animal welfare and environmental impact, inbreeding monitoring and minimum inbreeding management for the Reggiana breed.

The Parmigiano Reggiano Cheese Index is an indicator that allows selective choices to be made that can gradually increase milk production with particular attention to fat and protein percentages and, more generally, to quality preservation. As part of the Dual Breeding project, new genetic indices have been developed, such as the Somatic Cell Genetic Index, based on the somatic cells present in milk, and the Farrowing-Conception Genetic Index, a genetic indicator of each cow's ability to be fertile, that is, to conceive in the shortest possible time after calving (Lucarini, 2021; www.dualbreeding.com).

5.2 Rossa Reggiana breed milk characteristics

Although it was once defined as a triple-purpose breed, the Reggiana cow is now used for its predominant aptitude for milk production, as its milk is characterized by high quality parameters, particularly suitable for processing into Parmigiano Reggiano cheese. The most interesting and significant differences between the local cattle breeds (Reggiana, Bruna Alpina and Bianca Val Padana) and the "Frisona Pezzata Nera" have been known for more than 25 years. Studies have shown the superiority of Reggiana milk, which has higher contents of dry matter, casein, ash, calcium, phosphorus and citric acid. It also has a higher titratable acidity, good surfacing capacity and is characterized by lower chloride content.

The most frequent phenotypic frequencies of proteins evidenced to date using electrophoretic techniques are those homozygous for A, for B and heterozygous for AB. In particular, the B variants of β-casein and κ-casein are thought to be more advantageous for cheesemaking, as milk produced from cows with κ-casein BB has significantly better lactodynamometric and rheological parameters than milk characterized by the κ-casein AA phenotype. In fact, κ-casein BB leads to the formation of smaller and more numerous micelles among the different casein types. In terms of cheesemaking, this characteristic offers higher yields, better rheological properties, greater whey bleeding, with less occurrence of abnormal fermentations, and allows the cheese to mature better over the long term, improving the digestibility of its protein and lipid components. Milk studies carried out on the gene and genotype frequency on the whole population and continuously updated show that in the genetic heritage of Reggiana, compared to other breeds, there is a higher frequency of the B variant of κ-casein. They show that κ-casein variant B is present with a frequency of 27 %, which is considered one of the highest among dairy breeds. Milk is rich in protein, particularly κAB and κBB casein, and also has high levels of calcium and phosphorus, two elements that contribute to increased cheese yield and quality. In addition, the cholesterol level is very low. The diet set for Reggiana cattle includes fresh grass, green forages and hay from stable meadows or alfalfa, allowing milk with a lipid profile rich in polyunsaturated fatty acids and CLA (conjugated linoleic acid isomers). The breed's average production recorded by the "Associazione Italiana Allevatori" (AIA) in 2022 was 6. 271 kg, with 3,68 percent fat and 3.41 percent protein content. From this data, it can be said that the Reggiana breed produces on average 38 % less than the Friesian breed (10. 097 kg, 3,74% fat, 3,33% protein), which is a large difference. This difference in production explains why the Reggiana breed has been supplanted by the Holstein breed for Parmigiano Reggiano production. However, milk from the Reggiana cow contains more dry matter in the milk than Holsteins and has a higher percentage of caseins, making it more suitable for cheese production, given the excellent rennet coagulation properties. In addition, the lower milk yield is compensated by the higher economic yield, due to the milk-quality payment (Gandini et al., 2007). Some studies on lactation curves (Sabbioni et al. 2003) have also shown the ability of Reggiana cows to produce high amounts of milk (peak production from 22.17 kg at 1st calving to 28.72 kg at 5th calving), fat-corrected milk (peak production from 19.95 kg at 1st calving to 25.33 kg at 5th calving), fat (peak production from 0.75 kg at 1st calving to 0.97 kg at 5th calving) and protein (peak production from 0.70 kg at 1st" calving to 0.89 kg at

5th calving). Lactation persistence was higher in cows at 1st calving (73 d) than in subsequent cows (56-59 d) (Beretti et al., 2010).
The history of the Rossa Reggiana cow shows that the recovery of this breed was only possible through the link with the prince product of its district, the "Parmigiano Reggiano delle Vacche Rosse," on which two additional marks are placed in addition to those of Parmigiano Reggiano: the "Vacche Rosse, razza Reggiana" mark owned by Grana d'oro and the "Razza Reggiana" mark owned by AnaBoRaRe. In fact, in 1991 the Vacche Rosse consortium was established and in 1992 the dairy producer consortia started the production of a branded Parmigiano Reggiano cheese made exclusively from milk of Reggiane cows. Branded cheese is sold at higher prices than standard Parmigiano Reggiano and is revitalizing interest in Reggiane cows (Gandini et al., 2007). The premium price that this branded Parmigiano-Reggiano can achieve, when compared to undifferentiated Parmigiano-Reggiano, has guaranteed an economic remuneration to farmers that compensates for the lower productivity of Reggiana cows compared to that of other cosmopolitan dairy breeds. The resulting economic benefit to Reggiana cattle breeders has made it possible to sustain a successful conservation program for this genetic resource closely intertwined with its cheese (Bovo et al., 2021).

5.3 Rossa Reggiana cow feeding and milk production in summer

As stated in the article by Bovo et al. (2021), several studies have been conducted to characterize the Reggiana red breed at the genetic level. On the other hand, no study has been conducted to evaluate the impact that the feeding of these animals may have on their production and milk characteristics, especially during the summer period, when high temperatures and high humidity are present in the Padana Plain and in the hills of the Appennino Tosco-Emiliano, leading to the occurrence of heat stress in cattle raised in these areas. In the following experimental part, this issue will be investigated in depth, to obtain a characterization of milk quality and dairy aptitude, as well as to identify the ideal dietary and nutritional parameters for optimizing the productivity of Rossa Reggiana dairy cattle during summer season.

6. EXPERIMENTAL CONTRIBUTION

6.1 Introduction

Over the years, the scientific literature has focused on the study of how nutrition can influence the composition and dairy characteristics of milk in cosmopolitan, high-producing breeds, such as Holstein and, to a lesser extent, Brown Swiss and Jersey. Although genetics is the most effective method in modifying milk production and composition in the long-term, feeding offers a rapid and effective method for obtaining short-term changes (Sutton, 1989). Most studies have focused on the changes that feeding can induce on fat and protein, both parameters being subjected to milk-quality payment, but also on lactose, urea, and milk coagulation parameters. Roughage, forage to concentrate ratio, soluble carbohydrates, dietary lipids, dietary proteins, intake, and frequency of feeding are the main factors influencing fat in milk (Sutton, 1989), while forage to concentrate ratio, dietary protein amount and source and dietary fat amount and source are the dietary components that most influence its protein content (Jenkins and McGuire, 2006). The milk's lactose content depends on the animal's energy balance and the cereals fed, which vary the starch intake (Formigoni and Mordenti, 2004), while the protein content in feed rations and the energy intake provided by not-structural carbohydrates act on the urea content (Cevolani et al., 2014; Gulińsk et al., 2015). Finally, the milk coagulative parameters depend on the phenotype and amount of caseins present in milk (Buchberger and Dovč, 2000; Mariani, 2011) and on milk minerals, especially calcium (Stocco et al., 2021), parameters that are affected to a lesser extent by feeding.

Another important factor that affects the health and production of dairy cows in the summer period, especially in a hot and humid area such as the Padana Plain, is heat stress. It represents an increasingly relevant and topical issue due to ongoing climate change (Coccimiglio et al., 2009; Summer et al., 2019). Heat stress affects dairy cows in various ways, culminating in decreased milk production and general animal performance, and is measured by the Temperature Humidity Index (THI), which considers the humidity and temperature present in a specific area. When the THI exceeds a value of 72, cows can be considered under heat stress (Atrian and Shahryar, 2012).

In the Parmigiano Reggiano cheese production area, the Rossa Reggiana cow represents an indigenous and limited-spread breed with strong territorial linkage. Bred between the provinces of Reggio Emilia and Parma, this breed was born as a triple-purpose breed, but today it is employed almost exclusively for milk, which is totally directed to the PDO Parmigiano Reggiano cheese production of excellence, with the "Vacche Rosse" brand. While there have been numerous genetic studies aimed at safeguarding the Rossa Reggiana cow, there is a notable absence of nutrition studies in the scientific literature correlating diet composition with the milk characteristics of this breed. The field trial reported in this study aims to provide guidelines about the best feeding of the Rossa Reggiana cattle under heat stress condition, by evaluating the relationships present between the diet provided and the amount and composition of milk produced.

6.2 Materials and methods

The present field trial was realized in agreement with the "Associazione Nazionale Allevatori Bovini di Razza Reggiana" (ANABoRaRe) and the "Consorzio Vacche Rosse" on 9 farms located in the Parmigiano Reggiano cheese production area, which joined the project, sponsored by the "Consorzio Agrario di Parma". The analyses were conducted on feed, milk and faeces taken at the farm. Several parameters were measured at each sampling, such as average herd feed intake, average herd digestibility of different nutrients in the ration, average herd quantitative production, and milk quality and dairy aptitude.

6.2.1 Farms recruitment

Farm	Geographic coordinates	Territory	Housing type	Total cows	Milking cows	Milk production 2021 (q)
1	44.904037120484624, 10.095780668507434	Plain	Loose	331	144	10 326
2	44.91997342650956, 10.704075565175042	Plain	Loose	214	100	5 059
3	44.71926717368281, 10.750321123763051	Plain	Fixed	102	40	2 948
4	44.66326495812765, 10.700105097694978	Plain	Loose	177	95	7 844
5	44.64428649974956, 10.49049311732634	Foothills	Loose	230	107	6 625
6	44.716322873212384, 10.510294440026897	Plain	Loose	281	115	8 155
7	44.496547652849834, 10.53675354123176	Hill	Fixed	74	31	*n/a
8	44.484096841163606, 10.61670604001185	Hill	Fixed	85	44	3 384
9	44.56384167461867, 10.300219067002384	Hill	Loose	151	62	4 116

***Table 1**. Information on sampled farms (source https://www.razzareggiana.it/).*

*n/a: not available

The 9 farms involved in the field trial are located in the provinces of Reggio Emilia (farms 2, 3, 4, 5, 6, 7 and 8) and Parma (farms 1 and 9), in plain, hilly or piedmont territory. These are medium- and small-scale farms, partly loose-housing barns, and partly fixed-housing barns. Traditional feeding is used in all of them. Geographic coordinates and some information about the 9 visited barns are provided in Table 1.

6.2.2 Sampling and data collection

The 9 barns described above were sampled twice during the summer period in July 2022. The two samplings were conducted approximately 2 weeks apart. Each barn was visited 2 times per sampling on 2 consecutive days, for a total of 4 times.

During the first visit, data were collected on the type of housing, structure (length and number of troughs in cm, length of the trough front in m, number of self-catchers, number of cubicles, presence and number of fans, height difference to reach the milking parlour in %, distance between the milking parlour and the fence in m, length of the fence in m), animal conditions (animals exposed to the sun, coat height, coat cleanliness, fouling thickness, polypnea), number of milkings, frequency of feeding, time spent in the milking parlour over a day in h, time spent on treatments over a month in min, functional milk controls in previous months, and number of total and lactating animals. The 20% of the herd in each barn was measured, rating the BCS on a scale of 1 to 5, the chest circumference in cm, the live weight in kg, and the number of lactations, to establish the average size of the cows bred. The BCS was established by visual and tactile assessment, considering the fat cover at the level of specific anatomical region, as reported by Edmonson et al. (1989).

On each first day of sampling, all feeds supplied to the lactating cows in the feeding line over the next 24 h, including various types of hay and green fresh grass, were weighed using the farm weighbridge or a digital dynamometer. To estimate the amount of feed supplied, the average value provided by the self-feeders was considered summed with the amount supplied directly to the herd by the farmer. On each second day of sampling, the surplus of all forages left in the trough, put aside after each meal, before providing other forages, was weighed, and then summed. By subtracting the total weight of the feed provided from the total weight of the surplus, it was possible to calculate the total barn ingestion and, by dividing by the number of animals that had access to the feeding line, the average ingestion per head during the 24 h.

During the second day of sampling, a pull of faeces was also taken from the 30 % of the cows in the barn from the rectum or on the ground, to measure the instantaneous pH with a digital pH-meter, after mixing and homogenizing the collected faeces and evaluating the faecal score. The faecal score is rated on a scale of 1 to 4, based on the water content, consistency, thickness, and shape of the faeces on the ground (Kononoff et al., 2002).

At each sampling, hay and feed samples of all feed provided, the surplus and the faeces taken from the 30% cow pull were collected for laboratory wet-chemical analysis. The label of the commercial products included in the ration were also taken. A special core drill was used to take hay samples directly from the bales, mixing samples from 2 or 3 different portions of the bale, while fresh grass or surplus samples in the feeding line were taken in at least 5 points and then mixed. Concentrate was taken at several points from different self-feeders or from the container into which the feed was poured from the silos.

At each sampling, the amount (kg) of milk delivered into the tank and the amount of milk out of the tank was measured by estimating the number of cows in the tank and those out of the tank. By dividing the amount of milk in the tank by the number of cows that produced it, the average production per head was determined. Moreover, during the second day of sampling, two 250-mL samples of tank milk, one from the same morning's milking and one from the previous day's evening milking, were collected from the milkers and stored in the refrigerator. These samples were analysed by appropriate laboratory techniques to obtain the rheological characteristics and chemical composition of the milk itself.

6.2.3 Samples chemical analysis

Each sample brought to the laboratory was weighed with properly calibrated scale. Before analysis, the forage and faeces samples were dried in the dryer at 55 °C for 72 h, until a constant weight was obtained. After drying, the samples were weighed again with the initial scale, to estimate the pre-humidity. Once dried, all samples were ground separately in a Retch SK mill (Bauknecht, Stuttgart, Germany) and then passed through a 1-mm sieve. Dry matter (DM) content was determined by leaving the weighed samples in the stove at 103 °C overnight to subtract residual humidity. Ash content was measured gravimetrically by combustion in a muffle furnace at 550 °C (Simoni et al., 2020). Crude protein (CP) content was calculated as a percentage by multiplying the N content *6.25. Nitrogen was determined by chemical analysis performed by combustion digestion of the sample at 900 °C in excess of oxygen using the Dumatherm® machine

(Gerhardt GmbH & Co, Königswinter, Germany) (Mihaljev et al., 2015). Starch content was determined by the polarimetric method (European 29 Commission, 2009). The fibrous fractions aNDFom and ADFom were analysed according to the method of Van Soest et al, (1991), using a semiautomatic system for the boiling and filtration step (FI-WE Raw Fibre Extractor, VELP Scientifica, Usmate Velate, Italy). The aNDFom refers to neutral detergent fiber dosed with heat-stable amylase expressed as net of residual ash, while the ADFom refers to acid detergent fiber expressed as net of residual ash. Acid detergent lignin (ADL) was analysed following the system of sequential analysis (sequential NDF), using the method described by Van Soest et al. (1991), by the technique of solubilization of cellulose with sulfuric acid. The aNDF was determined using a heat-stable amylase, but without sodium sulfite, and was expressed by including residual ash. Residual ash was not determined on the fibrous fractions, except for ADL. Residual aNDF and ADF in the crucibles were collected and weighed for determination of nitrogen bound to the fibrous fractions (N-NDF and N-ADF) (Simoni et al., 2021).

Digestibility of the food fibrous fractions, surplus and faeces was calculated with the appropriate artificial rumen based on the modified Tilley and Terry method, to distinguish rapidly and slowly digestible fiber from not-digestible fiber. Fermentation was conducted in an in vitro batch system described by Goering and Van Soest (1970), with modifications reported by Righi et al. (2009), keeping the sample inside flasks at a constant temperature of 39° C in anaerobiosis, in presence of ruminal fluid, adequate pH and ruminal microorganisms (Simoni et al. 2020). Ruminal fluid was collected at the slaughterhouse from the rumen of 4 cows and processed as described by Simoni et al. (2020) and Simoni, et al. (2020). The digestibilities for each forage sample were calculated at 12, 30, 120 and 240 h, for each concentrate at 12, 72, 120 h, and for each surplus and faeces only at 240 h, obtaining the values of degradable NDF (NDFD) and indigestible NDF (uNDF).

The MilkoScan™ FT3 (FOSS Italy S.r.l., Padova, Italy) was used to determine the chemical composition of milk taken during sampling (fat, protein, lactose, TS, SNF, urea, casein). The Fossomatic™ 7 DC (FOSS Italy S.r.l., Padova, Italy) was used for somatic cell detection (SCC, DSCC). Finally, two MAPE System ldg 2.0s (Ma.Pe. System SRL, Firenze, Italy) were used for the milk coagulation properties analysis (RCT, A30, K20, A60, Amax).

6.2.4 Diets reconstruction and data analysis

The collected barns, animal, and ration data, as well as the data from chemical analyses and digestibility performed on the samples in laboratory, were subsequently entered into the dedicated professional Nutritional Dynamic System (NDS) program, a software created to support nutritionists in the development of ruminant rationing. This is a program developed by RUM&N, on the base of the CNCPS v6.5/v6.55 model, in collaboration with Cornell University, Department of Animal Science, which continuously developed and updated the model itself (https://www.rumen.it/it). Through this program, the diets fed to animals on all 9 farms sampled were reconstructed, obtaining a realistic estimation of the rations provided and predicting the production efficiency of the herd. Since the NDS software does not present the Rossa Reggiana cow as a predefined model, it was used the Simmental breed, a dual-purpose cow with very close production and characteristics.

6.2.5 Data and statistical analysis

The estimated chemical composition data of the 18 rations fed to cows obtained from the NDS software was entered into Microsoft Excel software (Microsoft Corporation, 2018). A database was created to compare the diet chemical composition data with milk analysis data, to establish feeding guidelines for the Rossa Reggiana cow, creating graphics and studying the regressions obtained. Finally, by entering the data into the IBM® SPSS® v.26 software platform, specific for data analysis, the correlations present between milk components and diet components were obtained and studied.

To prove that the cows during the sampling period were under heat stress conditions, the average temperature and humidity values present on the sampling days at the specific barn locations were taken by consulting the historical archive of the website www.ilmeteo.it. The formula used to calculate THI is: $[(1.8 \times T) + 32] - (0.55 - 0.55 \times RH) \times [(1.8 \times T) - 26]$, where T is temperature (°C) and RH is relative humidity (%) (NOAA, 1976). This represents an accurate estimation of reality.

6.3 Results and discussion

6.3.1 Diets characteristics

Diet composition	*Average amount*		*Min amount*		*Max amount*	
	Kg as fed	Kg DM	Kg as fed	Kg DM	Kg as fed	Kg DM
Alfalfa hay several cuts	7,76	6,83	5,40	4,73	12,82	11,19
Polyphytic/stable meadow hay	7,53	6,66	3,00	2,78	12,82	11,19
Mixed grass hay	6,02	5,36	4,06	3,53	10,68	9,62
Alfalfa-grass mixed hay	6,03	5,30	2,01	1,77	9,34	8,26
Fresh alfalfa grass	24,15	8,32	12,00	3,55	45,28	14,32
Polyphytic/stable meadow grass	18,68	3,98	10,58	2,99	30,29	5,26
Fresh shredded corn	14,83	2,77	6,76	1,36	23,00	4,53
Different types of concentrate	7,45	6,65	4,00	3,57	10,00	8,92
Hay	10,12	8,95	5,58	4,93	14,74	12,99
Fresh grass	20,86	6,12	6,76	1,36	45,28	14,32
Concentrate	7,45	6,65	4,00	3,57	10,00	8,92
Total raton fed	38,43	21,72	22,24	18,77	59,23	28,64

Table 2: diet composition during sampling days. The first part of the table shows different types of hay, fresh grass and concentrate fed to the cows on the various farms, while the second part considers hay, fresh grass and concentrates all together. Finally, the third part shows the total ration fed. The barns did not use all types of hay and fresh grass. The values reported refer to the farms that used that feed.

Estimated diet chemical composition	*Average amount*	*Min amount*	*Max amount*
Ration as fed (kg)	38,43	22,24	59,23
DMI (kg)	21,72	18,77	28,64
DM (%)	58,69	43,50	88,10
Forage (% DM)	69,07	55,88	82,71
Concentrate (% DM)	30,93	17,29	44,12
Forage/concentrate	2,45	1,27	4,78
CP (% DM)	14,17	11,07	17,17
Soluble CP (% DM)	4,65	3,46	5,72
RDP 3x Level 1 (% DM)	9,56	7,41	11,42
RUP 3x Level 1 (% DM)	4,66	3,03	5,98
MET (% DM)	0,21	0,16	0,26
LYS (% DM)	0,64	0,47	0,81
LYS/MET	3,05	2,70	3,45
aNDFom (% DM)	44,63	38,11	51,54
aNDFom forage (% DM)	38,73	28,59	47,97
ADF (% DM)	28,01	22,91	35,55
ADL (% DM)	5,36	3,88	8,97
CHO C uNDF (% DM)	18,26	14,16	29,32
Sugars (WSC) (% DM)	7,59	4,83	9,28
Starch (% DM)	13,99	7,51	21,08
RD CHO 3x Level 1 (% DM)	44,49	37,67	49,76
RD Starch 3x Level (% DM)	10,86	6,03	16,11
Soluble fiber (% DM)	5,90	1,75	10,42
NFC (% DM)	29,97	24,09	35,22
EE (% DM)	2,73	2,39	3,33
TFA (% DM)	1,97	1,70	2,62
C18:1C (% DM)	0,27	0,14	0,40
C18:2 (% DM)	0,78	0,51	1,06
C18:3 - ALA (% DM)	0,43	0,27	0,62
Others AGLC (% DM)	0,07	0,05	0,07
Ashes (% DM)	8,56	6,90	10,05
Ca (% DM)	0,84	0,57	1,11

P (% DM)	0,37	0,31	0,41
Mg (% DM)	0,31	0,23	0,41
K (% DM)	2,11	1,67	2,56
Na (% DM)	0,24	0,10	0,49
Cl (% DM)	0,60	0,43	0,69
Cu ppm	13,76	7,80	78,63
Fe ppm	303,81	234,72	440,66
Zn ppm	33,48	26,57	38,36
ME Mcal/kg	2,08	1,70	2,33
ENl 3x NRC Mcal/kg	1,28	1,01	1,46

Table 3: chemical composition of the diet provided during the sampling days estimated with NDS professional rationing software.

All herds sampled during the experimental field trial feed their cows a traditional diet consisting of hay, concentrate and fresh green grass, freshly cut. According to what is stated in Article 3 of the "Regolamento per il conferimento in uso dei marchi ANABoRaRe e di identificazione del Parmigiano Reggiano DOP di Razza Reggiana - Vacche Rosse" the use of the single or unifeed plate for feeding the Rossa Reggiana cow is prohibited. According to that rule, fresh grass must be obligatorily provided to Rossa Reggiana cows during the production period (spring, summer and partly autumn) to ensure the quality of the milk produced. Article 3 also states that hay fed to cows of this breed must be at least 50 % farm produced and at least 90 % from cultures produced within the Parmigiano Reggiano PDO area. In addition, it is reported that the concentrate used in the ration for lactating and dry cows must not exceed 50 % of dry matter and must be made from certified not-GMO cereals and legumes. Finally, there is a ban on the feeding of fresh chopped crops except for farms with an alfalfa-based forage orientation, whose crop plan is oriented at least 60 % to alfalfa (www.razzareggiana.it).

The farms sampled complied with the above regulation impositions. Due to the use of fresh grass, the amount of matter as ingested by the cows is high, as reported in Table 2 under "Total ration fed", while the average dry matter ingested is 21.72 kg, which is in line with what was reported by Dado and Allen (1995) about the Holstein breed and by National Research Council (2001) about large breed cows in mild lactation, at table 14-6. Only barn 9 at sampling 2 did not provide fresh green grass to the cows, in fact the ingestion of matter as fed was only 22 kg. In the rations provided, forage plays a predominant role compared to concentrate, and the ratio between these two parameters is never less than 1, in line with what Formigoni and Mordenti (2004) stated. The most used forages in the area where sampling was conducted are alfalfa, mixed grass, and stable meadow, as shown in Table 2, while by Article 5 of the cattle feeding regulations of the "Disciplinare di Produzione del Formaggio Parmigiano Reggiano " the use of silage is strictly forbidden. During the 18 samplings performed, only the barn 3 never used hay or fresh alfalfa. This demonstrates the importance of this forage in Parmigiano Reggiano cheese production area. Given its high protein content, alfalfa largely covers the protein requirements of cows, reducing the need to use protein supplements (Formigoni et al., 2010). The concentrates employed are commercial grain and legume mixtures and meal for lactating cows, that are mostly high in starch content. Only barn 9 uses a self-produced meal consisting of corn, barley, wheat and NaCl. The low concentrate diet allows cows to never be forced to their maximum productivity and to respect their physiological rumination activity. In addition, the high percentage of forages decreases the risk of ruminal and metabolic acidosis (Formigoni et al., 2010).

The estimated chemical composition of the ration reported in Table 3 derives from the NDS software, into which were input all data from the chemical analysis of single forages and concentrates conducted in the laboratory. The average values of Crude Protein (CP), Rumen Degradable Protein (RDP), and Rumen Undegradable Protein (RUP) are within normal ranges for a dairy cow ration, how is reported by National Research Council (2001) in table 14-6. Lysine and methionine are the main limiting amino acids in dairy cattle diets, and the ratio between them should be 3 to 1, as stated by Cevolani et al. (2014). In the diets analysed, the average ratio between the two parameters appears to be correct, while the minimum value, belonging to barn 6, is lower. Regarding structural and non-structural carbohydrates, NDF and ADF are higher than the reported reference ranges of Table 14-6 from the National Research Council (2001), while NFC appears to be slightly lower than the reference range, as well as starch. This testifies to the high use of forages in the rations studied. Sugars should be in the range of 4 to 8 %, so the estimated value in the diet is very close to the upper limit, while soluble fiber is in the correct range of 4 to 10 %. The diet fat content, indicated as ethereal extract (EE), is also quite low and never exceeds 6-7%, a limit reported by the National Research Council (2001) on page 32. This could be because high amounts of lipid supplements were not used in the rations studied, partly because the "Disciplinare di Produzione del Formaggio Parmigiano Reggiano" limits the total crude fat intake to a total of 300 grams/head/day. Calcium and phosphorus are the two main minerals in the diet of dairy cattle, and their ratio should not be too different from 2 to 1. The percentage of calcium should also not exceed 1 % of the ration, otherwise there is a risk of reduced performance and DMI (National Research Council, 2001). The average of the estimated mineral measured in the sampled rations meets the limit values, although the National Research Council (1989) recommends increasing the amount of Na from 1.2 % to 1.5 %, chlorine from 0.4 % to 0.6 %, and magnesium from 0.3 % to 0.35 % in dry matter for cows under heat stress. Sodium and magnesium do not meet these values. Finally, the estimated value of NEl is similar to that reported in Table 14-7 of the National Research Council (2001) for the Holstein breed.

The sampling was all conducted during the summer period in presence of heat stress. Several structural, management and feeding strategies should be employed at this time of year to reduce the suffering of dairy cows and to promote milk production. Feeds should be provided during the cooler hours of the day, and diets with higher concentrates and lower forage content should be favoured to reduce fiber intake, ruminal fermentations, and subsequent heat production. Concentrates with low starch content and high digestible sugar content, whose metabolizable energy is more readily utilized, with less heat production, should be used. In heat stress situation it would also be useful to increase the intake of crude protein, minerals, and vitamins (Kadzere et al., 2002; West, 1999). The analysed rations do not meet all these criteria, because the diet is heavily oriented toward forage ingestion and NFC levels are not very high. However, the Rossa Reggiana breed has much lower production than the Holstein breed and is more rustic, traits that could make it more resistant to heat stress. Therefore, such strategies might be useful but not necessary for this breed. Only by comparing the production obtained in the summer period with that obtained in other seasons in other studies it will be possible to determine how much heat stress affects the Rossa Reggiana cow production and try to understand what strategies can be implemented to reduce its impact.

6.3.2 Milk production and characteristics

Items	*Average*	*Min*	*Max*
MY (kg)	*19,36*	*16,97*	*23,87*
ECM (Kg)	*17,59*	*14,43*	*20,67*
Fat (% p/p)	*3,40*	*2,90*	*4,00*
Protein (% p/p)	*3,29*	*3,12*	*3,43*
Lactose (% p/p)	*4,69*	*4,55*	*4,79*
TS (% p/p)	*11,99*	*11,37*	*12,49*
SNF (% p/p)	*8,67*	*8,44*	*8,85*
Casein (% p/p)	*2,59*	*2,47*	*2,70*
RCT (min)	*14,37*	*10,34*	*16,62*
A30 (mm)	*40,14*	*33,69*	*45,23*
K20 (min)	*4,06*	*3,00*	*4,84*
A60 (mm)	*39,79*	*28,84*	*49,05*
Amax (mm)	*45,71*	*38,66*	*52,55*
SCC (x10^3/ml)	*265,69*	*114,00*	*648,50*
DSCC (x10^3/ml)	*75,94*	*64,80*	*82,00*
Urea (mg/dl)	*31,57*	*25,55*	*38,30*
Corrected Urea (mg/dl)	*26,50*	*21,31*	*32,30*
Delta (mg/dl)	*5,07*	*4,24*	*6,00*

Table 4: milk production and characteristics.

Considering the lactation duration of 305 days, the average production of Rossa Reggiana cows in the two lactations of the 18 samplings conducted is 5905 kg of milk, with an average fat content of 3.40 % and protein content of 3.29 %. The average production provided by AIA data (2022) for the Rossa Reggiana cow is 6,271 kg, with 3.68 % fat and 3.41 % protein, while the average production provided by www.consorziovaccherosse.it is 5,557 kg, with 3.45 % protein and 3.54 % fat. Therefore, the average production in kg is in line with the data provided, while the percentages of fat and protein are lower than the average. This could be attributed to the summer period, when there is normally a decrease in fat and protein content in milk due to heat stress. During the summer period protein production from the ruminal microbiota decreases, and this is reflected in reduced protein concentration in milk (Kadzere et al., 2002). The value of caseins in milk is also affected by the heat stress phenomenon and is lower than that reported in the "Relazione intermedia del Progetto dual breeding" for the period 2016-2019 conducted by the University of Bolzano, which shows a value of 2.75 %. Lactose, on the other hand, remains in the normal reference range.

RCT, k20 and a30 are three rheological parameters of milk, which show the milk cheesemaking aptitude. RCT measures rennet coagulation time, k20 represents curd firming time and a30 is curd firmness 30 minutes after rennet is added to milk. Short to medium milk rennet reactivity and higher curd firming capacity are desirable characteristics for cheesemaking and are associated with higher cheese yield (Visentin et al., 2017). The "Relazione intermedia del Progetto dual breeding" for the period 2016-2019 carried out by the University of Bolzano shows in the summer season, considering data from all breeds included in the project, values of a30 equal to 24.23 mm, RCT equal to 20.78 min and k20 equal to 5.11 min. Comparing these data with those shown in Table 4, it can be seen that RCT and K20 are lower, while a30 is significantly higher. Comparing these parameters with other data reported in scientist literature (Auldist et al., 2004; Pretto et al., 2012; Visentin et al., 2017), RCT and k20 are found to be consistently lower and a30 higher. This demonstrates the high dairy aptitude of Rossa Reggiana cow milk, which takes little time to coagulate and to firm up and achieves greater curd firmness after 30 minutes, compared to other breeds. The high dairy aptitude could be attributed to the favourable phenotypic composition of caseins and to the high content of κ-casein variant BB.

Somatic milk cells stay below the limit imposed by Presidential Decree 54/1997 of 400 000 cells per ml. However, the average value exceeds 200 000 and could indicate the presence in herds of cows with health problems, such as mastitis. The maximum value was measured during the second sampling in barn 5 and far exceeds the limit.

The optimal range of urea in milk is 20 to 30 mg/dl and is obtained by correct protein and fermentable carbohydrates intake, which promote the activity of ruminal bacteria and their production of microbial proteins (Cevolani et al., 2014). The average corrected urea of the analysed samples falls within the indicated range, demonstrating that protein metabolism of the Rossa Reggiana breed is maintained even under heat stress conditions on the farms sampled and in presence of high alfalfa content in the ration.

6.3.3 Temperature humidity index during sampling

Farm	*Sampling*	*RH (%)*	*T (°C)*	*THI*
1	1	50	29	**77**
	2	33	33	**79**
2	1	50	29	**77**
	2	43	29	**76**
3	1	47	29	**77**
	2	33	33	**79**
4	1	47	29	**77**
	2	58	27	**75**
5	1	47	29	**77**
	2	58	27	**75**
6	1	49	28	**76**
	2	33	33	**79**
7	1	52	27	**75**
	2	52	30	**79**
8	1	52	27	**75**
	2	52	30	**79**
9	1	52	27	**75**
	2	52	30	**79**
Average		48	29	**77**
Min		33	27	**75**
Max		58	33	**79**

Table 6: average THI calculated on the days of sampling.

The temperature humidity index (THI) is a parameter that indicates the level of animal heat stress by estimating the perceived ambient temperature in relation to relative air humidity values. It was calculated using the formula: [(1.8 x T) + 32] - (0.55 - 0.55 x RH) x [(1.8 x T) - 26], where T is temperature (°C) and RH is relative humidity (%) (NOAA, 1976).

During the days when samplings were conducted, the calculated daily average THI is always greater than 72, so the cows are always in a mild to moderate heat stress situation, as reported by Atrian and Shahryar (2012).
Temperature and humidity data were taken from the historical archive of www.ilmeteo.it. It is therefore an estimation made since the average temperatures and humidity recorded on the internet site and not collected manually. This does not specifically consider temperature differences that might be present between plains and hills, even if they are minimal. Nor does it consider strategies implemented within individual barns to reduce heat stress. However, this estimate makes it possible to establish that, on average, the animals in the sampled barns during the month of July 2022 did not live in favourable and physiological environmental conditions, impacting their productive and reproductive activity.

6.3.4 Herd characteristics

Farm	*Average weight kg*	*Min weight kg*	*Max weight kg*	*Average BCS*	*Min BCS*	*Max BCS*	*Average thorax CRF*	*Min thorax CRF*	*Max thorax CRF*
1	555,66	380,66	708,09	3,28	2,50	4,00	194,82	170	214
2	597,81	537,63	743,58	3,27	3,00	3,50	200,64	193	218
3	592,79	486,62	690,71	3,20	2,50	4,00	199,91	186	212
4	595,08	458,75	708,09	3,20	2,75	4,00	200,19	182	214
5	530,99	418,68	623,62	3,11	2,75	3,75	191,83	176	204
6	635,01	500,90	761,69	3,30	2,75	4,00	205,13	188	220
7	576,50	386,86	699,37	3,31	2,75	4,00	196,79	171	213
8	571,08	479,57	708,09	3,21	2,75	3,75	197,18	185	214
9	663,61	515,42	789,32	3,50	2,75	4,00	208,50	190	223
Average	590,95			3,27			199,44		

Table 5: herd characteristics.

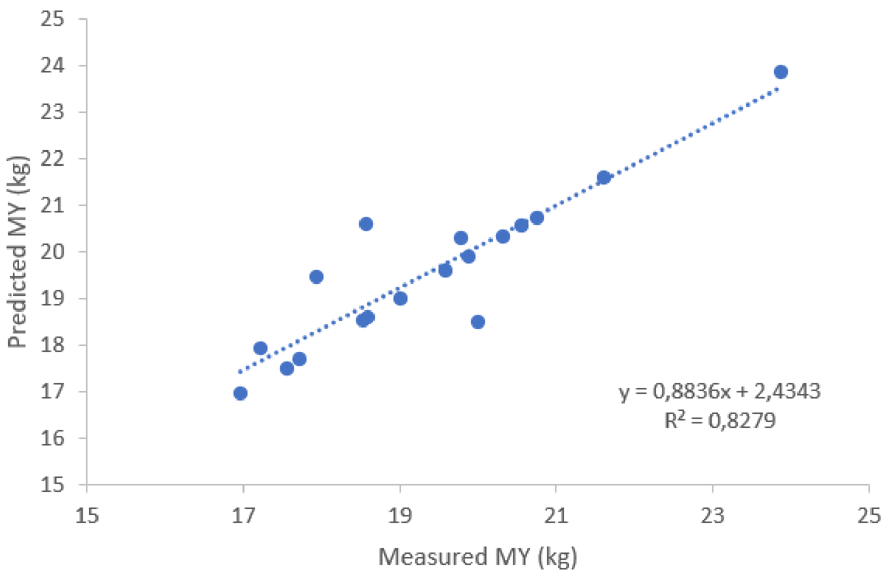
During sampling, were collected data on about 20 % of the herd, regarding weight, BCS and thorax circumference. The average Rossa Reggiana cattle in the sampled barns has a weight of 591 kg, a BCS of 3.3 and a thorax circumference of 200 cm. The average weight reported by www.razzareggiana.it under "Caratteri morfologici" is 6.5-7 quintals, so it appears to be higher than the weight measured. This could be due to high variability of cows at the time of sampling and to the presence of young animals, which have not yet finished development. The average BCS estimated on a scale of 1 to 5, as indicated by Edmonson et al. (1989), shows that these animals are in good nutrition status. The Rossa Reggiana cow was originally born as a triple-purpose cow and has limited milk production, so it has a less dairy conformation than the Holstein breed and converts more energy to meat and fat and less to milk, resulting in a greater fat cover panniculus and less angular shapes.

According to data from functional controls conducted in the 9 farms during the summer months of 2022, the average cows age at the control is 67 months, with an average lactations number of 3.2 and a primiparous cows percentage of 26. The average animals age at first conception is 30 months, while the average calving-conception interval is 130 days. First fertilization occurs on average 78 days after parturition, and the conception rate at first service is 49%.

These data provide an idea about the animals object of this study and about the field trial conducted. Some of these data were entered into the NDS rationing software to obtain a more truthful reconstruction of the herd to which rations were administered, bringing estimation of milk production and milk characteristics as close as possible to reality.

6.3.5 Relationships between diets characteristics and milk characteristics

6.3.5.1 Predicted milk yield and real milk yield

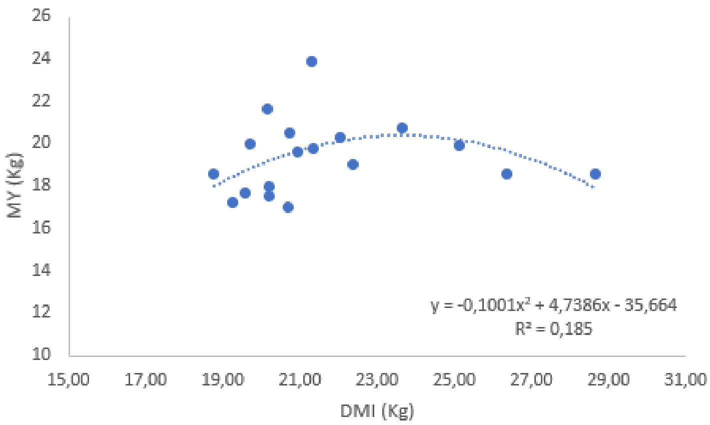


Graphic 1. Relationship between milk yield measured during sampling and milk yield predicted by NDS software. The correlation has the following parameters: r = 0.910; P < 0.01. The predicted Milk Yield was obtained as average between the metabolizable energy (ME) allowable milk and the metabolizable protein (MP) allowable milk.

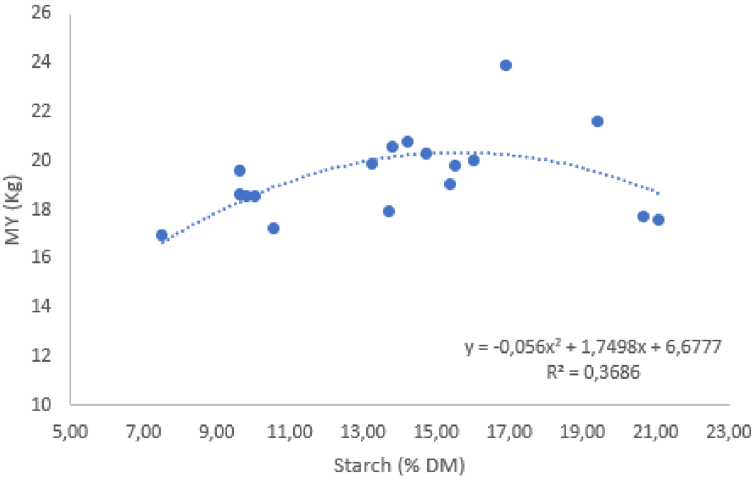
In the graphic 1 it is possible to observe the nearly linear relationship between the measured average milk production of cows during the days when sampling took place and the average milk production estimated by NDS based on the input data, regarding the amount, type and chemical analysis of the feed fed in the ration. As can be observed, the measured and estimated MY data sometimes coincide and the R^2 is very high. This confirms that the diets reconstruction was performed accurately and realistically, closely simulating what occurs in reality. Therefore, the diet recreated on the software predicts milk production very similarly to what occurred in practice. It is so possible to proceed by comparing ration components and milk characteristics, knowing that, although it is an estimation, the reconstruction of the diets was done correctly.

In contrast, fat and protein are data entered as input into the software, so no correlations can be established. However, the milk yield, protein and fat amounts produced are parameters that depend not only on feeding, but also on other external factors. Among these, genetics, management (Walker et al., 2004) and heat stress (Summer et al., 2019) play a key role.

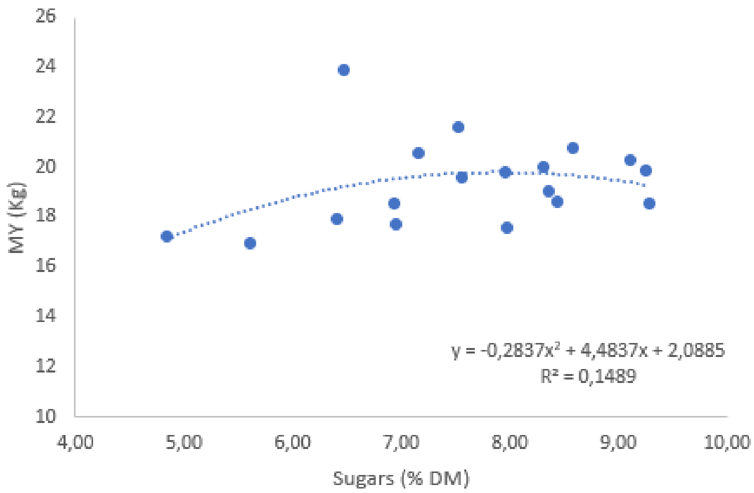
6.3.5.2 Milk yield and diet's components



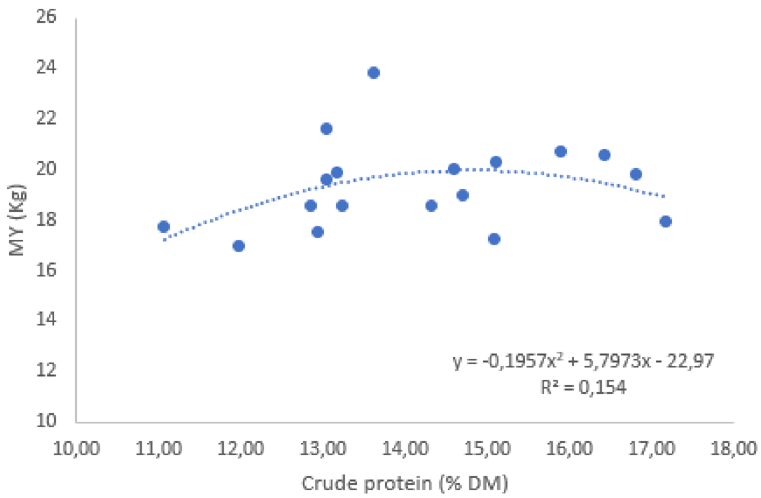
Graphic 2. Relationship between dry matter intake and measured milk yield. The correlation has the following parameters: r = 0.073; P = 0.386.



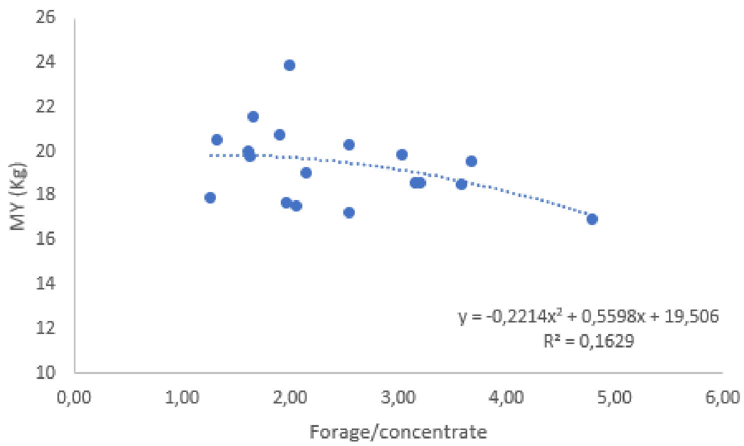
Graphic 3. Relationship between starch and measured milk yield. The correlation has the following parameters: r = 0.303; P = 0.111.



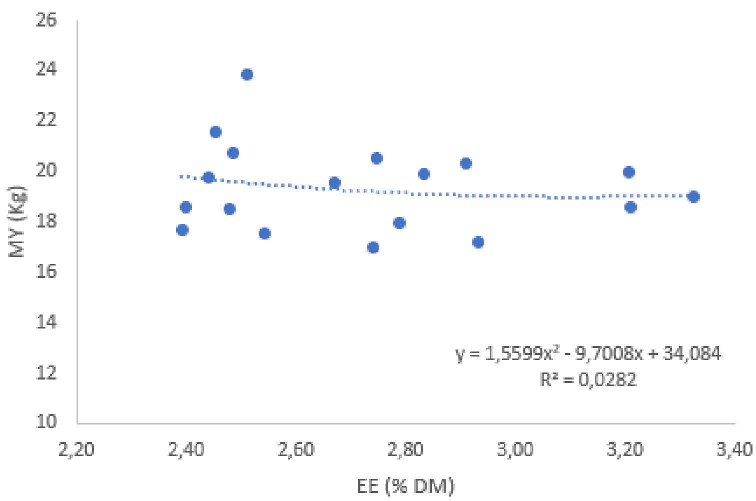
Graphic 4. Relationship between sugars and measured milk yield. The correlation has the following parameters: r = 0.259; P = 0.149.



Graphic 5. Relationship between crude protein and measured milk yield. The correlation has the following parameters: r = 0.190; P = 0.225.



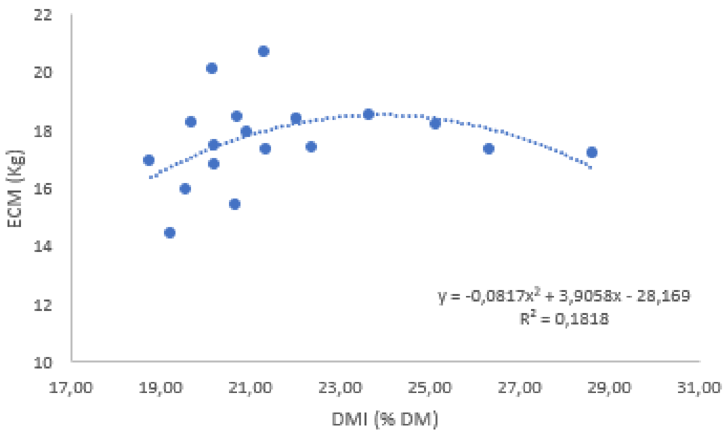
Graphic 6. Relationship between forage to concentrate and measured milk yield. The correlation has the following parameters: r = - 0.384; P = 0.058.



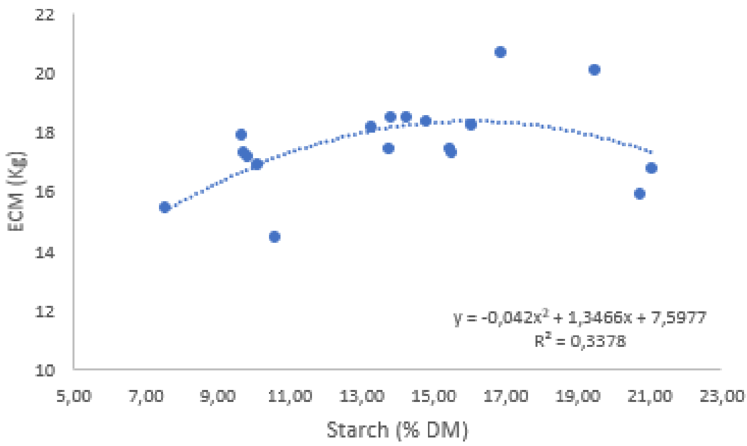
Graphic 7. Relationship between ethereal extract and measured milk yield. The correlation has the following parameters: r = - 0.150; P = 0.276.

Milk yield, according to what is observed in graphic 2, shows maximum values when the ingested dry matter content is between 23 and 25 kg, although the maximum production of 23.87 kg belongs to the second sampling of barn 4, where 21.3 kg of dry matter was fed. The starch value should be around 15 to 17 % of dry matter (graphic 3) and that of sugars should be around 8 % (graphic 4), although barn 4 gets the highest production with 6.47 % of sugars. Graphic 5 shows how crude protein should be around 15-16% to maximize milk yield. Finally, according to graphic 6, increasing the ratio of forage to concentrate leads to a progressive decrease in milk production, with an almost linear relationship, as supported by Jenkins and McGuire (2006). The forage-concentrate ratio should be between 1 and 2 to obtain maximum production. On the other hand, no interesting relationships were found between dietary lipids and the amount of milk produced (graphic 7), contrary to what is stated by Sutton (1989), probably because the percentage of lipids in the analysed diets varies too little to determine changes in the amount of milk produced.

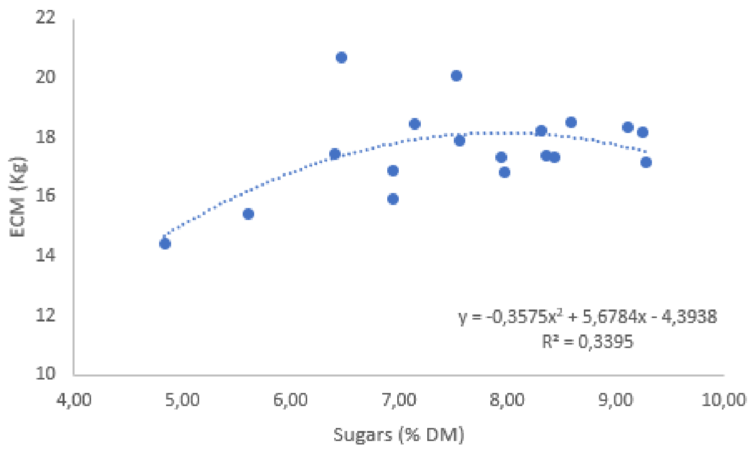
6.3.5.3 Energy corrected milk and diet's components



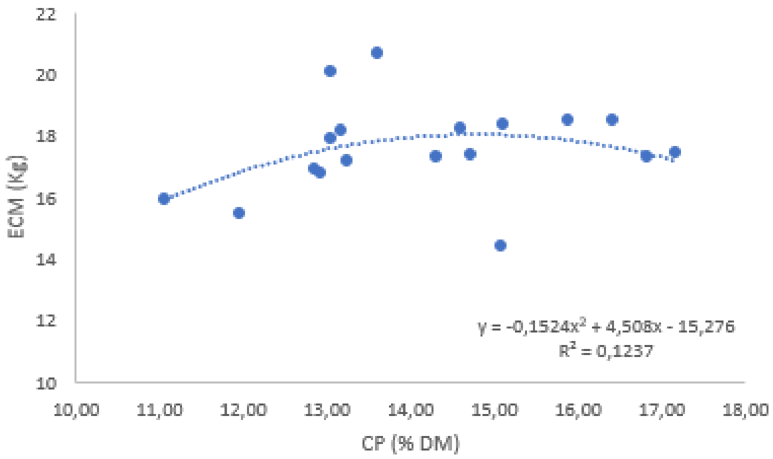
Graphic 8. Relationship between dry matter intake and energy corrected milk. The correlation has the following parameters: r = - 0.141; P = 0.288.



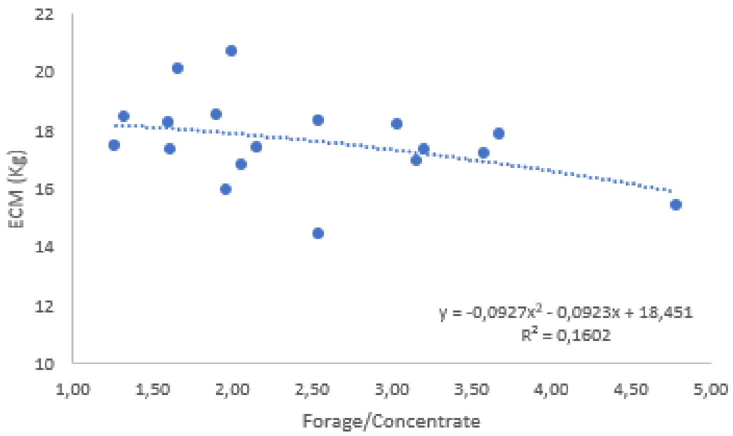
Graphic 9. Relationship between starch and energy corrected milk. The correlation has the following parameters: r = 0.357; P = 0.073.



Graphic 10. Relationship between sugars and energy corrected milk. The correlation has the following parameters: r = 0.405; P = 0.048.



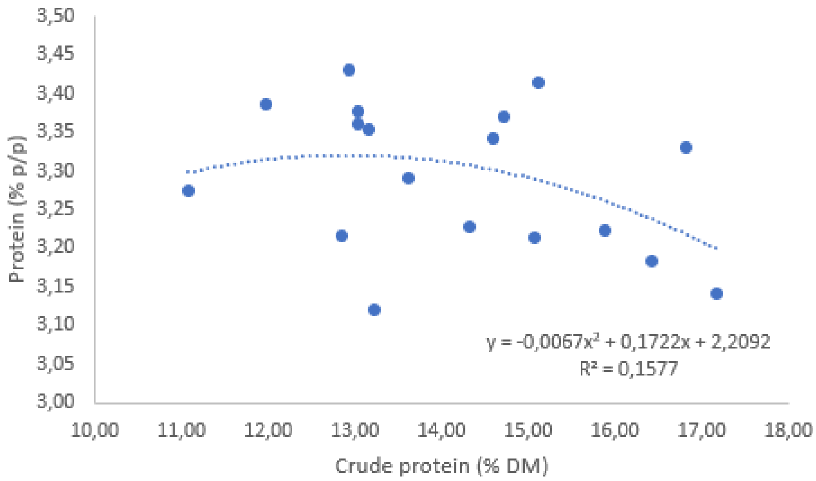
Graphic 11. Relationship between crude protein and energy corrected milk. The correlation has the following parameters: r = 0.164; P = 0.258.



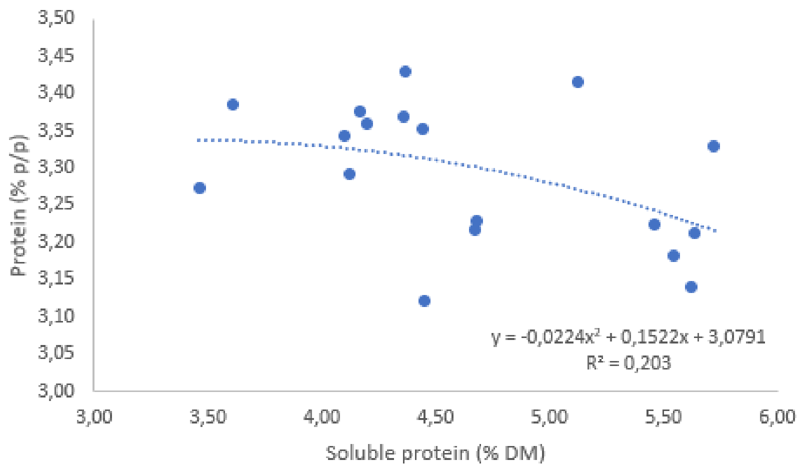
Graphic 12. Relationship between forage to concentrate and energy corrected milk. The correlation has the following parameters: r = - 0.396; P = 0.052.

The energy corrected milk or ECM determines the amount of milk produced adjusted to 3.5 % fat and 3.2 % protein and is calculated using the following formula: milk yield (kg) × [(38.30 × fat content (g/kg) + 24.20 × protein content (g/kg) + 16.54 × lactose content (g/ kg) + 20.7) / 3140), according to Sjaunja et al. (1990). Analysing graphics 8 to 12, it can be observed that ECM and different diet parameters interact with each other in a similar way as seen with milk production, since ECM and MY are correlated with each other (R = 0.929; P < 0.01). What is correlated more with ECM than with MY are sugars, whose ideal value for maximum ECM is always between 8-9% (graphic 10). To obtain maximum ECM, the ideal values of DMI, starch and crude protein are respectively 24 kg (graphic 8), 15-17 % (graphic 9) and 15 % (graphic 11). How is observed in graphic 12, as the ratio of forage to concentrate increases, ECM tends to decrease linearly, so the ideal range between 1 and 2 for these two parameters is confirmed. Also in this case, no relationship was found between ethereal extract and ECM.

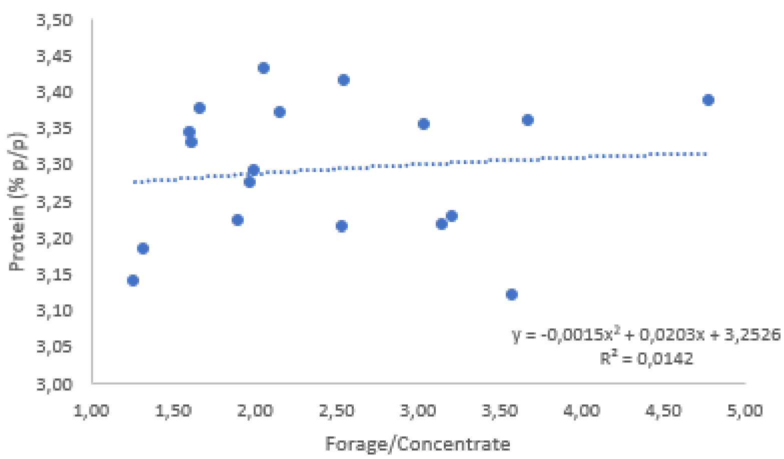
6.3.5.4 Milk protein and diet's components



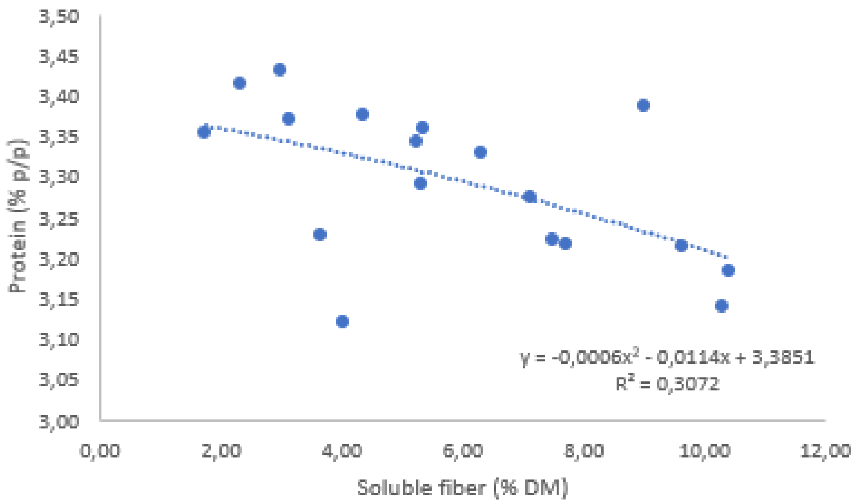
Graphic 13. Relationship between diet crude protein and milk protein. The correlation has the following parameters: r = - 0.340; P = 0.083.



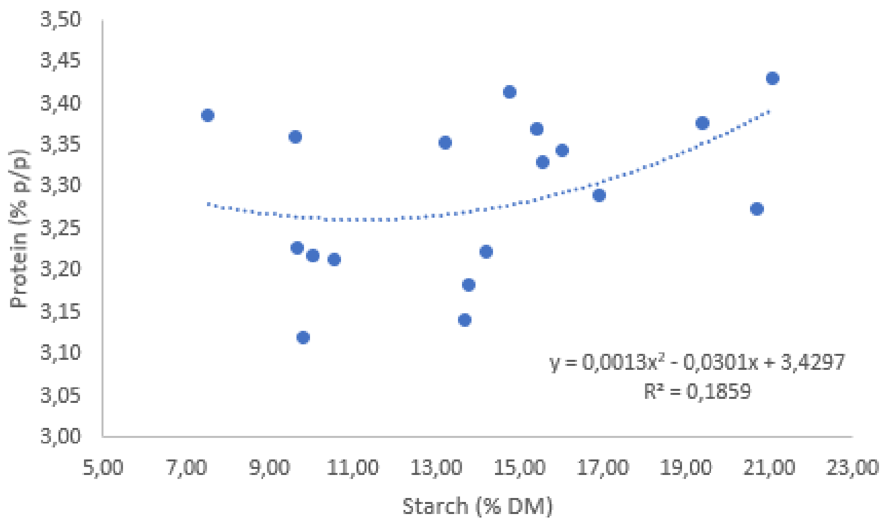
Graphic 14. Relationship between diet soluble protein and milk protein. The correlation has the following parameters: r = - 0.443; P = 0.033.



Graphic 15. Relationship between diet forage to concentrate and milk protein. The correlation has the following parameters: r = 0.128; P = 0.306.



Graphic 16. Relationship between diet soluble fiber and milk protein. The correlation has the following parameters: r = - 0.550; P = 0.009.



Graphic 17. Relationship between diet starch and milk protein. The correlation has the following parameters: r = 0.355; P = 0.074.

According to Jenkins and McGuire (2006), the dietary components that most influence the percentage of protein in milk and that have been most studied are the forage/concentrate ratio, the amount and source of protein in the diet, and the amount and source of fat in the diet. These components are investigated in this study, as shown in graphics 13 to 17. Graphics 13 and 14 highlight the relationship of crude protein and soluble protein in the diet with protein in milk. As the protein portion in the ration increases, there is a gradual decrease of protein in milk. Normally, the protein increase in the diet should promote the presence of protein in milk, although the diet induced change in % protein in milk is never high. The increase in milk protein content should be about 0.02 percentage units for every 1 percentage unit increase in dietary protein (Jenkins and

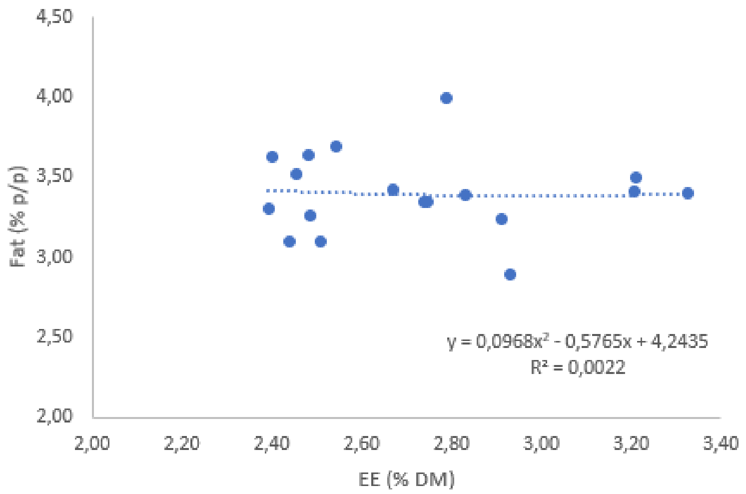
McGuire, 2006). In the examined case, the trend is reversed. It is possible that the Rossa Reggiana breed does not need a particularly high protein amount, or it is not very efficient at degrading ingested protein, resulting in greater nitrogen elimination through faeces and urine. The heat stress to which the tested cows are subjected could also decrease the efficiency of ruminal bacteria in microbial protein production or in the transfer and production of protein in milk by the udder. Many other variables, such as animal management and genetics, could also be involved, affecting protein transfer into milk. The ideal crude and soluble protein ranges in the diet to have a good percentage of protein in milk are 12 to 14 % and 3.5 to 4 %, respectively.

Graphic 15 shows that there is no significant relationship between the forage-concentrate ratio and protein in milk, although the trend line increases slightly as the forage-concentrate ratio increases. On the contrary, Jenkins and McGuire (2006) claim that reducing the forage ratio to 40 % of the ration DM would promote protein in milk. In the analysed diets, the forage percentage never falls below 55 %, so there is not much variability, and, according to the results obtained, maximum protein production in milk occurs with a forage percentage of 70 %. Instead, there is a clear negative correlation between soluble fiber in the diet and protein in milk (graphic 16). The value of soluble fiber should be between 2 and 4 % to maximize protein production in milk. Soluble fiber could negatively interact with microbial protein production or could reduce intestinal absorption. Graphic 17 illustrates the relationship between starch and protein in milk, which shows a positive correlation. As starch values increase, up to 21-22% DM, protein in milk increases. In fact, as stated by Tyasi et al. (2015), to ensure proper microbial protein production at the ruminal level, it is necessary to provide fermentable energy to the bacteria through starch supply.

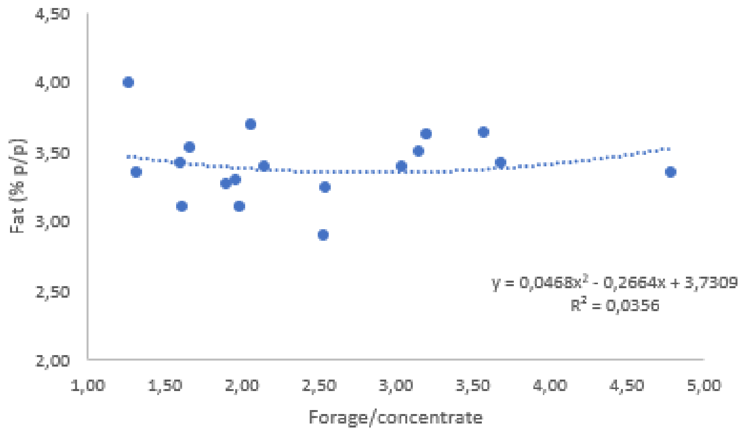
According to Formigoni and Mordenti (2004) and Jenkins and McGuire (2006), as fat in the diet increases there should be a decrease in protein in milk, due to an unclear dilution effect. The diets analysed in the current study have low and little variable fat percentages (EE between 2.39 and 3.33 % DM). Indeed, no clear relationships were found between the percentage of fat in the diet and the percentage of protein in milk.

Graphics regarding the relationships between casein and dietary components were not considered, as they behave very similarly to what has been described for protein. Caseins are in fact a subgroup of proteins in milk, and it is more important their phenotype, largely determined by genetics, than their quantity in milk.

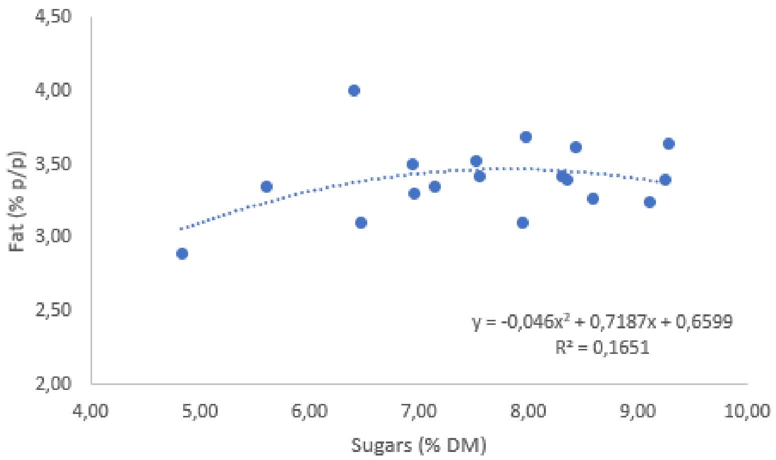
6.3.5.5 Milk fat and diet's components



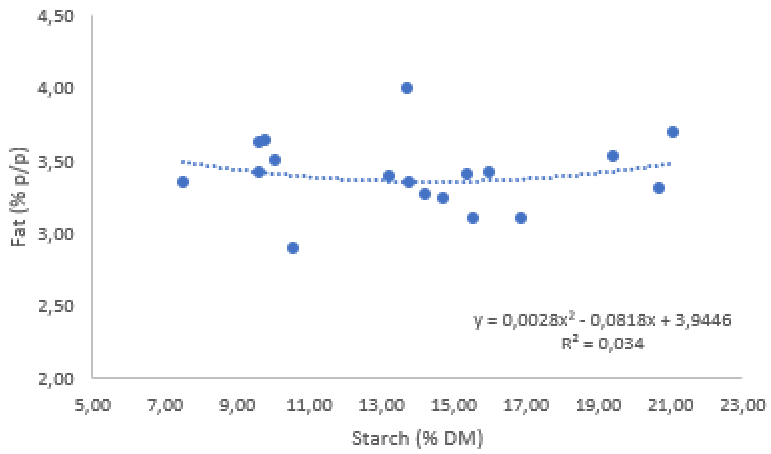
Graphic 18. Relationship between diet ethereal extract and milk fat. The correlation has the following parameters: r = - 0.032; P = 0.450.



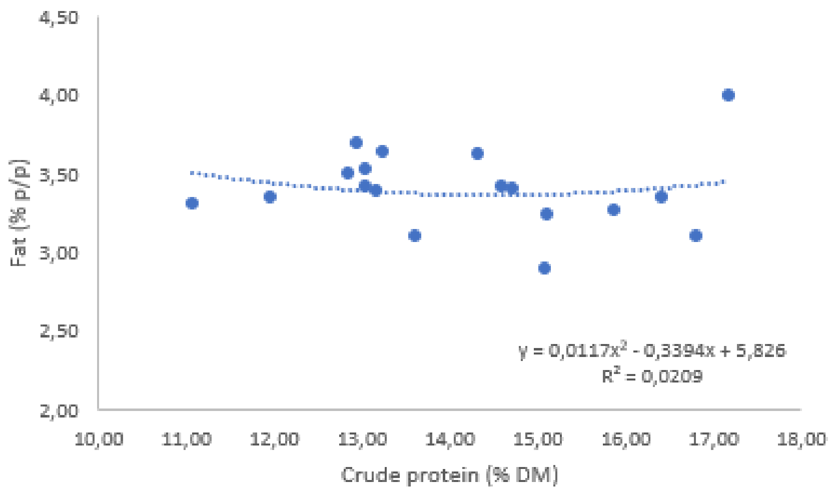
Graphic 19. Relationship between diet forage to concentrate and milk fat. The correlation has the following parameters: r = - 0.004; P = 0.493.



Graphic 20. Relationship between diet sugars and milk fat. The correlation has the following parameters: r = - 0.246; P = 0.162.



Graphic 21. Relationship between diet starch and milk fat. The correlation has the following parameters: r = - 0.003; P = 0.495.



Graphic 22. Relationship between diet crude protein and milk fat. The correlation has the following parameters: r = - 0.026; P = 0.460.

In the scientific literature is reported that fat in milk is the parameter most influenced by nutrition. According to Sutton (1989), the main contributors to the variation of fat in milk are roughage, forage to concentrate ratio, soluble carbohydrates, dietary lipids, dietary proteins, intake, and frequency of feeding. By changing these components in the diet, it is possible to vary the fat content of milk by up to 3 %.

As can be seen in graphics 18 to 22, no interesting relationships were found between dietary components and the percentage of fat in milk. Graphic 18 shows that there is no relationship between the amount of fat in the diet and the percentage of fat in milk. The reason could be the rather low percentage of fat in all the diets fed, which do not allow some variability to be observed. However, it is possible that there are different proportions and types of fatty acids in milk, depending on the different types of fat in the various diets.

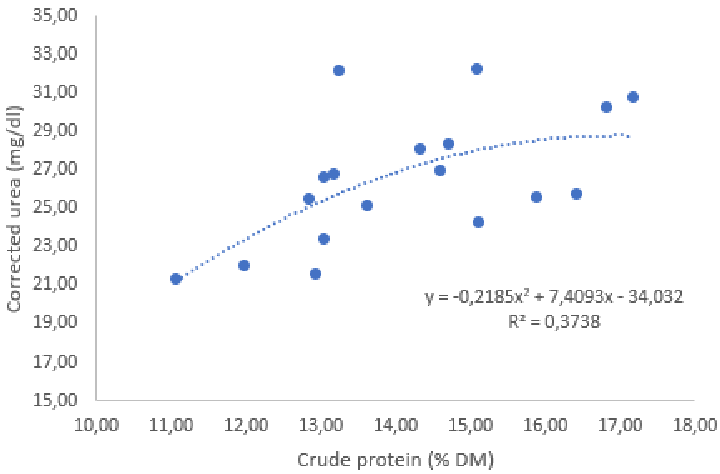
The forage-concentrate ratio (graphic 19) also shows no relationship with fat in milk. Sutton (1989) states that milk fat remains fairly stable as long as the forage ratio is 50 % of the ration. If forage declines, milk fat begins to fluctuate. The diets fed to the Rossa Reggiana cows in the 9 barns sampled never drop below 55 % forage. Therefore, it is difficult to determine whether the forage to concentrate ratio can determine the fluctuation of fat in milk. Barn 3 had the highest fat value of 4% at the first sampling, with a forage-concentrate ratio of 1.27, the lowest among all.

Sugars and starches (graphics 20 and 21 respectively) do not show relevant relationships with fat in milk. However, it seems that the optimal level of sugars for fat in milk is 8%, a value already observed when considering milk yield and ECM. It is possible to deduce that Rossa Reggiana cows need high levels of sugars to maximize their production.

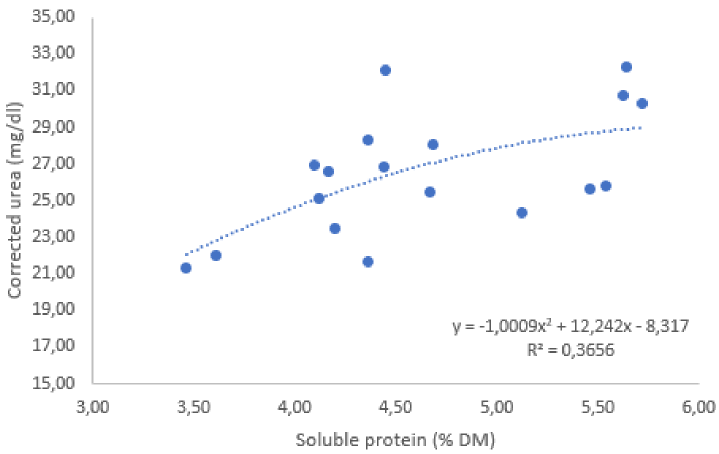
Formigoni and Mordenti (2004) and Sutton (1989) state that crude protein is not an effective way to modify fat in milk, despite the fact that this parameter may have a minimal influence on the amount of fat in milk. Figure 22 confirms this statement for the examined diets, as there is no relationship between crude protein and fat in milk, even if the highest percentage of fat in barn 3 was obtained with the highest percentage of crude protein in the diet and most of the values near 3.5 % of fat are found for a protein content on DM between 13 and 15 %.

It is possible that several dietary parameters must act concurrently to determine a significant change in milk fat, whereas in this study they were considered only individually. Other external factors may also affect the amount of fat in milk. For example, heat stress, a condition to which all cows are subjected during the sampling conducted, reduces the percentage of fat in milk, although the effects are conflicting (Summer et al., 2019).

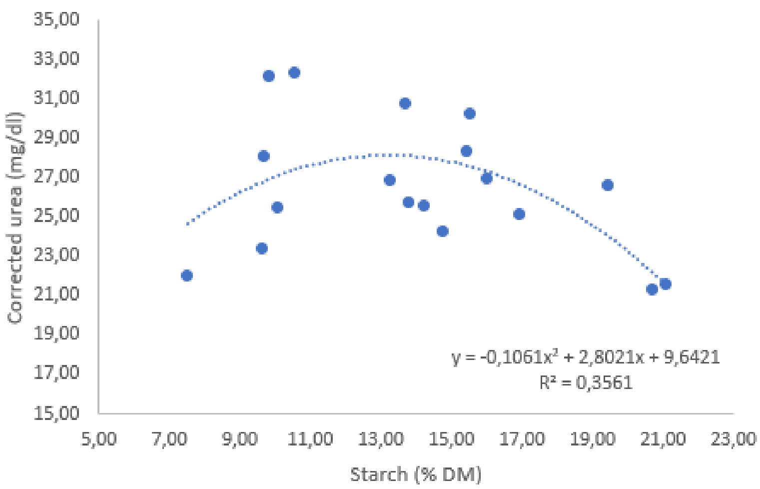
6.3.5.6 Milk corrected urea and diet's components



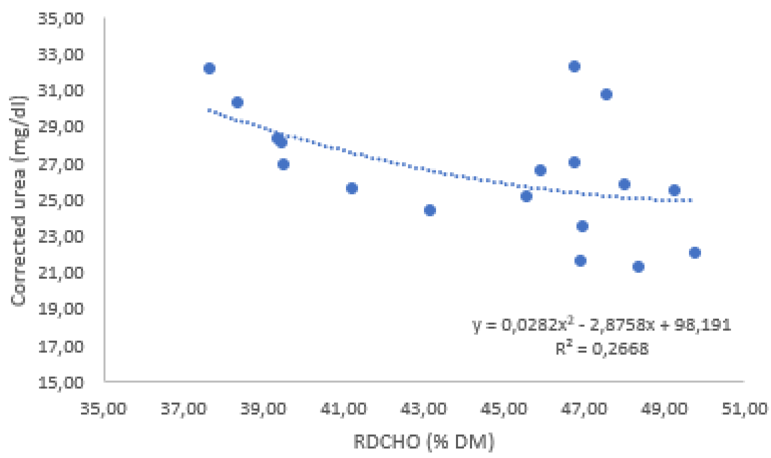
Graphic 23. Relationship between diet crude protein and milk corrected urea. The correlation has the following parameters: r = 0.579; P = 0.006.



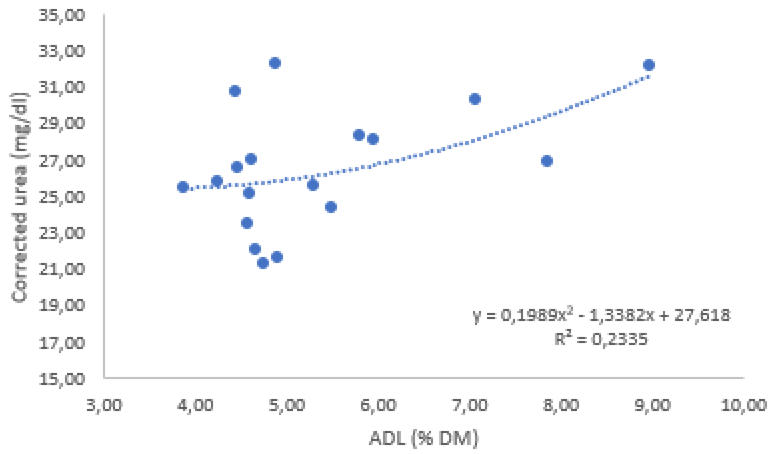
Graphic 24. Relationship between diet soluble protein and milk corrected urea. The correlation has the following parameters: r = 0.590; P = 0.005.



Graphic 25. Relationship between diet starch and milk corrected urea. The correlation has the following parameters: r = - 0.309; P = 0.106.



Graphic 26. Relationship between diet rumen degradable carbohydrates and milk corrected urea. The correlation has the following parameters: r = - 0.508; P = 0.016.



Graphic 27. Relationship between diet ADL and milk corrected urea. The correlation has the following parameters: r = 0.471; P = 0.024.

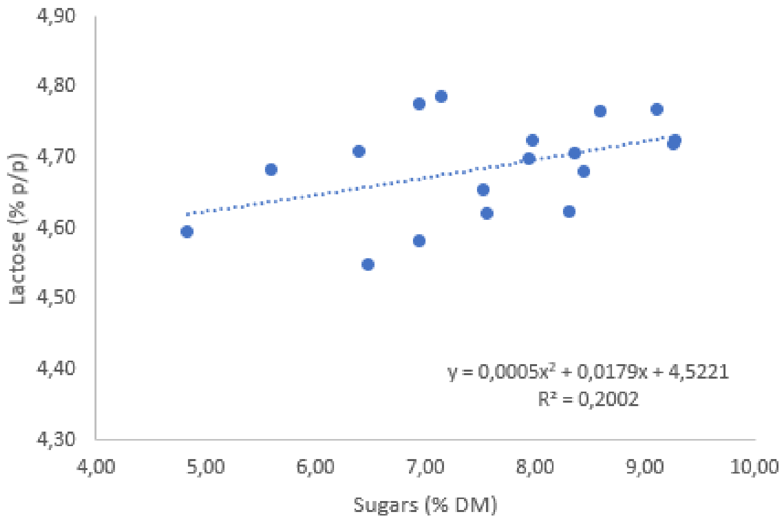
Urea is a body waste product excreted in milk and urine. The optimal urea amount in milk should be between 20 and 30 mg/dl. Causes that can increase urea in milk are protein excess in the diet and lack of energy for bacteria synthesizing microbial proteins in the rumen, provided by not-structural carbohydrates and especially starch (Cevolani et al., 2014).

Graphics 23 and 24 show a clear correlation between crude protein and corrected urea and between soluble protein and corrected urea, respectively. Increasing the amount of crude protein and soluble protein in the diet also progressively increases urea in milk, which only in a single sampling from barns 1, 2, 3 and 7 slightly exceeds values of 30 mg/dl. Therefore, the Rossa Reggiana cows from the 9 sampled barns are sensitive to increased protein in the diet and respond by increasing urea in milk. The protein content of the diets proves to be adequate, as values too far out of the normal range are not observed. The values of rumen undegradable protein (RUP), rumen degradable protein (RDP), methionine, and lysine are also positively correlated with corrected urea in the diet (R = 0.539 and P = 0.010, R = 0.557 and P = 0.008, R = 0.507 and P = 0.016, R = 0.619 and P = 0.003, respectively). This shows that ration estimation by NDS software occurred correctly.

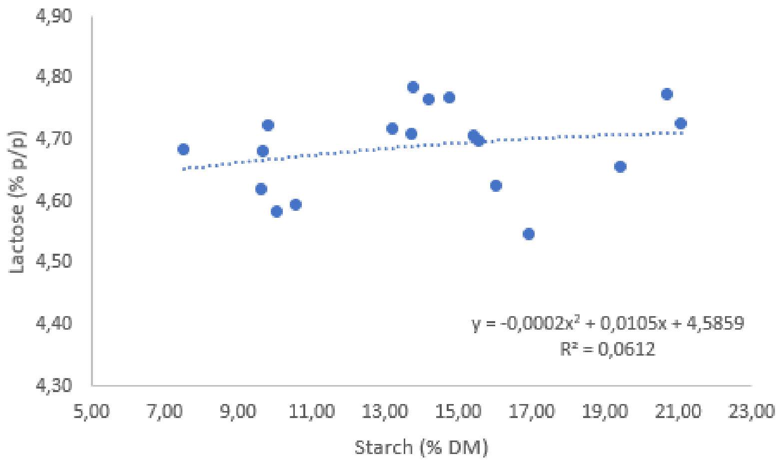
Therefore, for the breed examined, 17 % crude protein and 5.50 % soluble protein on diet dry matter should not be exceeded in the ration to avoid problems resulting from urea excess, such as hypofertility and embryonic death (Cevolani et al., 2014). Only barn 2 at the first sampling and barn 7 at the second sampling showed urea values above 30 for lower crude protein values (15 % and 13 %, respectively).

Graphic 25 shows the relationship between starch in the diet and corrected urea in milk. Although there are some low values of urea even with low levels of starch in the diet, tendency is that as starch increases, urea decreases, starting from values between 13 and 15 %, as more energy is supplied to the ruminal bacteria, which are essential for microbial protein production. In fact, the correlation between the two parameters is negative, indicating that one parameter's increase leads to the decrease of the other. A negative correlation was also found between degradable carbohydrates in the rumen (RDCHO) and urea in milk (graphic 26), because these carbohydrates also provide ready energy to ruminal bacteria. To keep low urea in milk, it is necessary to have a high level of RDCHO in the diet, between 47 and 50 % of DM, although barn 2 at the first sampling and barn 3 at the first sampling had urea values above 30 mg/dl for high levels of RDCHO. On the other hand, a positive correlation was found between ADL in the diet and urea in milk, probably because the increase in low-degradable fiber in the ration results in lower efficiency of ruminal bacteria and lower nitrogen fixation in the rumen. It is necessary to maintain low ADL values in the diet, around 4-5 %, even though barn 2 at the first sampling and barn 3 at the second sampling always have high urea values for low ADL levels.

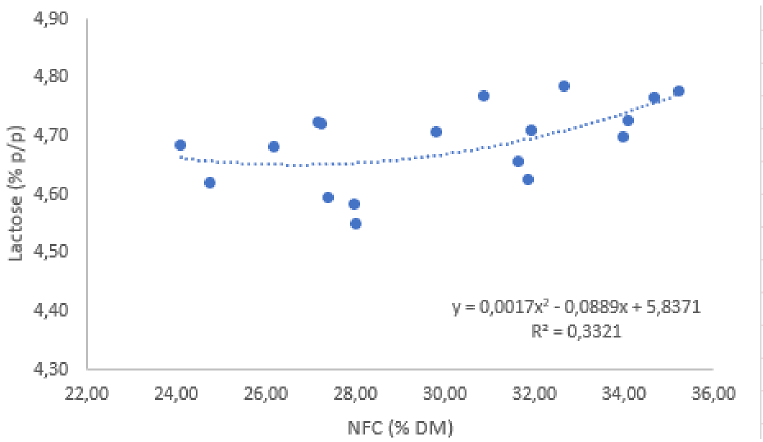
6.3.5.7 Milk lactose and diet's component



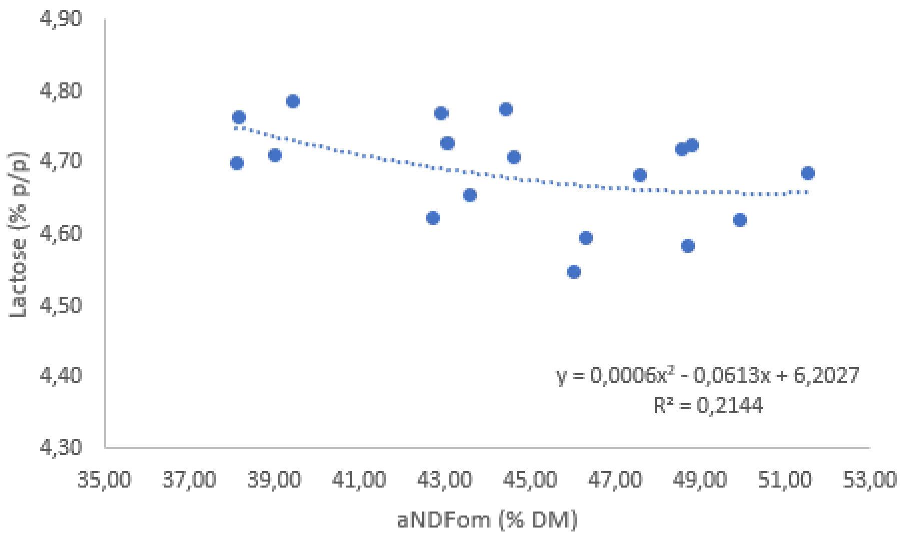
Graphic 28. Relationship between diet sugars and milk lactose. The correlation has the following parameters: r = 0.446; P = 0.032.



Graphic 29. Relationship between diet starch and milk lactose. The correlation has the following parameters: r = - 0.273; P = 0.137.



Graphic 30. Relationship between diet not fibrous carbohydrates and milk lactose. The correlation has the following parameters: r = 0.533; P = 0.011.

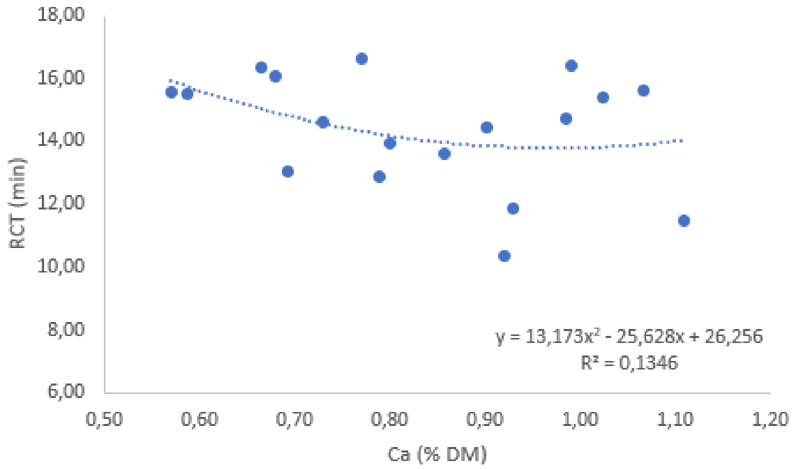


Graphic 31. Relationship between diet aNDFom and milk lactose. The correlation has the following parameters: r = - 0.448; P = 0.031.

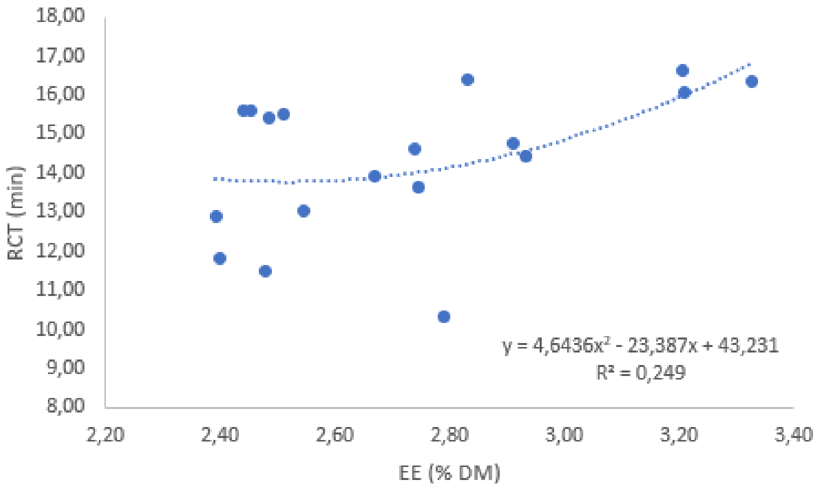
Lactose is the main sugar in milk, which allows milk secretion through its own osmotic effect. It gives flavour and aroma to freshly consumed milk, while it does not greatly influence the dairy aptitude of milk. The milk's lactose content tends to increase according to the animal's energy balance and the cereals fed. Corn, as the predominant starch, increases the lactose content of milk (Formigoni and Mordenti, 2004).

Looking at the data obtained, some interesting correlations are noted between milk lactose and some dietary parameters. Graphic 28 shows a positive correlation between sugars in the diet and lactose in milk. The Rossa Reggiana cow shows again to be sensitive to increased sugars in the diet, the optimal range of which is between 8 and 10 % on DM. These are high levels of sugars. Starch in the diet does not show such a strong correlation with lactose in milk (graphic 29). There is a slight increase in lactose as the percentage of starch in the diet increases, although at 15 % of starch there are several high lactose barns. Not-fibrous carbohydrates, containing starches and sugars, also have a positive correlation with lactose in the diet, as shown in graphic 30. To have a high amount of lactose in milk, it is important to have more than 32 % of NFC on DM in the diet. In contrast, graphic 31 shows a negative correlation between neutral detergent fiber and lactose in milk. NDF is part of the structural carbohydrates in the diet, the high percentage of which in the ration reduces lactose in milk. The value of aNDFom should be between 37 and 39 % to maintain lactose at proper levels. Negative correlation was also observed between milk lactose and dietary aNDFom contained in forages (R = -0.412; P = 0.045).

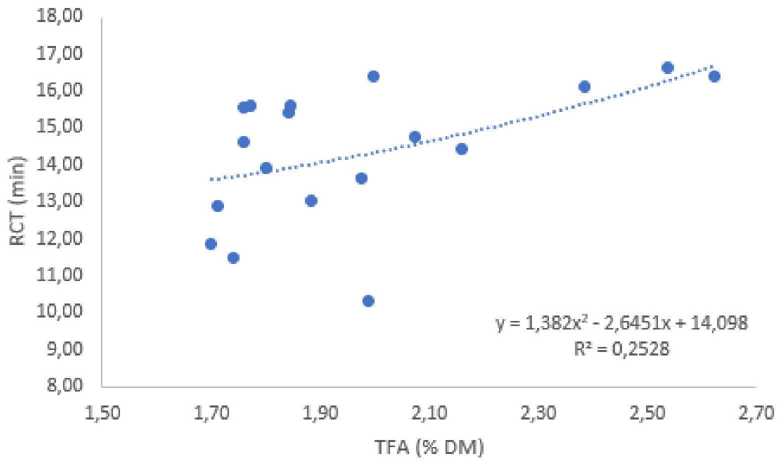
6.3.5.8 Milk rheological characteristics and diet's components



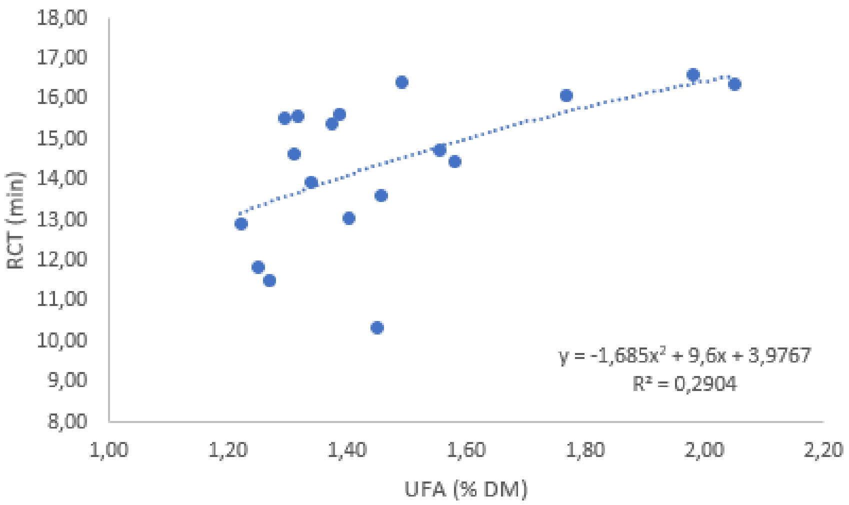
Graphic 32. Relationship between diet Ca and milk rennet coagulation time. The correlation has the following parameters: r = - 0.323; P = 0.096.



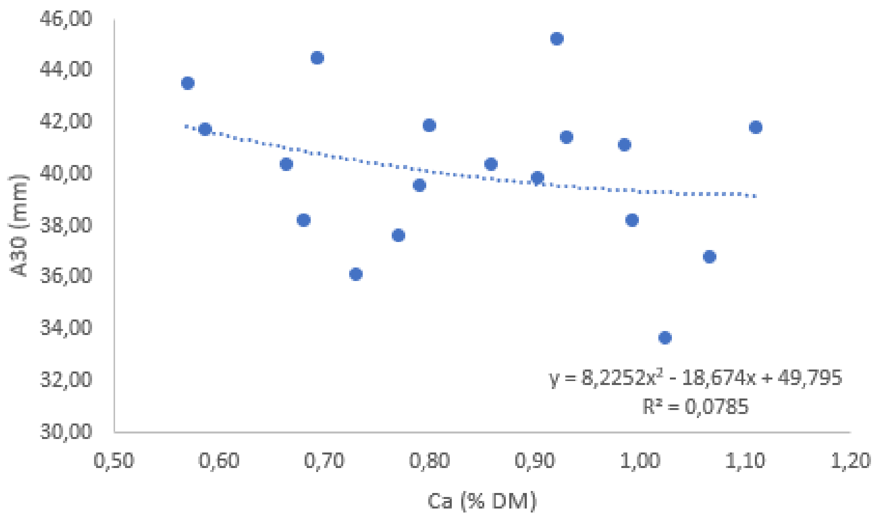
Graphic 33. Relationship between diet ethereal extract and milk rennet coagulation time. The correlation has the following parameters: r = 0.457; P = 0.028.



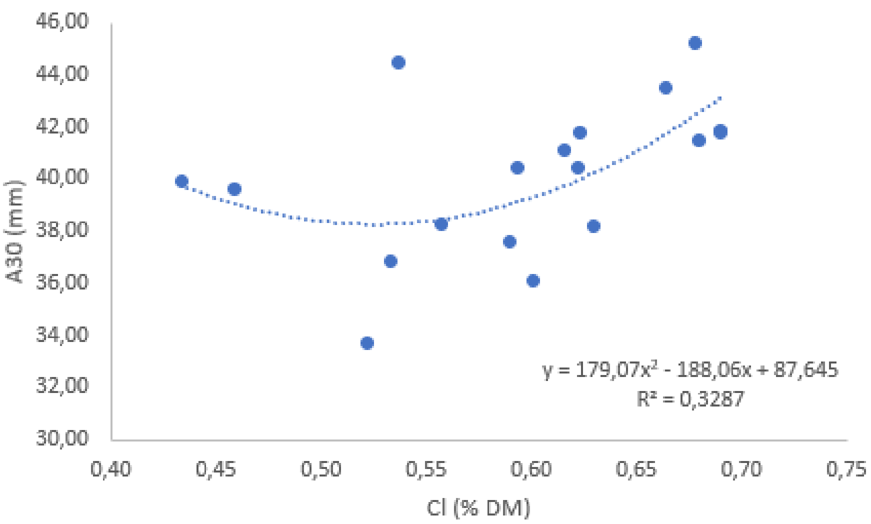
Graphic 34. Relationship between diet total fatty acids and milk rennet coagulation time. The correlation has the following parameters: r = 0.498; P = 0.018.



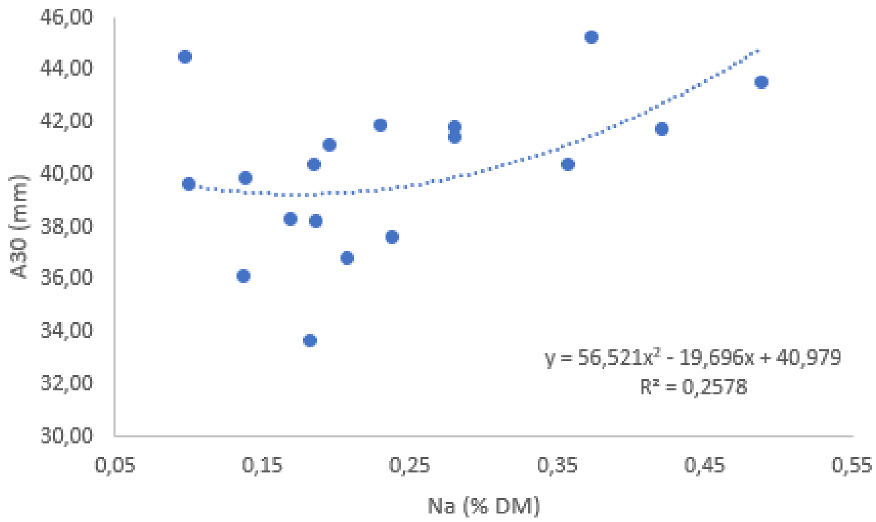
Graphic 35. Relationship between diet unsaturated fatty acids and milk rennet coagulation time. The correlation has the following parameters: r = 0.539; P = 0.010.



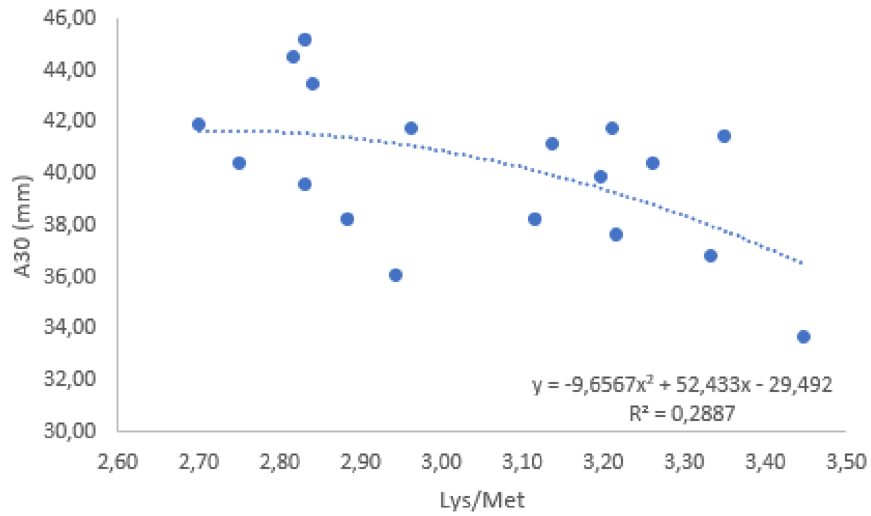
Graphic 36. Relationship between diet Ca and milk A30. The correlation has the following parameters: r = - 0.272; P = 0.138.



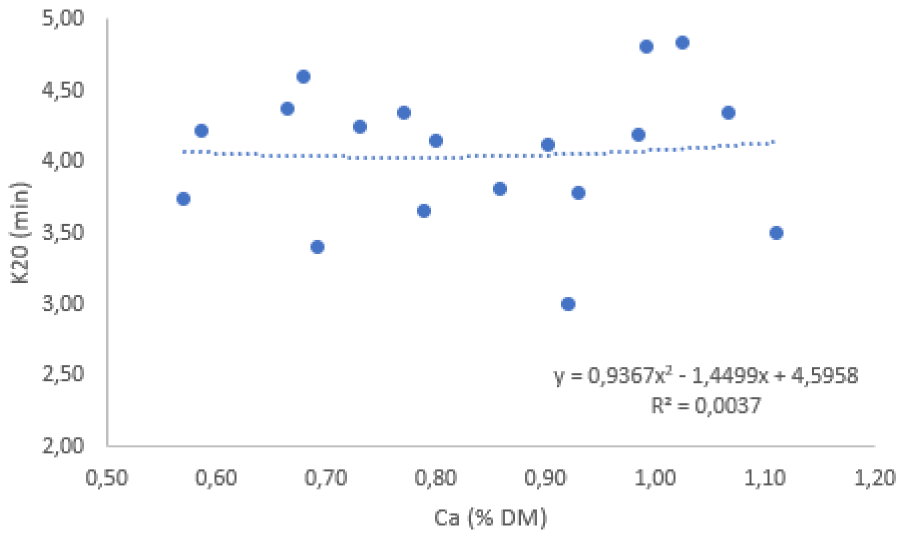
Graphic 37. Relationship between diet Cl and milk A30. The correlation has the following parameters: r = 0.446; P = 0.032.



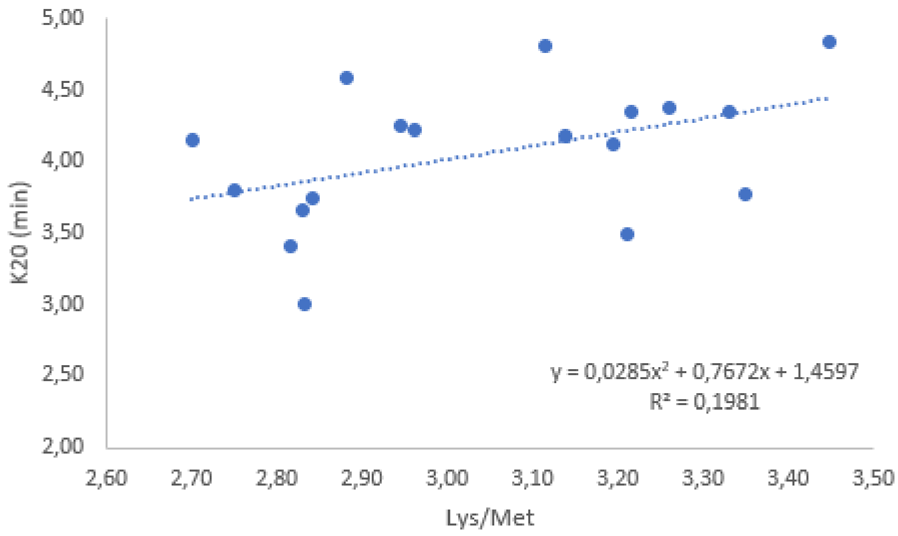
Graphic 38. Relationship between diet Na and milk A30. The correlation has the following parameters: r = 0.447; P = 0.032.



Graphic 39. Relationship between diet Lys/Met and milk A30. The correlation has the following parameters: r = - 0.518; P = 0.014.



Graphic 40. Relationship between diet Ca and milk K20. The correlation has the following parameters: r = 0.036; P = 0.443.



Graphic 41. Relationship between diet lysine/methionine and milk K20. The correlation has the following parameters: r = 0.444; P = 0.032.

Rennet coagulation time (RCT), curd firming time (K20), and curd firmness 30 or 60 min after addition of rennet to milk (A30 and A60, respectively) represent the main milk coagulation properties, which define the milk's aptitude for cheesemaking and the amount of cheese yielded (Visentin et al., 2017).

According to Stocco et al. (2021), there are relationships between coagulation capacity, mineral content, and dairy characteristics of cow's milk. Especially Ca actively participates in the milk coagulation process during cheesemaking, by binding to casein micelles. In the analysed data, no significant correlation was found between dietary calcium intake and milk coagulation properties, as shown in graphics 32, 36 and 40. In graphics 32 and 36, the correlation appears to be negative, which means that as calcium increases, RCT and A30 decrease, but the data dispersion in the graphics is too high to find a significant relationship. The decrease in RCT is a positive factor for the dairy attitude of milk, as it means that milk takes less time to coagulate. On the other hand, the decrease in A30 is a negative factor, because it means that in 30 minutes there is less curd firmness. The conclusions are that calcium provided in the diets analysed is not able to substantially change the amount of calcium present in milk, without important repercussions on the coagulation properties of milk. Probably much higher or lower values are needed than those observed. However, increasing calcium beyond values of 0.80 % on DM leads to a tending decrease in RCT.

In contrast, there is a positive correlation between diet supplied fat and RCT. Both increases in ethereal extract (graphic 33), total fatty acids (graphic 34) and unsaturated fatty acids (graphic

35) lead to an increase in rennet coagulation time. In agreement with Stocco et al. (2021), fat in milk plays a passive role during the coagulation process, as fat globules are trapped in the para-casein matrix and thus positively affects cheese yield and recovery of agitation time and energy in the curd. According to Sutton (1989), the dietary fat composition effects on fat synthesis in milk are complex. There may be increases or decreases in milk fat depending on the amount, physical form, and amino acid composition of fats fed. In the conducted study, the increase in dietary fat has a negative effect on RCT. To keep RCT as low as possible, EE values should be less than 3 %, TFA values should be less than 2 %, and UFA values should be less than 1.60 %. In any case, the maximum RCT value found is lower than that reported in the intermediate report on the Dual Breeding project, which is 20.78.

Two other positive correlations were observed between dietary Cl content and A30 (graphic 37) and dietary Na content and A30 (graphic 38). Na and Cl contribute to the ionic strength of milk (Stocco et al., 2021), but have no direct desirable effects on the coagulative properties of milk. However, these minerals allow maintenance of body homeostasis and of ruminal pH. Cows under heat stress require higher amounts of minerals, as they lose them through polypnea and sweating. In fact, these minerals play a key role in the thermoregulation of cows (Kadzere et al., 2002). Probably the higher amount of minerals in the diets of Rossa Reggiana cows permits the maintenance of ruminal pH and homeostasis in the body, favouring the transfer of useful substances for milk coagulation in milk, such as protein and calcium. Therefore, as Na and Cl increase, curd firmness increases. To increase A30, Cl should have values between 0.65 and 0.70 % on DM, and Na between 0.4 and 0.5 % on DM. A60 is also positively correlated with Cl and Na ($R = 0.421$ and $P = 0.041$, $R = 0.520$ and 0.013 respectively). A negative correlation was also noted between A30 and the ratio of lysine to methionine. This probably is caused by the influence of these two essentials and limiting amino acids on the production of proteins and especially caseins transferred into milk, which has a direct effect on the coagulation properties of milk. The ratio of lysine to methionine should not be more than 3:1.

Finally, a positive correlation was observed between methionine/lysine and curd firming time. The more the ratio increases, the more K20 increases (graphic 41). It is again shown that the ratio of lysine to methionine should be no higher than 3:1.

No other interesting correlations between diet contents and milk coagulation parameters were observed.

6.4 Conclusions

Comparing the estimated nutritional parameters of the diet with measured milk production and milk components, interesting results were found, which allow to provide some guidelines on the best feeding of Rossa Reggiana cattle under heat stress. To maximize milk yield and energy corrected milk, values of DMI between 23 and 25 kg, starch between 15 and 17 % on DM, sugars of 8 % on DM, crude protein of 15-16 % on DM, fodder/concentrate ratio between 1 and 2 would be required, while no interesting parameters were found for EE. To maximize the percentage of protein in milk, crude protein values of 12-14 % on DM, soluble protein of 3.5-4 % on DM, forage of 70 % on DM, soluble fiber of 2.4 % on DM, and starch of 21-22 % on DM should be present in the diet. No interesting parameters were found for maximizing milk fat content, probably because all the diets analysed have a high forage content, which itself maximizes milk fat content. Sugars around 8 % on DM and protein between 13 and 15 % on DM, however, seem to favour % of fat in milk. To prevent milk urea content from exceeding 30 mg/dl, crude protein should undergo 17 % on DM and soluble protein 5.5 % on DM, while starch should exceed 13-15 % on DM and rumen degradable carbohydrates 47-50 % on DM. ADL, on the other hand, should be maintained at values of 4-5 % on DM. To maximize lactose values, sugars should be between 8 and 10 % on DM, starch should be greater than 15 % on DM, NFC should be greater than 32 % on DM, and aNDFom should not exceed 37-39 % on DM. To reduce RCT, Ca should be greater than or equal to 0.80 % on DM, EE should not exceed 3 % on DM, TFA should be less than 2 % on DM, and UFA should be less than 1.60 % on DM. To increase A30, Cl % should be between 0.65 and 0.70 on DM, sodium % should be between 0.4 and 0.5 on DM, and the Lys to Met ratio should not exceed 3 to 1. Finally, also to reduce K20, the Lys/Met ratio should not exceed 3 to 1.

The study presented has some limitations, as the diets provided are all very high in forage and quite similar to each other, and the number of barns sampled is limited. The results obtained from the present study show that Rossa Reggiana cattle fed with the nutritional diet have a modest production with high levels of forage (55-82 %), high level of sugars (5-9,3 %), low levels of fat (2.39-3.5 %), modest levels of starch (7.5-21 %) and protein (11-17 %). It would be interesting to study how this breed performs with different and richer diets, for example without the use of fresh grass and/or with more concentrate. Also, by comparing the production obtained in the summer period with that obtained in other seasons, in future studies it will be possible to determine how much heat stress affects production, to assess the rusticity of this breed.

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