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CICLO XXXII

ITALIAN LOCAL GOAT BREEDS: PHENOTYPIC VARIATION OF MILK MINERAL AND FATTY ACID COMPOSITION AND MILK TECHNOLOGICAL TRAITS

LE RAZZE ITALIANE DI CAPRA: VARIAZIONE FENOTIPICA DELLA COMPOSIZIONE DEL PROFILO MINERALE, DEGLI ACIDI GRASSI E DELLE CARATTERISTICHE TECNOLOGICHE DEL LATTE

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DECLARATION

I declare that the present thesis has not been previously submitted as an exercise for a degree at University of Padova, or any other University, and I further declare that work embodied is my own.

Contents

Abstract

Studies on goat milk have mainly focused on dairy cosmopolitan breeds and very limited information is available on local breeds. Generally, the cosmopolitan breeds received major attention by farmers and dairy industries for their greater milk yield than local breeds. However, the refuse of locals in favour of more productive breeds implies a gradual decrease in number of animals and may lead to the extinction of the local breed causing the genetic erosion of the species. Hence, preserving local breeds from extinction is a significant action that aims not only to maintain ancient local traditions but also to support local economies in marginal areas. Moreover, local breeds are well adapted to their original environment, showing a better resistance to disease; in addition, several studies found that local breeds yield milk with better milk composition than cosmopolitan breeds.

The overall objectives of the present thesis were to assess the phenotypic variation of i) yield, chemical composition and somatic cell; ii) minerals composition iii) fatty acids composition; and iv) technological traits in milk of five Italian local goat breeds (Garganica, GA; Girgentana, GI; Jonica, JO; Maltese, MA; and Mediterranean Red, MR) and one cosmopolitan breed (Saanen, SA). The main source of variation were breed, lactation phase and parity order.

The first study examined the phenotypic variation of milk yield and composition. From February to September (every 2-3 weeks) individual milk yield was recorded for 60 does, and a total of 840 individual milk samples were collected. Local breeds produced less milk than cosmopolitan breed; however, local milks were poorer in somatic cell score but richer in fat and lactose than SA breed. The variation in yield and milk quality traits through lactation followed the general pattern described for small ruminants. Milk yield peaked around the 4^{th} week of

lactation for all breeds; however, local breeds showed a greater persistency of lactation (until 23rd week of lactation) compared to Saanen breed (until 16th week of lactation). The better milk composition was observed in late lactation.

The second and third contribute investigated the variation in milk mineral and fatty acids composition of the pervious goat breeds by investigating a subset of original dataset (n = 252; 7 does per breed). Breed affected P, Mg and Zn and C4:0, from C14:0 to C18:0, C16:1, desaturation index of C16:0 and atherogenic index; however, SA mineral profile was similar to that one of some local breeds, whereas its fatty acids profile was less healthy. Week of lactation highly affect major and trace milk minerals contents. Due to the concentration effect, the greatest content for almost all the minerals was found in late than early lactation. On the other hand, fatty acids were affected by feeding ratio adjusted according lactation phases; in detail, short and medium chain of fatty acids were greater in early than late lactation, and milk of late lactation was richer in n3, n6, conjugated of linoleic acid and unsaturated fatty acids.

The fourth research examined the milk composition and coagulation properties of samples collected monthly from GA, GI, MA, MR and SA breeds between May and August (n = 178; 8-10 does per breed). Breed significant affected milk coagulation properties, protein and lactose percentage, and pH; whereas, month of lactation affected all the studied traits. Milk of local breeds were richer in protein that reflected in shorter time of coagulation and curding firming time than SA breed. Therefore, SA milk showed a curd firmness different only to GI breed. Rennet coagulation time and curd firmness decreased toward the end of lactation, observing significant difference between May and August, whereas curd- firming time differs between May and June-July.

Riassunto

Nel corso degli ultimi anni le razze caprine cosmopolite sono state preferite alle razze locali grazie alla loro maggiore produttività. La sostituzione delle razze locali a favore delle razze più produttive ha favorito una graduale diminuzione del numero di animali legati alle razze locali causando l'erosione genetica della specie. Preservare le razze locali rappresenta una azione prioritaria che mira a tutelare le antiche tradizioni e a sostenere le economie locali e delle aree marginali. Inoltre, le razze endemiche mostrano un miglior adattamento al loro ambiente originale e una migliore resistenza alle malattie; in aggiunta, diversi studi riportano una migliore qualità del latte delle razze locali rispetto alle cosmopolite.

Gli obiettivi di questa tesi di dottorato sono stati quelli di studiare le variazioni fenotipiche di alcune caratteristiche qualitative del latte di cinque razze caprine locali italiane (Garganica, GA; Girgentana, GI; Jonica, JO; Maltese, MA; e Rossa Mediterranea, MR) e in una razza cosmopolita (Saanen, SA) quali: i) produzione di latte, composizione chimica e contenuto di cellule somatiche; ii) composizione dei minerale iii) composizione degli acidi grassi; e iv) caratteristiche tecnologiche.

Il primo studio ha posto l'attenzione alla produzione di latte e alla composizione chimica del latte. Mensilmente da Febbraio a Settembre è stata registrata la quantità di latte riferita a 60 capre in lattazione (10 per razza) e complessivamente sono stati raccolti 840 campioni individuali di latte. Le razze locali hanno prodotto significativamente meno latte, con un minor punteggio di cellule somatiche e un maggior contenuto di grasso e lattosio rispetto alla SA. Tutte le razze hanno raggiunto il picco di produzione intorno alla quarta settimana di lattazione; tuttavia, le razze locali hanno mostrato una maggiore persistenza di lattazione (fino alla 23^{a} settimana di

lattazione) rispetto alla razza SA (fino alla 16^{a} settimana di lattazione) e come atteso la miglior composizione chimica è stata osservata a fine lattazione.

Il secondo e il terzo studio hanno valutato la variazione della composizione minerale e del profilo degli acidi grassi nel latte delle precedenti razze caprine esaminando un sottoinsieme del dataset originale (n = 252; 7 capre per razza). La razza ha influenzato il P, Mg e Zn e il C4:0, dal C14:0 al C18:0, C16:1, l'indice aterogenico e quello di desaturazione del C16:0. La composizione in minerali della SA è risultato simile a quello delle razze locali, mentre il profilo acidico è risultato meno in linea con i requisiti salutistici dell'uomo. I giorni di lattazione hanno fortemente influenzato il contenuto dei minerali, infatti per quasi tutti i minerali è stato riscontrato a fine lattazione un contenuto più elevato degli stessi probabilmente a causa di un effetto di concentrazione. Come atteso il profilo degli acidi grassi è stato influenzato dall'alimentazione modificata e somministrata a secondo delle diverse fasi di lattazione; in particolare, gli acidi grassi a corta e media catena sono risultati maggiori a inizio lattazione, e i contenuti di n3, n6, coniugati dell'acido linoleico e acidi grassi insaturi sono risultati maggiori a fine lattazione.

Il quarto contributo ha infine studiato le qualità di coagulazione del latte analizzando dei campioni di latte (n = 178; 8-10 capre per razza) raccolti mensilmente tra Maggio e Agosto da capre di razza GA, GI, MA, MR e SA. La razza ha significativamente influenzato le proprietà di coagulazione del latte, il contenuto di proteine e il pH; mentre, lo stadio di lattazione ha influenzato tutti i caratteri studiati. Le razze locali hanno prodotto un latte con maggior contenuto proteico ottenendo minori tempi di coagulazione e di rassodamento rispetto alla SA. Il tempo di coagulazione e la consistenza del coagulo sono diminuiti da Maggio ad Agosto, mentre il tempo di rassodamento della cagliata è diminuito da Maggio a Giugno-Luglio.

Chapter 1

General introduction

1.1. Biodiversity: genetic erosion and safeguard of local breeds

In zootechny, the term *biodiversity* defines the genetic diversity within domestic species. There are more than 50,000 bird and mammal species worldwide; however, only 38 species are classified as domestic species and just 5 of them (cattle, chicken, sheep, goat and pig) are mainly reared for production reasons (Cappelloni, 2006). Approximatively, there are 8,800 livestock breeds in 183 countries with 7.64% of them not at risk of extinction, 27.14% at risk of extinction whereas the status of the 65.21% is still unknown (DAD-IS, 2019). The erosion of genetic animal resource is a consequence of farmers and industries' interest in rearing more productive breeds (milk, meat, eggs; FAO, 2005). In detail, the endangered status is unknown for c. 63% of breeds in goat, chicken and pig species, whereas is equal to 55% and 52% in cattle and sheep species, respectively (DAD-IS, 2019). On the other hand, amongst goats and sheep, only the 10% of their breeds are not at risk of extinction; whereas in cattle, chicken and pig breeds such percentage is 8, 7, and 3%, respectively. The greatest percentage of breeds at risk of extinction was found in chicken (13%) and sheep (12%) followed by cattle, goat and pig species with the risk ranging between 9.6% and 8.5% (DAD-IS, 2019).

FAO database (DAD-IS, 2019) reported that North America is the region with the highest number of breeds at risk of extinction (68.4%; 93 breeds), whereas Europe and Caucasus regions have the highest percentage of breeds not at risk of extinction (9.3%; 314 breeds). In EU, Italy offers a broad variability within species with 55, 53, 40 and 19 local breeds in goat, cattle, pig and chicken species, respectively (DAD-IS, 2019). Specifically, Italy has the largest variability

among European countries in goat species with c. 61% of their local breeds at risk of extinction, 10% not at risk and 29% of them still unknown. In 1963 the Ministry of Agriculture, Food, Forestry and Tourism established the Italian institution of National Association of shepherding (ASSONAPA) to preserve the biodiversity of goat species (P.D. of 28/10/1963, n. 1871). This body deals with the management of herd books and breeding registers. The herd book is specific for each breed and contains information on production aptitude, morphological traits and ascendant of every breeding animal. Whereas, the breeding registers are simplified form of the herd books. They contain information of yearlings and breeding animals that match with specific standard defined by breed specifications and technical standards.

Currently, Italy developed just 3 herd books for Sarda, Saanen and Camosciata delle Alpi goat breeds (ASSONAPA; www.assonapa.com). The first (Sarda breed), was activated in 2002, while the other two books (Saanen and Camosciata delle Alpi) in 1973 (ASSONAPA; www.assonapa.com). However, general information of all Italian local goat breeds are included in the breeding registers managed by ASSONAPA (www.assonapa.com).

The characterisation and identification of economic and unique components of each breed may become an efficient tool to preserve and safeguard the animal genetic resource of native breeds that are well adapted to specific environmental conditions of their native regions. Another component to take into account are the local communities, which may play their part thanks to a sustainable employment and the conservation of such resources. Therefore, it is important to highlight that the role given to such local breeds in food production and in the cultural preservation of ancient local traditions, considered a heritage at both local and national level, can be the key to prevent their loss (Gandini and Villa, 2003; FAO, 2005).

1.2. Italian goat breeds

Italy supplies only 1% of the European goat milk production while France (21%), Greece (20%) and Spain (17%; FAOSTAT, 2019) are the main European producers of goat milk. Overall, the 95% of goat milk produced in Europe is used for cheese manufacturing (Boyazoglu and Morand-Fehr, 2001). Despite a limited contribution within the European goat milk production, Italy showed an increase of 29% in milk production from 2009 to 2017 and of 42% in goat cheese production between 2009 and 2014 at national level (FAOSTAT, 2019). This species is the main one reared by developing countries to satisfy their meat, dairy and skin needs, while assuming a different role for developed countries (Haenlein, 2004; Kumar et al., 2016). Indeed, a growth in goat dairy production by developed countries is attributable mainly to goat milk nutritional aspects, its lower allergenicity compared to cow milk and to the growing demand by connoisseurs interested in those products (Haenlein, 2004).

Studies on goat milk yield and its composition refer mainly to cosmopolitan breeds such as Alpine, Murciano-Granadine, Saanen, Toggenburg and Anglo-Nubian breed (Sung et al., 1999; Damián et al., 2009; Rojo-Rubio et al., 2016). Those breeds received great attention by farmers and dairy industries for their higher milk yield compared to local breeds. However, there is a lack of information on milk yield and composition of local breeds due to the larger use of the aforesaid more productive cosmopolitan goat breeds.

Italy, among European countries, shows the greatest number of local goat breeds (n = 55) and these breeds are mainly reared in the South of Italy. The Italian National Statistics Institute (ISTAT; 2019) reports a total of 466,817 goats reared in 17,072 goat farms in the South of Italy; whereas, in the North and Middle of Italy there is a total of 233,782 and 63,210 animals reared in 10,732 and 2,811 farms, respectively. The most relevant goat breeds reared in the South of Italy

are Garganica, Girgentana, Jonica, Maltese and Mediterranean Red breeds. The next paragraph reports the origin and a general description of those breeds (Garganica, Girgentana, Jonica, Maltese, Mediterranean Red and Saanen breeds) which will be object of study of the present thesis.

Figure 1. Garganica breed

The Garganica breed is native of Apulia region. It is a medium-size goat characterised by a black-red mantle, an average female body weight (BW) of 35 kg, 95% of fertility rate (no. of kidding does/ no of inseminated does, %), 1.6 of prolificacy (no. kids/kidding) and an expected first lactation at 18 months of age. During a conventional lactation (210 days; Noè et al., 2005) primiparous and multiparous does yield an average of 117 and 162 kg/lactation of milk, respectively. This breed is reared in medium-big herds under extensive or semi-intensive conditions (www.capre.it).

Figure 2. Girgentana breed

The Girgentana breed is native of Sicily region. It is a medium-size goat characterised by a white mantle and spiral horns. The average female BW it is around 46 kg with a 95% of fertility rate, 1.8 of prolificacy and an expected first lactation at 15 months of age. During a conventional lactation (210 days) (Di Trana et al., 2015) primiparous does yield an average of 282 kg of milk with

3.86% of fat and 3.48% of proteins, whereas, multiparous does yield an average of 343 kg of milk per lactation with 4.09% of fat and 3.45% of proteins. It is mainly reared in small-medium herds under semi-intensive conditions (www.capre.it).

Figure 3. Jonica breed

The Jonica breed is native of Apulia region. It is a mid-big size goat characterised by a white mantle with long ears, an average female BW of 48 kg, 97% of fertility rate, 2.17 of prolificacy and an expected first lactation at 15 months of age. Primiparous and multiparous does yield an average of 158 and 372 kg of milk in a conventional lactation, respectively (210 days; Di Trana et al., 2015). It is mainly reared in small-medium herds under semi-intensive conditions (www.capre.it).

Figure 4. Maltese breed

The origin of Maltese breed is still unknown; however, there are hypotheses that it may have been originally from Malta island as a result of a cross breeding of North African and Mediterranean goat breeds (Fontanesi et al., 2009). This breed is a medium-size goat characterised by a white mantle and black ears, cheeks and/or neck. It is well adapted to intensive or extensive conditions, shows an average female BW of 46 kg, 95% of fertility rate, 1.8 of prolificacy and an expected first lactation at 18 months of age. The period of lactation is c. 210 days (Di Trana et al., 2015). Primiparous does yield an average of 242 kg of milk/lactation with

4.07% of fat and 3.57% of proteins, whereas multiparous does yield ≥ 300 kg of milk/lactation with 4.28% and 3.67% of fat and proteins, respectively (www.capre.it).

Figure 5. Mediterranean Red breed

The origin of Mediterranean Red breed (known also as Derivata di Siria) is still unknown. The hypothesis is that it may have been originally from Middle East (Fontanesi et al., 2009). This breed is a medium-size goat characterised by a red mantle, an average female BW of 48 kg, 95% of fertility rate, 2.1 of prolificacy and an expected first lactation at 15 months of age. During a conventional lactation (210 days; Di Trana et al., 2015) primiparous does yield an average of 121 kg of milk, whereas multiparous does yield an average of 395 kg with 4.12% of fat and 3.50% of proteins (www.capre.it). It is mainly reared in small and mid-herd under extensive or semi-intensive conditions (www.capre.it).

Figure 6. Saanen breed

Saanen breed is native of Saanen valley (Switzerland) and it is one of the most reared goats among cosmopolitan breeds in 68 countries. This breed is a big-medium size goat characterised by a white mantle, an average female BW of 60 kg, 90% of fertility rate and an expected first lactation at 12 months of age. The period of lactation is c. 280 days (Amicabile, 2016). Primiparous does yield an average of 373 kg of milk/lactation with 3.18% of fat and 3.09% of proteins, whereas multiparous does yield c. 591 kg of milk/lactation with 3.19% of fat and 3.12% of proteins (www.capre.it). It is reared in medium-big herds under intensive or semi-intensive conditions.

1.3. Goat milk: chemical and technological traits

Goat milk is a functional food known for its significant nutritional properties. In fact, it is considered a valid substitute for infants who cannot be fed by breast-feeding (Kumar et al., 2016). Goat milk is also characterised by a particular composition, which makes this milk an optimal alternative to traditional cow milk and dairy products. Considering milk composition, goat and cow species show some analogies in protein and total casein contents. Whereas, they differ for lactose and fat percentages, fat globule dimension, fatty acids and mineral composition, amino acids and casein fractions contents and pH (Park et al., 2007; Ranadheera et al., 2019). Despite the lower lactose content present in goat milk compared to cow milk (4.16% vs. 4.76%), the first one is richer in oligosaccharides derived from lactose which show prebiotic and anti-infective properties for human health (Ranadheera et al., 2019).

Differences found in milk composition of these two dairy species are related to their fat content, dimension and fatty acids profile. Specifically, goat milk shows smaller globule fat dimension than cow milk (2.5-3 vs. 3-4 μm, respectively) which promotes a greater fat globule dispersion in the emulsion of milk-lipid and a greater digestibility in human beings than cow milk (Ranadheera et al., 2019). From a technological point of view, the combination of the small fat globule dimension and the absence of agglutinin explains the poorer creaming ability of goat milk compared to cow milk (Ranadheera et al., 2019). Goat milk fatty acids profile contains greater proportion of short and medium chain fatty acids (16.6 vs. 9.2 % of total fatty acids; Ranadheera et al., 2019) than cow milk. In detail, goat milk is rich in fatty acids from C4:0 to C16:0, C18:2 but lower in C18:0 and C18:2. The nomenclature of C6:0, C8:0 and C10:0 fatty acids comes from the name of the caprine species due to the high amount presents in goat milk and are known as caproic, caprylic and capric acid, respectively (Haenlein, 2004). Short and

medium chain fatty acids are a source of direct energy, showing beneficial effects on human health and providing a significant contribution for the prevention of coronary heart disease (Haenlein, 2004; Kumar et al., 2016). In addition, medium fatty acids affect the texture and flavour of dairy products. For instance, the high amount of caproic, caprylic and capric acid in goat milk increases the "goaty" taste of the product which is a trait highly appreciated by connoisseurs consumers (Ranadheera et al., 2019).

Although the total milk protein content is similar in both species, some differences can be found in their casein fraction composition and ratios. Indeed, the main protein detected in goat milk is the β-casein, followed by αs2 and κ-casein whereas αs1-casein is almost absent (Ambrosoli et al., 1988; Sanz Ceballos et al., 2009; Clark et al., 2017). Such a reduced amount of αs1-casein in goat milk makes goat dairy products more suitable for consumers who show symptoms of allergy or intolerance to cow milk protein. On the other hand, presence of αs1-casein affects the coagulation ability of milk. Therefore, milk with low concentrations of αs1-casein shows shorter rennet coagulation time and curding firming time but smaller curd firmness than milk rich in αs1-casein (Ambrosoli et al., 1988; Clark and Sherbon, 2000). Indeed, a lack or low content of αs1-casein in goat milk is the cause of the softer consistency of the curd compared to cow milk. Another difference is the presence of six amino acids (threonine, lysine, isoleucine, cysteine, tyrosine, and valine) which can only be found in goat milk (Ranadheera et al., 2019).

Sanz Ceballos et al. (2009) and Kumar et al. (2016) reported that goat milk contains greater amount in calcium, potassium, phosphorus and a lower level of sodium and sulphur than cow milk (Ranadheera et al., 2019). Goat milk consumption improves the metabolism of calcium and phosphorus and promotes the bioavailability of iron and copper in humans with anaemia more than cow milk (Barrionuevo et al., 2002; Haenlein, 2004). Calcium and phosphorus may also influence milk coagulation properties such as during the reaction of aggregation, the

reduction of rennet coagulation time and the increase of gel strength (Lucey and Fox, 1993). Furthermore, goat milk presents a slight acidity compared to cow milk (6.47 vs. 6.58; Ranadheera et al., 2019) which contributes to promote the reduction of rennet coagulation time in goat milk (Park, 2007). The main differences between milk gross composition and quality traits are due to the diversity of the two species and their own milk secretion system. Indeed, goat milk secretion is characterised by a release of epithelial cells in their milk (apocrine process), whereas this does not occur in cow milk secretion (merocrine process; Haenlein, 2002; Raynal-Ljutovac et al., 2007). This peculiar secretion system explains the greater amount of somatic cells found in goat milk than cow milk which is the reason why it cannot be used as a reliable indicator of mastitis as for cows (Sandrucci et al., 2019). Finally, differences in their oestrous cycle, with goat and cow being respectively seasonal and annual polyestrous species, (Solaiman, 2010) may affect market milk composition (Park et al., 2007).

1.4. Breed and lactation effect on goat milk

Milk composition, yield and sensory qualities are affected by intrinsic (i.e. species, breed, genotype, pregnancy and lactation stage) and extrinsic factors (i.e. environment, feeding regime, management; Fekadu et al., 2005; Chilliard et al., 2007). Species is the main intrinsic factor affecting milk composition and yield followed by breed and lactation phase (Damián et al., 2009; Vacca et al., 2018; Sandrucci et al., 2019). For instance, Damián et al. (2009) found better milk quality in Anglo-Nubian than Saanen breed which are two dairy cosmopolitan goat breeds. Vacca et al. (2018) and Sandrucci et al. (2019) studies compared milk yield and composition of Alpine breeds (Alpine and Saanen) with Mediterranean breeds (Murciano-Granadina, Maltese, Sarda,

and Sarda Primitiva) and Italian local breeds (Bionda dell'Adamello, Frisa, Nera di Verzasca, and Orobica), respectively. Both studies showed that Alpine breeds were greater in milk yield than their counterparts, whereas Mediterranean breeds showed better milk quality than Alpine breeds. To our knowledge only few studies explored gross composition, detailed minerals and fatty acids profile and milk coagulation properties of local goat breeds. For instance, differences in minerals profile were detected between milk of local Portuguese goat breeds (Serrana, Serpentina, Charnequeira and Algarvia) and milk of Saanen breed by Trancoso et al. (2010) who reported the effect of breed on Ca, P, Mg, Na, K and Fe contents. However, it is important to highlight that these differences may be related to the different feeding strategies applied (e.g. goats were reared in different regions). Differences in minerals content of the milk were also found in another study between Anglo-Nubian and French Alpine goat breeds. The authors reported a greater overall mean of K compared to Na (Park and Chukwu, 1988).

Yurchenko et al. (2018) reported the effect of breed on milk fatty acids profile in Saanen and Swedish Landrance goats. They found a difference on fatty acids from C4:0 to C16:0, C16:1, C18:1, in atherogenic index and desaturation index of C16:0, showing a healthier milk fatty acids profile in the latter breed than the first. Pizzillo et al. (2004) reported a breed effect on fatty acids profile from C4:0 to C16:0, from C18:0 to C20:0, saturated and unsaturated fatty acids which were detected in ricotta cheese produced by using milk of Girgentana, Maltese, Mediterranean Red and local goat breeds.

From a technological point of view, better milk quality composition found in local than cosmopolitan breeds may help to explain their greater milk coagulation properties. In general, an intensive breed selection and the consequent high milk yield leads to a dilution effect of components (Serradilla, 2001) as seen in different studies where milk of high yield breeds showed reduced coagulation ability (Poulsen et al., 2013; Penasa et al., 2014; Vacca et al., 2018).

Several studies also explored the effect of the phase of lactation on milk yield and gross composition (Carnicella et al., 2008; Goetsch et al., 2011; León et al., 2012), on minerals (Park and Chukwu, 1988; Kondyli et al., 2007; StrzałKowska et al., 2008; Mestawet et al., 2012; Antunović et al., 2018), fatty acids composition (Strzalkowska et al., 2009; Kuchtík et al., 2015) and on milk coagulation properties (Pazzola et al., 2017; Vacca et al., 2018).

In a conventional lactation, the production of colostrum goes until the 5^{th} day after parturition while production of mature milk goes from the 6^{th} day after parturition to the end of lactation. Milk yield reaches its maximum peak around days 43 and 50 of milking followed by one phase of persistence and one of decline (Gipson and Grossman, 1989). The lower milk yield of late (decline phase) than early lactation is attributable to the reduction of mammary gland efficiency (Albenzio et al., 2016). Concerning milk components, lactose shows the lowest variability through lactation and its trend follows the one of milk yield with a peak matching the one of the milk (Costa et al., 2019). The effect of concentration as a result of the drying off, led to a higher amount of milk protein, fat contents and somatic cell count during late lactation compared to early lactation (Fekadu et al., 2005; Albenzio et al., 2016). The greater percentage of proteins found in late than early lactation can explain the decrease of pH at the end of lactation (Bonfatti et al., 2013; Vacca et al., 2018).

The effect of lactation on minerals pattern was studied by several authors (Park and Chukwu, 1988; Mestawet et al., 2012; Antunović et al., 2018) who reported a general greater content of minerals at the end of lactation than during early lactation. This can be explained by a concentration effect as well as by extrinsic factors such as seasonal wheatear conditions, soil composition and quality phenological state of plants. Qeshlagh et al. (2016) found a greater content of Ca, P and Na in ewe milk during summer season compared to spring season.

Contrary to milk gross composition, the clear effect of lactation phase on milk fatty acids composition has not been explained yet (Craninx et al., 2008). Indeed, the results presented in some studies on the effect of lactation on milk fatty acids are quite controversial. For instance, Kuchtík et al. (2015) found that individuals, groups and indices of fatty acids were highly affected by days of lactation while Strzalkowska et al. (2009) reported that no effect of lactation was found for C4:0, C14:0, C16:0, C17:0 and medium fatty acids chain. Milk fatty acids composition is mainly influenced by feeding regime (Griinari et al., 1998; Gama et al., 2008; Schmidely and Andrade, 2011). However, feeding is adjusted according to the lactation phase (Solaiman, 2010) which makes difficult to independently evaluate the effect of lactation and feeding on milk fatty acids profile. In detail, about 40% of individual fatty acids are synthesised *de novo* by mammary glands (from C4:0 to C14:0, all odd fatty acids and 50% of C16:0), whereas the other 60% comes from the mobilisation of adipose tissue or from feed intake (50% of C16:0 and from C18:0 onwards). However, a correct feeding regime which suits animal requirements permits not only to maintain stable its energy balance but also to reduce the onset of metabolic disease which may induce milk fat depression syndrome. In detail, a decrease in ruminal pH due to milk fat depression syndrome had an effect on microbial population and fatty acids profile (Griinari et al., 1998; Gama et al., 2008).

Looking at milk technological traits, the main factors affecting milk coagulation properties are milk gross composition, minerals content (mainly Ca, P and Mg) and pH (Malacarne et al., 2014; Vacca et al., 2018). There are studies reporting that protein and minerals increase at the end of lactation mainly due to the lower amount of milk produced when compared to early lactation. Moreover, the high content of proteins found in late lactation promotes a reduction in pH. Indeed, milk with optimal gross composition (protein, fat, lactose) and minerals content and with a low pH showed low time of rennet coagulation and curding firming whereby

providing great curd firmness (Malacarne et al., 2014) as seen in Vacca et al. (2018) who observed better milk coagulation properties in late lactation than early lactation.

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Chapter 2

Aims of the thesis

The present thesis aimed to develop phenotypic characterization of milk yield, milk composition and rheological properties of five Italian local goat breeds and one cosmopolitan breed under the same management conditions.

The specific objectives were:

i. To assess the week of lactation and breed effects on milk yield, composition and somatic cell score by comparing five endangered Italian local breeds (Garganica, Girgentana, Jonica, Maltese, Mediterranean Red) and one goat specialised cosmopolitan breed (Saanen) reared under similar management conditions;

ii. To define milk mineral contents of five endangered Italian local breeds (Garganica, Girgentana, Jonica, Maltese, Mediterranean Red) and one cosmopolitan breed (Saanen) during the entire lactation reared under similar management conditions;

iii. To characterize fatty acids profile during a complete lactation in Garganica, Girgentana, Jonica, Maltese, Mediterranean Red and Saanen breeds reared under similar management conditions;

iv. To describe the phenotypic variation of milk coagulation properties in Garganica, Girgentana, Maltese, Mediterranean Red and Saanen goat milk breeds reared under similar management conditions, and to evaluate the rennetability of milk produced during mid and late lactation;

Chapter 3

Autochthonous dairy goat breeds showed better milk quality than Saanen under the same environmental conditions

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ABSTRACT

Studies on goat milk have mainly focused on cosmopolitan breeds and very limited information is available on local breeds, which is important for biodiversity preservation and local cheese production. The aim of this study was to evaluate the breed effect on milk yield, composition and somatic cell score (SCS) of five local Italian goat breeds (Garganica, Girgentana, Jonica, Maltese and Mediterranean Red) compared with a cosmopolitan specialized dairy breed (Saanen). A total of 60 goats (10 per breed) from an experimental farm were enrolled in the study. Milk yield, composition and SCS were recorded and analyzed every 2 weeks during the entire lactation. Data were analyzed using a mixed model with repeated measures. Saanen yielded between 0.27 and 0.62 kg day $\leq$ 1 more milk than the local breeds. Among local breeds, Maltese and Jonica were the most productive, with an average of 1.28 and 1.25 kg day^{-1}, respectively, while Mediterranean Red, Garganica and Girgentana produced $\leq$ 1 kg day^{-1}. Saanen had the highest SCS (6.81) and the lowest fat content (3.26 %). In relation to protein, Garganica showed the greatest content (3.71 %), and Saanen had a similar content to other local breeds (3.42%) except for Maltese, which was lower (3.11%). Saanen and Garganica had the lowest lactose percentage (4.28% and 4.26%, respectively). All breeds followed a similar pattern across lactation: SCS and fat and protein content peaked at the end of the lactation, whereas lactose percentage was highest at the beginning of the lactation. Differences between Saanen and the local breeds for milk yield, composition and SCS were consistent across lactation. In conclusion, local breeds produced less milk but with lower SCS and greater fat and lactose content than the Saanen cosmopolitan breed, suggesting a better milk quality.

INTRODUCTION

In the last 10 years, goat (Capra hircus) milk world production has increased by 27.9 %, from 14×10^6 t in 2004 to more than 18×10^6 t in 2014, and goat cheese manufacture has increased by 17 %, from 446×10^3 t in 2004 to 523×10^3 t in 2014 (FAOSTAT, 2019). The goat milk casein profile is more similar to human milk (Haenlein, 2004; Albenzio et al., 2012) than cow's milk is. Moreover, goat milk has greater protein micelles and smaller fat globules and a more favorable fat composition (Park, 1994; Williams, 2000; Faye and Konuspayeva, 2012), which is beneficial for digestibility and energy uptake (Park, 1994; Williams, 2000) as well as for cheese manufacture (Faye and Konuspayeva, 2012). However, the goat milk industry has put great efforts into in- creasing milk production, which has drastically eroded genetic variability in this species because farmers have often replaced local populations with genetically improved breeds to increase farm profitability.

Studies on milk yield and composition have mainly focused on cosmopolitan dairy-specialized goat breeds (Gipson and Grossman, 1989; Sung et al., 1999; Goetsch et al., 2011), and only few studies have investigated Italian local breeds (Tripaldi et al., 1998; Sacchi et al., 2005; Carnicella et al., 2008). The lack of information on potential milk yield and composition of autochthonous breeds is probably a major reason for the substitution of local with more productive cosmopolitan breeds such as Alpine, Murciano-Granadina and Saanen (Benjelloun et al., 2015). Currently, and according to official national data reported by the FAO (DAD-IS, 2019), Italy has 55 goat breeds considered of regional or local distribution, 34 of them being at risk of extinction and 16 having an unknown risk. The five most important breeds reared in south Italy are the Garganica, Girgentana, Jonica, Maltese and Mediterranean Red breeds, which are all at risk of extinction except for the Garganica, for which the risk level is unknown. Therefore, the aim of the present study was to evaluate the breed effect on milk yield, composition and somatic cell score (SCS) of five local goat breeds (Garganica, Girgentana, Jonica, Maltese and

Mediterranean Red) compared with a cosmopolitan dairy specialized goat breed (Saanen) reared on the same experimental farm.

MATERIALS AND METHODS

Animals and Management Condition

The study was carried out at the experimental farm of the Council for Agricultural Research and Economics (CREA, Potenza, Italy) from February to September 2016 and included Garganica (GA), Girgentana (GI), Jonica (JO), Maltese (MA), Mediterranean Red (MR) and Saanen (SA) goat breeds reared under the same management and feeding conditions. To our knowledge, there are not previous studies that included six different breeds reared under the same conditions allowing a direct comparison of their performance. A general description of the six breeds is reported in Table 1. Experimental procedures and animal care conditions followed the recommendations of European Union directive 86/609/EEC (CEU, 1986). A total of 60 dairy goats (10 does per breed) with a body condition score between 2.5 and 3.0 (1, very thin, to 5, very fat, with a 0.5 point increment; Villaquiran et al., 2005) and a body weight of 48 ± 4 kg for GA, 42 ± 6 kg for GI, 47 ± 6 kg for JO, 46 ± 5 kg for MA, 48 ± 3 kg for MR and 64 ± 7 kg for SA breeds at the beginning of the study were enrolled in the study. All goats and breeds grazed together during the day (8 h day^{-1}) in a natural pasture and received hay (composition: 60%–65% of grasses and 40%–35% of legumes and others; chemical composition: 89.10% of dry matter, 15.10% of crude protein, 52.60% of neutral detergent fibre and 1.10 Mcal kg^{-1} of net energy of lactation) *ad libitum* as a complement in the shelter. Moreover, goats were supplemented with commercial concentrate (chemical composition: 88.20% of dry matter, 21.70% of crude protein, 23.00% of neutral detergent fibre and 1.77 Mcal kg^{-1} of net energy of lactation) in the milking

parlour according to their requirements considering the mean body weight and mean milk production for each breed following NRC (2007) recommendations; this was adjusted every 15 days throughout lactation. All selected does kidded twins in February and parities from 1 to 5 were balanced between breeds (i.e., the same number of goats for each parity for each breed). Kids were kept with their dams until 40 days from birth and temporally separated from their dams 24 h before every sampling day. All goats were mechanically milked twice a day (07:30 and 17:30 LT) in a double 24-stall herringbone low-line milk pipeline milking parlour (Alfa Laval Agri; Monza, Italy) equipped with recording jars and electronic pulsators at a vacuum of 38 kPa, 90 pulses min^{-1} and a 60% pulsation ratio. Pre-milking included only forestripping, without any preparation of udder and teats. None of the does presented mastitis throughout the study.

Sample Collection and Analysis

The milk yield (kg day^{-1}) of individual does, as the sum of morning and evening milkings, was recorded every 2–3 weeks from 2 to 30 weeks of lactation using the recording jars in the milking parlour and an individual milk sample (50 mL; n = 840) collected during the morning milking. Thereafter, milk samples were stored in portable refrigerators (4 °C) and transferred to the milk laboratory of the Breeders Association of the Basilicata region (Potenza, Italy). Samples were warmed at 37 °C in a water bath prior to milk analysis for fat, protein and lactose percentages with MilkoScan FT6000 (Foss Electric, Hillerød, Denmark). Fat-corrected milk at 3.5% (FCM, kg day^{-1}) was calculated according to Pulina et al. (1991) following Eq. (1):

$$FCM = \text{milk yield} \times (0.634 + 0.1046 \times \text{fat}). \quad (1)$$

Somatic cell count (SCC, cells mL^{-1}) was determined using Fossomatic FC (Foss Electric, Hillerød, Denmark) and transformed to SCS according to Wiggans and Shook (1987) using the following Eq. (2):

$$SCS = 3 + \log_2 (SCC/ 100{,}000). \quad (2)$$

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS v. 9.4 (SAS Institute Inc., Cary, NC), with repeated measures. The statistical analyses included breed, week of lactation, and the interaction between breed and week of lactation as fixed effects and animal effect nested within breed and residual as random effects. Residual distributions from the model for each trait were examined and outliers were removed. The final mixed model was performed on 815 records. When the F ratio was significant, multiple comparisons of least squares means (LSMs) were performed using Bonferroni's test adjustment. Values are shown as LSM ± SE and significance was declared at $p < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

The analysis of variance revealed that milk yield, composition and SCS were affected ($p < 0.001$) by breed and week of lactation. The interaction between breed and week of lactation was significant for all the studied traits with the exception of SCS. Least squares means of the studied traits for breed effect are shown in Fig. 1. Saanen yielded between 0.27 and 0.62 kg day^{-1} more milk than the local breeds ($p < 0.05$). Among local breeds, MA and JO were the most productive, with an average of 1.28 ± 0.05 and 1.25 ± 0.05 kg day^{-1}, respectively, while MR, GA and GI produced ≤ 1.00 kg day^{-1}.

Saanen is one of the most specialized dairy breeds world- wide, and it has been subjected to intensive genetic improvement for milk yield resulting in more days in milk and greater milk yield than other breeds (Serradilla, 2001). Serradilla (2001) has reported that usually local breeds exhibit lower lactation milk yield than cosmopolitan dairy specialized breeds. However, after standardizing milk production with a 3.5% title of fat, MA and JO production was similar to that of SA (Fig. 1). The greatest SCS was observed for SA (6.81 ± 0.18; $p < 0.05$) and among local breeds GA (5.92 ± 0.18) showed higher SCS than GI and MA (5.03 ± 0.18; $p < 0.05$; Fig. 1). Higher values of SCC in goat milk compared to cow milk is physiological because goat milk protein secretion follows an apocrine process resulting in the release of the apical part of epithelial cells (Jiménez-Granado et al., 2014), while cows follow a merocrine process. In small ruminants, Raynal- Ljutovac et al. (2007) and Jiménez-Granado et al. (2014) reported that non-pathological factors such as breed, parity, stage of lactation, type of birth, oestrus, and diurnal, monthly and seasonal variations are responsible for 48% of SCC variance in dairy sheep and goats. However, studies on goat milk seem to confirm a negative relationship between high SCC and milk yield and quality which could affect milk rennet ability and cheese yield (Raynal-Ljutovac et al., 2007).

Fat, protein and lactose content (Fig. 1) were in agreement with those reported by FAO (2013) for goat milk. Milk fat content was similar among local breeds, whereas SA produced approximately 20% less milk fat than the average of the local breeds ($p < 0.05$; Fig. 1). The lower milk fat content of SA goats compared to the Italian local breeds is in agreement with Landau et al. (1995) and Donkin et al. (1996), who compared SA with Israeli and South African local goat breeds, respectively. As reviewed by Goetsch et al. (2011), the lower fat content in milk of SA (3.26 ± 0.12%; Fig. 1) compared with local breeds could be partially explained by a dilution effect, where SA yielded on average 30% more milk per day than the local breeds. Additionally,

Prasad et al. (2005) reported that the higher the milk production level, the lower the concentration of fat. Among local breeds, milk of GA had the greatest protein content (3.71 ± 0.07%; $p < 0.05$). Moreover, SA showed a protein content (3.42 ± 0.07%) similar to the local breeds, with the exception of MA (3.11 ± 0.07%; $p < 0.05$), which was lower (Fig. 1). These results disagreed with Tripaldi et al. (1998) and Serradilla (2001), who reported a lower protein content for SA compared to other local breeds. Moreover, Tripaldi et al. (1998) reported no differences in the protein content in milk of GA, MA and MR breeds. Lactose content was similar among GI, IO, MA and MR (4.51 ± 0.04%), while GA and SA (4.26 ± 0.03%) presented a lower content ($p < 0.05$; Fig. 1). The goat breeds which showed the greatest lactose content were the ones with the lowest SCS, in agreement with Sung et al. (1999), who reported a negative correlation be- tween SCS and lactose content among cosmopolitan breeds in Taiwan. The decrease in lactose percentage is usually related to mastitis occurrence due to the decrease in the synthesis function of the mammary gland (Raynal-Ljutovac et al., 2007). Nevertheless, although some authors have reported a decrease in lactose concentration with an increase in SCC in goat milk (Zeng et al., 1997; Sung et al., 1999), others have not observed any effects (Raynal-Ljutovac et al., 2007). The variation in milk yield and quality traits across weeks of lactation followed the general pattern described for small ruminants. In particular, milk yield increased in early lactation until the peak at approximately 4 weeks after kidding, followed by a decline towards the end of lactation (after 24 weeks) as shown in Fig. 2. The pattern of milk yield during the lactation was quite similar among breeds, with SA producing more milk between week 6 and 14 compared with local breeds ($p < 0.05$). Differences after the peak of lactation between the SA and the local breeds were less evident when comparing FCM (Fig. 2). Gipson and Grossman (1989) reported slightly earlier peak milk yield in Alpine (43.4 days) and Toggenburg (50.7 days) goat breeds compared with our findings (28 days). Girgentana and MR breeds after peak milk yield maintained a persistent

lactation, keeping their production stable until 23 weeks of lactation, followed by SA and GA breeds (16 and 20 weeks, respectively). The breeds that were less persistent after peak milk yield were JO and MA. et al., 2011; Mestawet et al., 2012; Jiménez-Granado et al., 2014). Although SA showed greater SCS than local breeds throughout lactation, these differences were rarely significant (Fig. 2). Fat content of SA was significantly lower during mid and late lactation compared to the local breeds (Fig. 2). Protein content showed significant differences only in late lactation with the greatest content for GA breed (Fig. 2). Lactose content decreased gradually until the end of lactation (Fig. 2), being this decrease in late lactation (from the 18th to 30th week) less marked for GI, JO, MA and MR than for GA and SA goat breeds. The decrease in lactose content at the end of lactation has been also reported by Prasad et al. (2005) in Indian goat breeds. However, Zeng et al. (1997) have reported a more constant lactose content through the lactation of Alpine goats. Overall, differences between SA and local breeds for milk yield, SCS and milk composition were consistent across lactation.

The increase in SCS, fat and protein towards the end of the lactation, when milk volume is low (Fig. 2), has been explain by several authors in part as a concentration effect (Goetsch et al., 2011; Mestawet et al., 2012; Jiménez-Granado et al., 2014). Although SA showed greater SCS than local breeds throughout lactation, these differences were rarely significant (Fig. 2). Fat content of SA was significantly lower during mid and late lactation compared to the local breeds (Fig. 2). Protein content showed significant differences only in late lactation with the greatest content for GA breed (Fig. 2). Lactose content decreased gradually until the end of lactation (Fig. 2), being this decrease in late lactation (from the 18th to 30th week) less marked for GI, JO, MA and MR than for GA and SA goat breeds. The decrease in lactose content at the end of lactation has been also reported by Prasad et al. (2005) in Indian goat breeds. However, Zeng et al. (1997) have reported a more constant lactose content through the lactation of Alpine goats. Overall,

differences between SA and local breeds for milk yield, SCS and milk composition were consistent across lactation.

CONCLUSIONS

Our results contributed to the characterization of milk yield and composition of local Italian goat breeds. Results indicated that breed and week of lactation were the main factors responsible for the variation in the studied traits. Local breeds produced less milk but with lower SCS, greater fat and lactose content than the SA cosmopolitan breed. Overall, no differences in protein percentage were observed between SA and local breeds, which is an important trait for cheese transformation. Therefore, the variability of milk yield and composition traits reported in the present study are of interest to preserve the biodiversity of local goat breeds and for the dairy industry to balance milk volume and component concentration for cheese manufacturing. Further studies on the milk fat profile and mineral composition of local goat breeds would be beneficial due to their relationships with human health and their milk rennetability.

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Table 1. Origin and description of goat breeds included in the study retrieved from Capre (2019) unless otherwise indicated.

Trait	Garganica	Girgentana	Jonica	Maltese	Mediterranean red	Saanen
Origin	Italy	Italy	Italy	Italy	Italy	Switzerland
Herd	Big and medium	Medium and small	Medium and small	Big, medium and small	Medium and small	Big and medium
Morphology	Black mantle	White mantle and spiral horns	White mantle and long ears	White mantle with black head and neck	Red mantle	White mantle
Female BW, kg	35	46	48	46	48	60
Fertility rate, %[a]	95	95	97	95	95	90
Prolificacy[b]	1.6	1.8	2.2	1.8	2.1	1.6
Age at 1st kidding, months	18	15	15	18	15	12
Days in milk	210[c]	210[d]	210[d]	210[d]	210[d]	280[e]
Milk yield, kg/lactation[c]	Parity 1 = 117 Parity 2 = 126 Parity ≥ 3 = 162	Parity 1 = 282 Parity 2 = 327 Parity ≥ 3 = 359	Parity 1 = 158 Parity 2 = 407 Parity ≥ 3 = 336	Parity 1 = 242 Parity 2 = 307 Parity ≥ 3 = 358	Parity 1 = 121 Parity 2 = 337 Parity ≥ 3 = 452	Parity 1 = 373 Parity 2 = 569 Parity ≥ 3 = 613

BW = body weight.

[a] Fertility rate = number of kidding does/number of inseminated does.

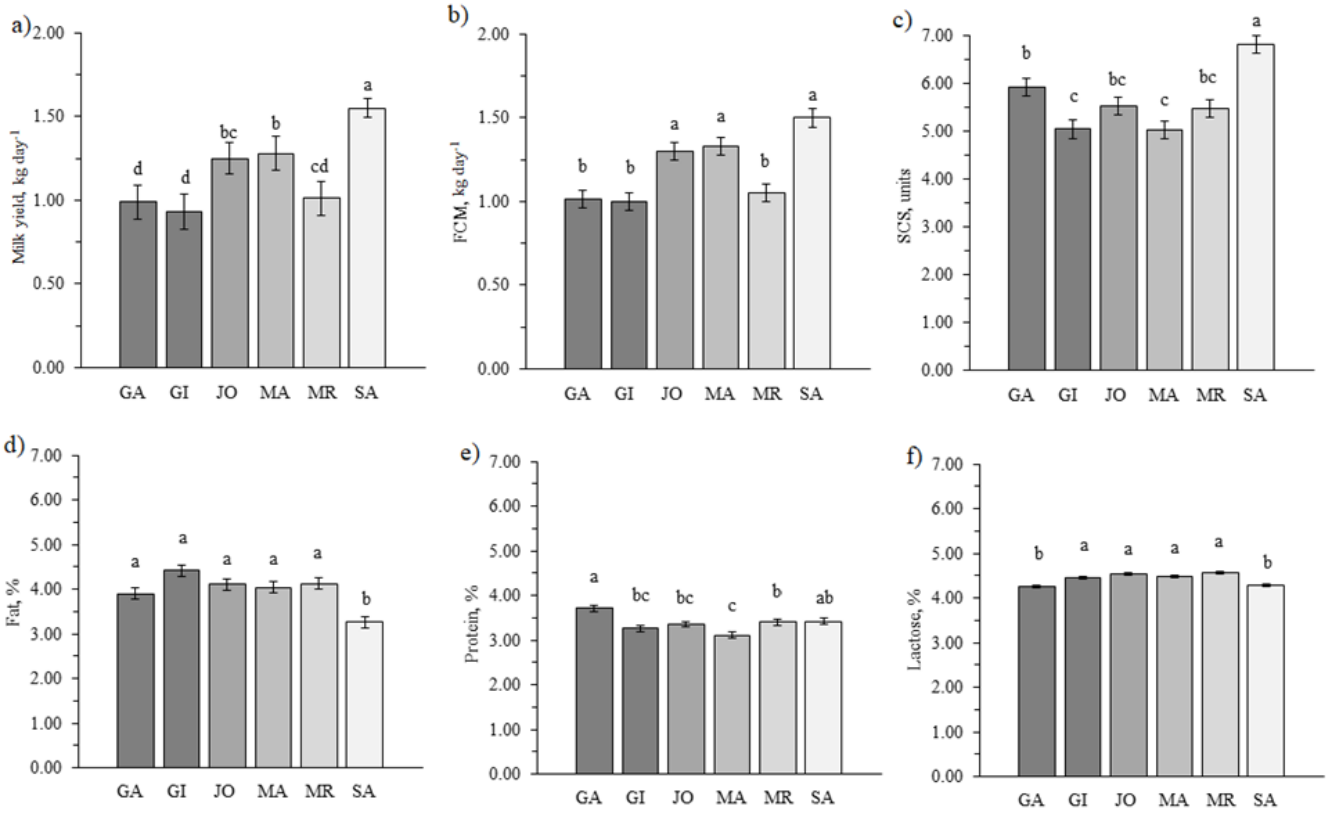
[b] Prolificacy = number kids/kidding.

[c] Information retrieved from Noè et al. (2005).

[d] Information retrieved from Di Trana et al. (2015).

[e] Information retrieved from Amicabile (2016).

Figure 1. Least squares means (with SE) for (a) milk yield, (b) fat-corrected milk at 3.5% (FCM), (c) somatic cell score (SCS), (d) fat percentage, (e) protein percentage and (f) lactose percentage for Garganica (GA), Girgentana (GI), Jonica (JO), Maltese (MA), Mediterranean Red (MR) and Saanen (SA) goat breeds. Means with different letters within a trait are significantly different ($p < 0.05$).



Chapter 4

Differences in the detailed milk mineral composition of Italian local and Saanen goat breeds

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SIMPLE SUMMARY

This study quantified major and trace minerals in milk of five Italian local goat breeds and a cosmopolitan goat breed throughout lactation. Significant differences were detected in milk minerals composition across week of lactation, with greater concentration at the end than at the beginning of the lactation for almost all minerals, while only P, Mg and Zn milk content differed among breeds. Due to the growing interest of consumers in goat milk and derived products, the characterisation of milk mineral contents could contribute to valorise autochthonous breeds.

ABSTRACT

Very little information about local breed goat milk is available, which is relevant for biodiversity preservation and local cheese production. This study aimed to evaluate the effect of breed and week of lactation on milk mineral profile of five Italian local breeds (Garganica, Girgentana, Jonica, Maltese and Mediterranean Red) and a cosmopolitan breed (Saanen). Sixty goats (10 per breed) from an experimental farm were enrolled in the study and sampled every 2 weeks for milk gross composition analysis. In addition, an individual milk sample was collected monthly from 42 goats (seven goats per breed) for mineral determination through inductively coupled plasma optical emission spectrometry. Data were analysed using a mixed linear model with repeated measures, including breed and week of lactation as fixed effects. Week of lactation affected mineral contents, except for B, being lower in early than late lactation, whereas, breed affected only P, Mg and Zn. Mediterranean Red and Jonica breeds' milk was richer in P than Maltese, and in Zn than Maltese, Girgentana and Saanen breeds. For Mg, only Saanen differed from Maltese. Such information might be useful for the valorisation of goat milk produced by autochthonous breeds.

Key words: doe; indigenous breed; lactation; major mineral; trace element

INTRODUCTION

Europe produces approximately 15% (2.8×10^6 tons) of the world's goat milk (18.6×10^6 tons; (FAOSTAT, 2019) and 95% of this amount is transformed into dairy products (Boyazoglu and Morand-Fehr, 2001). Goat cheese production corresponds to 2.3% (52×10^4 tons) of worldwide cheese production (23×10^6 tons; FAOSTAT, 2019), and several goat cheeses have protected designation status. In the last decade, the Italian goat milk and cheese production has increased 17% (24.9 to 29.2 $\times 10^3$ tons) and 32% (3.4 to 4.5 $\times 10^3$ tons), respectively (FAOSTAT, 2019). Consumers' interest in goat milk and derived products is mainly related to its better digestibility for infants, the elderly and patients with gastrointestinal disorders (Sanz Ceballos et al., 2009; Kumar et al., 2016) compared with cow milk; the smaller fat globule size and lower αs1-casein content of goat compared with cow milk are the major reasons of the difference in digestibility and allergenicity (Sanz Ceballos et al., 2009; Zenebe et al., 2014). Sanz Ceballos et al. (2009) reported greater amounts of Ca, P, Mg and Cu in goat compared with cow milk under identical environmental conditions. In addition, goat milk shows lower lactose content with a greater abundance of oligosaccharides derived from lactose, that positively affect human health for their prebiotic and anti-infective nature (Ranadheera et al., 2019). Moreover, goat milk contains more short fatty acids, n3, n6 and vitamin A than cow milk. Furthermore, fermented goat milk has a probiotic component that is maintained quite constant during the whole shelf life of the product for the low pH (6.47) and the buffering effect; in fact, fermented goat milk is considered a means to provide and improve probiotic intake in the human diet (Ranadheera et al., 2019).

Milk minerals play an important role in human health (Zenebe et al., 2014; Kumar et al., 2016) and milk coagulation ability (Lucey and Fox, 1993). In particular, Ca and P affect bone and teeth structure and muscular function, and Zn influences skin health and carbohydrate metabolism (Zenebe et al., 2014). Copper and Fe are involved in the haemoglobin synthesis and transportation (Barrionuevo et al., 2002). Moreover, Barrionuevo et al. (2002) reported that goat milk consumption favoured Fe and Cu absorption in healthy rats and in those with malabsorption syndrome compared with cow milk. From a technological point of view, Ca and P strongly affect the milk coagulation ability and the firmness of the coagulum at the end of the coagulation process (Lucey and Fox, 1993).

According to the literature, breed and stage of lactation affect mineral content of goat milk (Park and Chukwu, 1988; Khan et al., 2006). However, studies are mainly focused on cosmopolitan breeds such as Saanen, Toggenburg, Alpine and Anglo-Nubian (Trancoso et al., 2010; Mestawet et al., 2012), whereas local breeds are often neglected. In the last decades, local breeds have been progressively replaced with cosmopolitan breeds with the aim of increasing milk production. Nevertheless, studies on local breeds have revealed that milk gross composition is generally better in local than cosmopolitan breeds (Trancoso et al., 2010). Moreover, the replacement of native with high productive breeds is responsible for the reduction of the variability within species, the loss of niche products related to specific breeds and the loss of historical heritage of the country (Gandini and Villa, 2003). The FAO database (DAD-IS, 2019) reported that among the 55 Italian goat breeds, 61% are endangered and 29% are in unknown conditions of risk.

Nowadays, the assessment of milk quality is not only based on traditional components (i.e., fat and protein contents), but also on more specific compounds such as protein profile, fatty acid profile, and mineral content. However, information on those compounds and differences

among breeds is very scarce. Due to the growing interest of consumers towards goat milk and dairy products, mineral and fatty acid composition of milk from local goat breeds might be useful to valorise their productions. Because some minerals are correlated with protein and fat content in milk (Park, 1994), and differences in fat and protein content have been reported between Saanen and local breeds (Trancoso et al., 2010), we expect that breeds present differences in milk mineral content, in particular when local are compared with cosmopolitan breeds. Therefore, the aim of the present study was to characterise milk mineral contents of 5 local Italian goat breeds and to compare them with the cosmopolitan Saanen breed.

MATERIALS AND METHODS

Animals and Management Conditions

This research was conducted from February to August 2016 in the experimental farm of the Council for Agricultural Research and Analysis of Agricultural Economy Analysis, Research Unit of Extensive Animal Production (CRA-ZOE, Potenza, Italy). Experimental procedures and animal care conditions followed the recommendations of European Union directive 86/609/EEC. All animals were in the same farm under the same management conditions. Breeds included in the study were Garganica (GA), Girgentana (GI), Jonica (JO), Maltese (MA), Mediterranean Red (MR) and Saanen (SA). A general description of the 6 breeds is reported in Currò et al. (2019).

A total of 60 does (10 per breed) that kidded twins in February 2016 were enrolled in the study. Animals had similar body condition score at parturition (between 2.5 and 3.0; 1 = very thin to 5 = very fat, with 0.5 point-increment; Villaquiran et al., 2004), were from parity 1 to 5 (balanced among breeds) and their BW at the beginning of lactation averaged 48 ± 4 kg for GA, 42 ± 6 kg for GI, 47 ± 6 kg for JO, 46 ± 5 kg for MA, 48 ± 3 kg for MR and 64 ± 7 kg for SA.

Kids were kept with their dams until 40 days after birth and temporarily separated 24 h before every sampling day. Does were milked twice a day (morning and evening) in a double 24-stall herringbone low-line milk pipeline milking parlour (Alfa Laval Agri; Monza, Italy) equipped with recording jars and electronic pulsators at a vacuum of 38 kPa, 90 pulses/min and 60% pulsation ratio. The pre-milking phase included only fore stripping without any preparation of udder and teats. None of the does presented mastitis events throughout the trial.

During the study, does grazed together during the day in a natural pasture (8h/day) and were supplemented with polyphite hay *ad libitum* in the shelter, composed of 60–65% of grasses (mainly *Avena sativa L.*) and 35–40% of legumes (mainly *Vicia sativa L.*) [chemical composition: 89.10% of dry matter (DM), 15.10% of crude protein on DM, 52.60% of neutral detergent fibre on DM, and 1.10 Mcal/kg of net energy of lactation]. In addition, a commercial concentrate was offered to each doe in the milking parlour according to their requirements, considering the mean body weight and mean milk production every 15 days for each breed, following National Research Council recommendations (National Research Council, 2007). Saanen received between 0.8 and 1.3 kg/day, and local breeds received between 0.5 and 1.0 kg/day, being the greatest amount at the beginning and the lowest at the end of the lactation. Throughout the study all goats consumed the total amount of concentrated offered, and no spillage was observed. Concentrate included maize, wheat bran and flour, maize and sunflower germ flours, sugar beet molasses, soybean meal (48% crude protein), calcium carbonate, sodium chloride, sodium bicarbonate, I (5 mg/kg), Mn (50 mg/kg) and Zn (125 mg/kg). The chemical composition of the commercial concentrate was 88.20% of DM, 21.70% of crude protein on DM, 23.00% of neutral detergent fibre on DM and 1.77 Mcal/kg of net energy of lactation.

Sample Collection and Chemical Analysis Individual

Individual milk yield (kg/day) was recorded for each doe as the sum of morning and evening milkings from 2 to 30 weeks of lactation using the recording jars in the milking parlour. A total of 840 individual milk samples (50 mL each) were collected every two weeks and analysed for gross composition. Moreover, every month an additional milk sample from 42 out of the 60 goats (50 mL; n = 252; 7 goats per breed) was collected for mineral contents analysis.

Milk samples used for milk gross composition were stored at 4°C and analysed in the milk laboratory of the Breeders Association of Basilicata region (Potenza, Italy). Fat-corrected milk at 3.5% (FCM3.5%, kg/day) was calculated according to Pulina et al. (1991):

$$\text{FCM3.5\%} = \text{milk yield (kg/day)} \times (0.634 + 0.1046 \times \text{fat\%}). \quad (1)$$

Fat, protein and lactose percentages were determined using MilkoScan FT6000 (Foss Electric, Hillerød, Denmark). Somatic cell count (SCC, cells/mL) was assessed by Fossomatic FC (Foss Electric,) and transformed to somatic cell score (SCS) through the following formula (Wiggans and Shook, 1987):

$$\text{SCS} = 3 + \log_2(\text{SCC}/100\,000). \quad (2)$$

Milk samples used for minerals determination were stored at -80°C and analysed in the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy). Major (K, Ca, P, Na, S and Mg) and trace minerals (Zn, B, Sr, Ba, Fe, Al, As, Cr, Cuand Li) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES), Ciros Vision EOP (Spectro Analytical Instruments GmbH, Kleve, Germany) after mineralisation of the sample with nitric acid in closed vessels by a microwave system (Ethos 1600 Milestone S.r.l., Sorisole, Italy), following the procedures described in Manuelian et al. (2017). Instrument operating parameters (sample aspiration rate of 2

mL/min, plasma power 1350 W, coolant flow 11 L/min, auxiliary flow 0.60 L/min, nebulizer flow 0.75 L/min and integration time of 28 s) were optimized for acid solution.

Calibration standards were prepared from single element solutions (Inorganic Ventures, Christiansburg, VA, USA) in a concentration range between 0 and 100 mg/L and matched with 5% HNO3 (vol/vol) solution using 65% HNO3 Suprapur (100441, Merck, Darmstadt, Germany). The wavelengths used to determine the minerals were: 766.941 nm for K, 317.933 nm for Ca, 178.287 nm for P, 589.592 nm for Na, 182.034 nm for S, 285.213 nm for Mg, 213.856 nm for Zn, 249.677 nm for B, 407.771 for Sr, 455.404 nm for Ba, 259.941 nm for Fe, 167.078 nm for Al, 189.042 nm for As, 267.716 nm for Cr, 324.754 nm for Cu and 670.780 nm for Li. However, Al, As, Cr, Cu and Li were below the limit of detection of the instrument (0.01 µg/kg of DM) and were not further considered in this study.

Statistical Analysis

The final dataset consisted of 815 records for milk yield, FCM3.5%, SCS and gross milk composition, and 217 records for milk mineral composition. Sources of variation of milk yield, FCM3.5%, SCS, and gross and mineral composition were investigated using the MIXED procedure of SAS v9.4 (SAS Inst. Inc., Cary, NC, USA) with repeated measures, according to the following mixed linear model:

$$y_{ijk} = \mu + Breed_i + Week_j + (Breed \times Week)_{ij} + Goat_k(Breed_i) + \varepsilon_{ijk}, \quad (3)$$

where y_{ijk} is the dependent variable (milk yield, FCM3.5%, SCS, fat, protein, lactose or each mineral); µ is the overall intercept of the model; $Breed_i$ is the fixed effect of the *i*th breed (*i* = GA, GI, JO, MA, MR, SA); $Week_j$ is the fixed effect of the *j*th week of lactation (*j* = 1 to 14 for milk yield, FCM3.5%, SCS, fat, protein and lactose, corresponding to every 2-week sampling; *j* =

1 to 6 for each mineral, corresponding to every 4-week sampling); $(\text{Breed} \times \text{Week})_{ij}$ is the fixed interaction effect between breed and week of lactation; $\text{Goat}_k(\text{Breed}_i)$ is the random effect of the *k*th goat nested within the *i*th breed ~N (0, σ2 Goat(Breed)); and ε_{ijk} is the random residual ~N(0, σ2 $_\varepsilon$). In a preliminary analysis, the interactions Parity × Week of lactation and Breed × Parity were not significant and thus they were removed from the final model. Multiple comparisons of least squares means were performed for the main effects of breed and week of lactation using Tukey's test adjustment. Values are shown as least squares means ± standard error and significance was declared at $p < 0.05$, unless otherwise indicated.

RESULTS

Descriptive Statistics

Descriptive statistics for milk yield, FCM3.5%, SCS, fat, protein, lactose, major and trace minerals are reported in Table 1. Milk yield, SCS, fat, protein and lactose averaged 1.18 kg/day, 5.61 units, 3.97%, 3.36% and 4.48%, respectively. As expected, milk yield and FCM3.5% were the most variable traits [coefficient of variation (CV) = 45% and 41%, respectively] followed by SCS (CV = 34%). A lower variation was observed for fat (CV = 27%), protein (CV = 17%) and lactose (CV = 7%).

The most abundant mineral in goat milk was K (1662 mg/kg) followed by Ca (1067 mg/kg) and P (796 mg/kg; Table 1). The other major minerals had an overall concentration between 107 mg/kg (Mg) and 348 mg/kg (Na; Table 1). Among trace minerals, the most abundant was Zn (2.70 µg/g) followed by B (1.42 µg/g). The other trace minerals ranged from an overall mean of 0.31 µg/g (Fe) to 0.81 µg/g (Sr). The CV was lower for major than trace

minerals: in particular, the CV of major minerals ranged from 14% (K) to 20% (Na and Mg), and CV of trace minerals from 27% (Zn) to 91% (Ba).

Breed Effect

Breed strongly affected milk yield, FCM3.5%, fat, protein, lactose and SCS ($p < 0.01$; Table 2). Saanen had greater milk yield and SCS but lower fat content compared with the local breeds. However, in terms of FCM3.5% SA had similar milk production than JO and MA. Moreover, milk protein content of SA differed only from MA, and lactose content was similar between the SA and the GA. The order of abundance of major and trace minerals was similar in the different breeds (Table 2). Also, mineral contents slightly differed among breeds, and differences concerned only P ($p < 0.001$), Mg ($p = 0.048$) and Zn ($p < 0.001$). The greatest P content was detected for MR and JO, whose milk had on average 121 mg/kg more P than MA and GI breeds ($p < 0.001$). No differences between SA and the local breeds were observed for P. Regarding Mg content, the only significant difference was observed between SA and MA (+25 mg/kg for SA; $p < 0.05$). Mediterranean Red and JO breeds had the greatest Zn content, producing about 0.90, 0.72 and 0.69 µg/g more Zn than MA, GI and SA breeds, respectively.

Effect of Stage of Lactation

Week of lactation affected ($p < 0.001$) milk yield, FCM3.5%, fat, protein, lactose and SCS. Milk yield, FCM3.5% and lactose decreased by 67%, 63% and 11%, respectively, across lactation, whereas SCS, fat and protein increased by 60%, 44% and 37%, respectively. Week of lactation affected also major and trace minerals ($p < 0.001$; $p = 0.023$ for Zn) with the exception of B ($p = 0.83$). The lowest milk contents of K (1500 mg/kg), Ca (989 mg/kg), P (747 mg/kg), Na (304

mg/kg), S (256 mg/kg) and Mg (96 mg/kg) were observed in early lactation (4th and 8th week of lactation), which corresponded to the peak of lactation (Figure 1). In particular, the lowest K amount was detected in the 4th week of lactation (1500 mg/kg), whereas its amount increased in the 8th week of lactation (+12.9%) and remained stable until the end of lactation. Conversely to K trend, the content of Ca, P and Na was quite stable from 4th to 16th week of lactation, incremented +10% for Ca and P and 15% for Na between the 16th and late lactation (20th and 24th week of lactation). Moreover, S and Mg showed the lowest content in early lactation and increased of 15% during mid lactation (12th and 16th week of lactation) reporting the greatest content (Ca, 1156 mg/kg; P, 889 mg/kg; Na, 404 mg/kg; S, 329 mg/kg; Mg, 122 mg/kg) in late lactation (20th and 24th week of lactation). The greatest Zn content was found at the 4th week of lactation (2.94 µg/g), which differed significantly from the 8th week of lactation (2.47 µg/g). Intermediate values of Zn content were observed from the 12th to 24th week of lactation (2.64 to 2.80 µg/g). Strontium showed the greatest content in early lactation (1.00 µg/g from the 4th to 8th week of lactation), decreased by 37% until the 16th week (0.63 µg/g) and increased thereafter again until the 24th week of lactation (0.83 µg/g).

Barium showed an erratic pattern throughout lactation: the greatest values were obtained in early (4th and 8th week of lactation) and in the 20th week of lactation (0.40 µg/g), whereas the lowest were observed in mid (12th and 16th week of lactation) and in the 24th week of lactation (0.27 µg/g). Iron showed the lowest amount between the 4th and 16th week of lactation and increased in late lactation, where it reached the greatest content (0.39 µg/g).

DISCUSSION

Means and Variation of Milk Gross Composition and Mineral Content

Overall, means of milk yield and fat, protein and lactose contents were consistent with values of goat milk production and composition reported by Muehlhoff et al. (2013). The average SCS observed in our study (SCS = 5.61) was similar to that reported by Niero et al., (2018) for goat milk (SCS = 5.74). The greatest variability of milk yield was expected because the present study dealt with milk samples collected across a complete lactation and included several breeds. The variation observed for FCM3.5% (CV = 41%) was greater than the one reported by Bonanno et al. (2008) for GI (CV = 29%) when evaluating the feed effect on 37 goats during 3 months (from April to May). The variability of SCS (CV = 34%) was in agreement with Vacca et al. (2018), who reported a similar variation in individual milk samples of six goat breeds (SA, Camosciata delle Alpi, Murciano-Granadina, MA, Sarda and Sarda primitive). Also, the variability observed for fat, protein and lactose contents agreed with findings of Niero et al. (2018).

Regarding milk minerals, the greater content of K and Ca compared with other minerals in goat milk was in agreement with Kondyli et al. (2007) in local Greek goats and StrzałKowska et al. (2008) in Polish White improved goats. Nevertheless, Park and Chukwu (1988) reported greater content of P (1410 mg/L) than Ca (1389 ml/L) and K (989 mg/L) in milk of French Alpine and Anglo-Nubian goat breeds during the first 5 months of lactation. Milk of local goats of Canary Island (García et al., 2006) had greater Ca (1340 mg/kg), Na (510 mg/kg) and Mg contents (120 mg/kg) and lower K content (1240 mg/kg) compared with the present study. The variability observed for K, P and Ca in our study was similar to the CV reported by StrzałKowska et al. (2008) in Polish White improved goats. On the other hand, the variability reported by García et al. (2006) for Ca (CV = 18%) and K (CV = 16%) was similar to the CV reported in the present study, whereas they observed a greater variability for Mg (CV = 25%).

Regarding trace element, the overall Zn content was lower than Zn contents reported by García et al. (3.20 µg/g; 2006), Kondyli et al. (3.80 µg/g; 2007) and Güler (4.68 µg/g; 2007), and the B content was lower than that reported by Güler (16.9 µg/g; 2007), and Şanal et al. (8.09 µg/g; 2011) in a Turkish's local goat breed. The lower contents of B, Sr, Ba and Fe in the present study compared with Güler (16.9, 1.10, 0.99 and 3.88 µg/g, respectively; 2007) could be related to the period of lactation considered; indeed, we studied the complete lactation whereas Güler (2007) considered milk from late lactation, where milk yield decreases and milk components become more concentrated.

Breed Effect on Milk Mineral Content

The differences of milk gross composition among breeds have been previously discussed in Currò et al. (2019). Very few studies have assessed the mineral composition of goat milk and the differences among breeds. In fact, milk from Anglo-Nubian and French Alpine goats differed in Na and K content (Park and Chukwu, 1988). Trancoso et al. (2010) found breed differences for Ca, P, Mg, Na, K and Fe among several Portuguese breeds (Serrana, Serpentina, Charnequeira and Algarvia) and SA reared in different regions. Moreover, those authors observed significant differences between the two different ecotypes (Trasmontana and Ribatejana) of Serrana breed, which suggested that those differences were likely related to the feeding. On the other hand, Mestawet et al. (2012) did not observe significant variation for Ca, P, K, Mg, Na, Zn and Fe content among four Ethiopian goat genotypes (Somali, Arsi-Bale, Boer and Toggenburg×Arsi-bale crossbred). A greater content of P and Mg in milk (being part of the casein micelles) could indicate better milk coagulation capacity Malacarne et al. (2014). However, to compare milk minerals content among studies, differences in botanical species of grazing area, feeding

strategies and drinking water composition (Diana et al., 2009), and the different analytical methods used in each study should be considered because they have an impact on milk mineral contents (Lante et al., 2006).

Effect of Stage of Lactation on Milk Mineral Content

The differences of milk gross composition among week of lactation have been previously discussed in Currò et al. (2019). The effect of week of lactation on mineral profile in goat milk has been reported by other authors. Nevertheless, the pattern described in the present study for K was opposite compared with findings of Park and Chukwu (1988). Those authors observed the greatest K amount at the 4th week of lactation for Anglo-Nubian goats (1322 mg/L) and at the 8th week of lactation for French Alpine goats (1505 mg/L), with decreasing values thereafter until the 21st week of lactation (530 mg/L at and 675 mg/L, respectively). A possible reason to explain differences in the described pattern for K content between our study and Park and Chukwu (1988) could be the *ad libitum* access to a ration rich in K (1.82% of DM) until the 12th week of lactation in the latter study. On the other hand, the pattern observed for Ca, P, Na, S and Mg in the present study agreed with results described in Ethiopian goat breeds by Mestawet et al. (2012). Antunović et al. (2018) and Park and Chukwu (1988) detected the greatest Ca content at the end of the lactation in Croatian (1589 mg/kg) and Anglo-Nubian (1538 mg/kg) goat breeds, respectively.

Zinc trend reported in our study was in agreement with Antunović et al. (2018) who reported the lowest Zn content (2.40 µg/g) at the 8th week of lactation and an increment thereafter until the 21st week of lactation (3.60 µg/g) in Croatian goats. However, a different trend for Zn was reported by Kondyli et al. (2007) in Greek local goats with a grazing period

from April to July. Those authors observed the greatest Zn content at the 6th week of lactation (4.6 µg/g) and the lowest at the end of lactation (3.1 µg/g). Contrary to the pattern of Fe described in the present study, Kondyli et al. (2007) and Antunović et al. (2018) reported a stable Fe content in milk through the whole lactation in Greek and Croatian goats, respectively. In addition, StrzaŁkowska et al. (2008) observed in White Polish goats an erratic trend of milk Fe content through lactation with maximum values in the 1st, 7th and 10th weeks of lactation (1.21 µg/mL) and the lowest contents at the 24th week of lactation (1.04 µg/mL). To the best of our knowledge, this is the first study investigating Sr and Ba milk content variation through lactation and for this reason comparison with other studies dealing with this topic is not possible.

The increase of major mineral contents in milk through lactation could be the consequence of the concentration effect due to low milk yield at the end of lactation (Albenzio et al., 2016). However, the greater concentration of minerals at the end than at the beginning of lactation could also depend on extrinsic factors such as seasonal conditions (temperature and rainfall), soil and phenological state of plant as reported by Qeshlagh et al. (2016) who detected greater Ca, P and Na contents in ewe milk in summer than spring grazing season. The greatest B sources are food and drinking water (Diana et al., 2009). In this study, the B content fluctuate across lactation. The greater mineral content in late lactation could result in better milk coagulation properties than in early lactation; indeed, Malacarne et al. (2014) affirmed that milk richer in Ca, P and Mg influence positively milk coagulation properties (low rennet coagulation time and curding firming time with great curd firmness) in agreement with Vacca et al. (2018) who found that goat milk at the end of lactation showed better rennet coagulation time, shorter curd-firming time and a greater curd firmness compared with milk at the beginning of lactation.

CONCLUSIONS

The results of the present study contribute to the characterisation of milk from Italian local goat breeds with regards to mineral content and compare the milk mineral profiles of local breeds and the cosmopolitan Saanen breed. Small differences among breeds were observed for major and trace minerals in milk, being significant only for P, Mg and Zn. Although SA yielded more milk with greater SCS and lower fat concentration than the local breeds, this breed had the same P, Mg and Zn milk content than local breeds. Week of lactation affected significantly all major and trace minerals in milk (except for B), and the greatest contents for almost all the minerals were observed at the end of lactation, likely due to a concentration effect. Mineral fraction in milk is important for a technological point of view as well as for human health, thus the characterisation of the mineral profile of local goat breeds is a possible strategy to valorise the autochthonous breeds and preserve the biodiversity.

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Table 1. Descriptive statistics of goat milk yield, composition and mineral contents.

Trait	N. of observation	Mean	SD	Minimum	Maximum
Milk yield (kg/day)	815	1.18	0.53	0.20	3.25
FCM3.5% (kg/day)[1]	815	1.21	0.50	0.22	3.18
Fat (%)	815	3.97	1.06	1.90	8.81
Protein (%)	815	3.36	0.56	2.10	5.61
Lactose (%)	815	4.48	0.33	3.30	5.41
SCS (units)[2]	815	5.61	1.90	0.36	10.17
Major minerals (mg/kg)					
K	217	1662	231	1117	2278
Ca	217	1067	193	480	1593
P	217	796	127	462	1122
Na	217	348	71	241	650
S	217	293	50	150	411
Mg	217	107	22	64	169
Trace elements (µg/g)					
Zn	217	2.70	0.74	0.67	4.74
B	217	1.42	0.80	0.04	3.71
Sr	217	0.81	0.28	0.05	1.52
Ba	217	0.35	0.32	0.06	1.92
Fe	217	0.31	0.16	0.06	0.98

[1] FCM3.5% = milk yield (kg/day) × (0.634 + 0.1046 × fat%); [2] $SCS = 3 + \log_2(SCC/100{,}000)$.

Table 2. Least squares means of milk yield, composition and mineral contents of 6 goat breeds.

Traits	Breed[1]						Overall	
	GA	GI	JO	MA	MR	SA	SEM	*p*
Milk yield (kg/day)	0.99^{d}	0.93^{d}	1.25^{bc}	1.28^{b}	1.01^{cd}	1.55^{a}	0.10	***
FCM3.5% (kg/day)[2]	1.01^{b}	1.00^{b}	1.30^{a}	1.33^{a}	1.05^{b}	1.50^{a}	0.08	***
Fat (%)	3.90^{a}	4.42^{a}	4.10^{a}	4.04^{a}	4.13^{a}	3.26^{b}	0.16	***
Protein (%)	3.71^{a}	3.27^{bc}	3.36^{bc}	3.11^{c}	3.40^{b}	3.42^{ab}	0.08	***
Lactose (%)	4.26^{b}	4.47^{a}	4.53^{a}	4.49^{a}	4.58^{a}	4.28^{b}	0.04	***
SCS (units)[3]	5.90^{b}	5.05^{c}	5.54^{bc}	5.04^{c}	5.46^{bc}	6.78^{a}	0.27	***
Major minerals (mg/kg)								
K	1753	1753	1605	1587	1585	1667	32	
Ca	1073	975	1144	1041	1100	1093	24	
P	784^{ab}	756^{b}	833^{a}	716^{b}	882^{a}	817^{ab}	24	***
Na	374	332	353	340	326	359	7	
S	305	277	302	277	308	288	6	
Mg	114^{ab}	102^{ab}	107^{ab}	95^{b}	111^{ab}	120^{a}	4	*
Trace elements (µg/g)								
Zn	2.65^{ab}	2.46^{b}	3.17^{a}	2.29^{b}	3.19^{a}	2.49^{b}	0.16	***
B	1.32	1.33	1.36	1.57	1.32	1.56	0.05	
Sr	0.75	0.75	0.97	0.80	0.80	0.80	0.03	
Ba	0.25	0.35	0.42	0.24	0.36	0.40	0.03	
Fe	0.33	0.31	0.30	0.27	0.33	0.35	0.04	

[1] GA = Garganica; GI = Girgentana; JO = Jonica; MA = Maltese; MR = Mediterranean Red; SA = Saanen; [2] FCM3.5% = milk yield (kg/day) × (0.634 + 0.1046 × fat%); [3] SCS = 3 + $\log_2$(SCC/100,000); [abc] Least squares means with different superscripts within a row differ significantly ($p < 0.05$); *$p < 0.05$,**$p < 0.01$, ***$p < 0.001$.

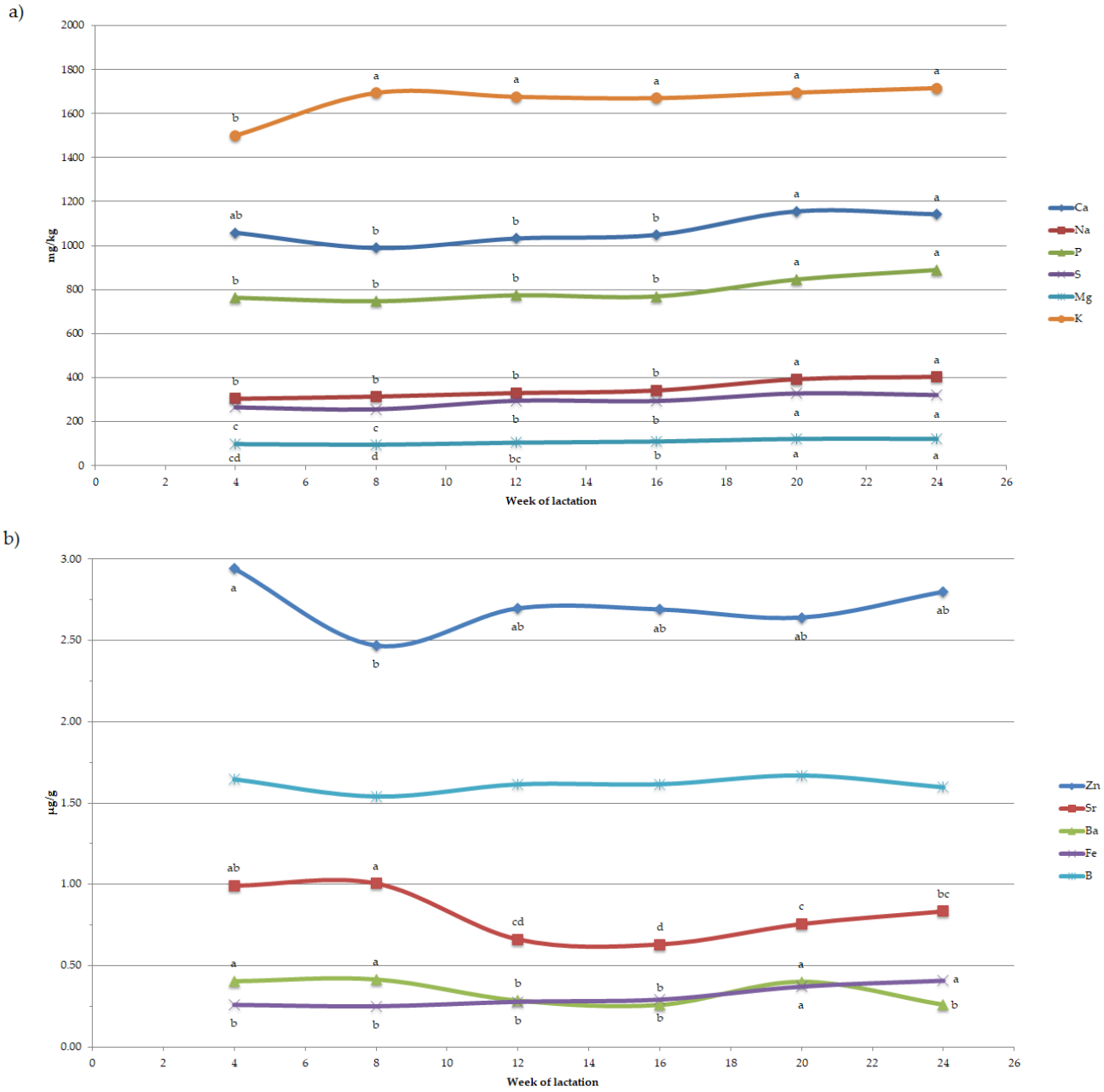

Figure 1. Least squares means of (a) major and (b) trace minerals of goat milk during lactation.

Least squares means with different superscripts within a mineral differ significantly ($p < 0.05$).

Chapter 5

Effects of Breed and Stage of Lactation on Milk Fatty Acid Composition of Italian Goat Breeds

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SIMPLE SUMMARY

Milk fatty acid composition affects human health and dairy products flavor. In particular, some saturated fatty acids increase the risk of cardiovascular diseases, whereas conjugated linoleic acid inhibits carcinogenesis and reduces atherosclerosis and diabetes. Moreover, a greater amount of some short chain fatty acids increase the goaty flavor of dairy products. The objective of this study was to evaluate the breed and week of lactation effects on milk fatty acid profile of 5 Italian local goat breeds and a cosmopolitan breed reared in the same farm. Results showed that the fatty acid profile was mainly affected by the week of lactation. Saturated fatty acids were abundant in early lactation and unsaturated fatty acids were abundant in late lactation. Local goat breeds produced milk with lower concentration of saturated fatty acids than the cosmopolitan breed. This study may contribute to valorize milk of Italian local goat breeds which seems to have a healthier profile than milk of the cosmopolitan breed.

ABSTRACT

Fatty acid (FA) profile plays an important role on human health and on sensory quality of dairy products. There is few information about breed influence on milk FA profile of local goat breeds. This study aimed to characterise and compare the milk FA profile of 5 local endangered goat breeds (Garganica, Girgentana, Jonica, Maltese and Mediterranean Red) and a cosmopolitan breed (Saanen) reared in the same farm during a complete lactation. A total of 252 milk samples were collected monthly from 42 goats (7 goats per breed) and analysed for gross composition and FA profile. Individual FA were determined using gas-chromatography. Data were analysed using a mixed model with repeated measures with breed and week of lactation as fixed effects. Results showed that the FA profile was significantly affected by week of lactation and only few FA by

breed effect ($p < 0.05$). Overall, the main differences were found between Saanen and local breeds. This study contributed to the characterisation of goat milk FA profile, and it may be of interest for the valorisation of milk from local goat breeds which seems to have a healthier profile than milk of the cosmopolitan breed.

Key words: atherogenicity index; conjugated linoleic acids; desaturation index; long chain fatty acids; local goat breeds; medium chain fatty acids; short chain fatty acids.

INTRODUCTION

From 2007 to 2017, goat milk production in Europe increased by 5% (FAOSTAT, 2019). In 2017, goat milk production represented 1.24% of the total European milk production (227 × 106 t), with France (21%), Greece (20%), and Spain (17%) being the main producers (FAOSTAT, 2019). In Europe, 95% of goat milk is transformed into cheese (Boyazoglu and Morand-Fehr, 2001). The increase of goat milk production reflects the consumer's trend towards an increased intake of goat milk and dairy products. Generally, milk shows a neutral effect on cardiovascular health, whereas fermented milk and cheese may have also a positive effect; indeed, the fermented dairy food consumption increases the intestinal microbiota, reduces the low density lipoprotein cholesterol, hypertension risk, and cardiovascular diseases (Lordan et al., 2018). Goat milk is richer in unsaturated fatty acids (UFA), short chain fatty acids (SCFA), and medium chain fatty acids (MCFA) than cow milk (Jirillo et al., 2010; Faye and Konuspayeva, 2012), and thus, it is of great interest for nutritional and beneficial aspects in human diet and health (Lordan et al., 2018). Goat dairy products consumption provides some antithrombotic effects and a potential reduction in platelet aggregation (Lordan et al., 2018). Moreover, it is easier to digest due to the smaller fat globule dimension (3.19 to 3.50 μm) (Park, 1994; Jirillo et al., 2010; Faye and Konuspayeva,

2012) and it is less allergenic because of the lower amount of αS1 casein than cow milk (Park, 1994).

Fatty acids (FA) play an important role in human health (Calder, 2015; Manuelian et al., 2017). It has been reported that polyunsaturated FA (PUFA) protect against heart attack risk, improve brain function, and reduce risk of dementia (Calder, 2015), and conjugated linoleic acids (CLA) inhibit the carcinogenesis and reduce the risk of atherosclerosis and diabetes (Poppitt et al., 2002; Walther et al., 2008). Recently, the negative role attributed to saturated fatty acids on human health was re-examined (Lordan et al., 2018); indeed, not all individual SFA have a negative impact on human health. In particular, SCFA and MCFA (composed mainly of SFA) are a source of direct energy available for the enterocytes, whereas long chain fatty acids (LCFA) are stored in adipose tissues (Van Schalkwijk et al., 2014; Schönfeld and Wojtczak, 2016). About 60% of milk FA derive from the mobilization of adipose tissue or from feed intake, and the remaining 40% are synthesized de novo by the mammary gland using the acetate and β-hydroxybutyrate from the rumen as substrates (Chilliard et al., 2000; Palmquist, 2009; Benedet et al., 2019). De novo FA are from C4:0 to C14:0, 50% of C16:0 and all odd FA, whereas 50% of C16:0 and all FA from C18:0 onwards derive from the arterial blood (Chilliard et al., 2000). Moreover, the mammary gland shows an important Δ-9 desaturase activity which adds a double bond in the cis Δ-9 position converting C14:0 into C14:1, C16:0 into C16:1, and C18:0 into C18:1 and C18:2 (Chilliard et al., 2000).

Milk FA composition is mainly affected by feeding (Griinari et al., 1998; Gama et al., 2008); however it is difficult to evaluate the effect of feeding and stage of lactation separately (Nantapo et al., 2014), being feeding closely connected to the season and lactation stage (Solaiman, 2010). Overall, diets richer in concentrate than forage cause the reduction of the ruminal pH and the activity of cellulolytic bacteria; this leads to the increase of trans-10, cis-12 CLA synthesis in

milk which could induce milk fat depression syndrome (Griinari et al., 1998; Gama et al., 2008). Milk FA profile is affected also by breed and lactation stage. For example, Strzalkowska et al. (2009) reported differences of FA profile in early, mid, and late lactation of Polish White Improved goats. Yurchenko et al. (2018) demonstrated that milk from Saanen and Landrance goat breeds fed the same ration differed from C4:0 to C14:0, C16:0, C16:1, and C18:1. However, the existing literature about FA profile characterization is limited to a restricted number of breeds and to our knowledge few studies have investigated the milk FA composition of older, endangered goat breeds.

Although the endemic breeds are well adapted to the environmental conditions showing a greater rusticity and resistance to diseases than cosmopolitan breeds (Di Trana et al., 2015; Malhado et al., 2009), they have undergone a massive substitution with more yielding breeds (Benjelloun et al., 2015). Among European countries, Italy has the greatest number of local goat breeds (55), from which 61% are at risk of extinction, 10% are not at risk, and the risk status of 29% of the breeds is unknown (DAD-IS, 2019). To maintain the variability among breeds it is important to ensure conservation of livestock genetic resources as part of socio-cultural and livestock heritage of the country (Gandini and Villa, 2003; Malhado et al., 2009). Recent studies have investigated milk (Albenzio et al., 2006; Tufarelli et al., 2009; Currò et al., 2019a) and dairy products (Pizzillo et al., 2004; Albenzio et al., 2006) gross composition and characterized the casein haplotypes (Sacchi et al., 2005) and minerals (Currò et al., 2019b) on some Italian local breeds. The aim of the present study was to characterize and compare milk FA composition of 5 local endangered goat breeds of South Italy (Garganica, Girgentana, Jonica, Maltese, and Mediterranean Red) and a cosmopolitan dairy specialized goat breed (Saanen) during a complete lactation under the same farm conditions.

MATERIALS AND METHODS

Animals and Management Conditions

The study was conducted from March to August 2016 in the experimental farm of the Council for Agricultural Research and Agricultural Economy Analysis (CREA, Potenza, Italy). Experimental procedures and animal care conditions followed the recommendations of European Union directive 86/609/EEC. A general description of the breeds used in the study can be retrieved from Currò et al. (2019a). Forty-two dairy goats of 6 breeds (7 does per breed; Garganica, GA; Girgentana, GI; Jonica, JO; Maltese, MA; Mediterranean Red, MR; and Saanen, SA) of parity order from 1 to 5, similar body condition score (between 2.5 and 3.0; 1 = very thin to 5 = very fat, with 0.5 point-increment (Villaquiran et al., 2005)) and body weight at the beginning of lactation of 47 ± 3 kg for GA, 44 ± 5 kg for GI, 45 ± 5 kg for JO, 45 ± 5 kg for MA, 47 ± 3 kg for MR, and 61 ± 6 kg for SA were enrolled in the study. All does were under the same managerial conditions and kidded twins in February. Kids were kept with their mothers until 40 days of age but, during that period, dams were separated from their kids 24 h prior to sampling. Does were milked twice a day (morning and evening) in a double 24-stall herringbone low-line milk pipeline milking parlor (Alfa Laval Agri; Monza, Italy) equipped with recording jars and electronic pulsators at a vacuum of 38 kPa, 90 pulses/min, and 60% pulsation ratio. The pre-milking phase consisted of forestripping only, without any preparation of udder and teats.

During the whole study, does grazed together 8 h/day in a natural pasture and were supplemented *ad libitum* with polyphite hay (60%–65% of grasses, mainly *Avena sativa L*. and 35–40% of legumes, mainly *Vicia sativa L*.) in the shelter. Moreover, goats received a commercial concentrate in the milking parlor composed of maize, wheat bran and flour, maize flour, sunflower germ flour, sugar beet molasses, soybean meal (48% crude protein), calcium

carbonate, sodium chloride, sodium bicarbonate, I (5 mg/kg), Mn (50 mg/kg), and Zn (125 mg/kg). The chemical composition and nutritional value of the hay and the concentrate are reported in Table 1. The amount of concentrate administered to the animals was adjusted every 15 days throughout lactation considering mean body weight and mean milk production per breed according to NRC (National Research Council, 2007) requirements. During the whole study, the amount of concentrate administration ranged from 0.6 to 1.0 kg/day for local breeds and 0.9 to 1.3 kg/day for SA breed, being the greatest amount at the beginning and the lowest at the end of lactation.

Sample Collection and Chemical Analysis

From 4 to 24 weeks of lactation, individual milk yield (kg/day) was recorded using the recording jars in the milking parlor as the sum of morning and evening milkings. In addition, individual milk samples (50 mL; n = 252) were collected and divided in two aliquots. One aliquot was stored at 4 °C and transferred to the milk laboratory of the Breeders Association of Basilicata region (Potenza, Italy) for the determination of fat, protein, and lactose percentages using MilkoScan FT6000 (Foss Electric, Hillerød, Denmark). Fat-corrected milk at 3.5% (FCM 3.5%) was estimated according to Pulina et al. (1991):

$$\text{FCM 3.5\%} = \text{milk yield} \times (0.634 + 0.1046 \times \text{fat\%}) \quad (1).$$

Somatic cell count (SCC; cells/mL) was determined using Fossomatic FC (Foss Electric, Hillerød, Denmark) and transformed to somatic cell score (SCS) through the formula (Wiggans and Shook, 1987):

$$SCS = 3 + \log_2(SCC/100{,}000) \quad (2).$$

The second aliquot was stored at −80 °C and transferred to the laboratory of the Department of

Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Italy) for the FA profile analysis. Milk fat was extracted from a milk subsample (5 mL) following the accelerated solvent extraction method by ASE 200 (Dionex Corp., Sunnyvale, CA, USA). Samples were put in 22 mL stainless steel extraction cells for fat extraction using hexane:isopropanol (2:1) as solvent. After the solvent evaporation, 40 mg of fat was transferred into tubes for the FA transesterification and methylation following an internal method adapted from Christie (1993). Transesterification and methylation processes were performed adding 2 mL of normal hexane and 100 μL of sodium methylate 1 M in the tube in which were contained 40 mg of fat. After 20 min of reaction time in a stirrer, 150 μL of oxalic acid in ethyl ether and 4 mL of sodium sulphate (0.47 M) were added in the tube. Successively, fatty acids methyl esters solution was centrifuged at 693 × g for 10 min at 10 °C. The FA gas-chromatographic analysis was performed using an Agilent 7820A GC System (Agilent Technologies, Santa Clara, CA, USA) equipped with an automatic sampler G4567A (Agilent Technologies) and flame-ionization detector. An Omegawax capillary GC column (24,136 Supelco; Sigma-Aldrich, Castle Hill, Australia), with a long of 30 m, inner diameter of 0.25 mm, and film thickness 0.25 μm, was used. Hydrogen was used as a carrier gas at a constant flow rate at 100 °C with an average speed of 30 cm/s. A split injection sleeve was used. The injector and detector temperature was set at 250 °C. The initial temperature of oven was at 50 °C for 2 min, and then increased from 4 °C/min to 220 °C and held for 18 min. Each individual FA was identified by comparing its retention time with that of a standard FA (FAME mix C4-C24 #18919-1AMP and octadecadienoic acid conjugated methyl ester; Supelco, Sigma-Aldrich). Individual FAs were calculated using GC/MSD ChemStation Software (Agilent Technologies) and expressed as percentage of total identified FA.

Identified individual FA were grouped in the following relevant FA groups: C14:0, which included C14:0 and C14:0 iso form; C15:0, which included C15:0, and C15:0 iso and anteiso forms; C16:0, which included C16:0 and C16:0 iso form; C17:0, which included C17:0, and C17:0 iso and anteiso forms; C18:0, which included C18:0 and C18:0 iso form; C18:1 which included C18:1n7 and C18:1n9; C18:2n6 which included C18:2n6 and C18:2n6 trans; n3, which included C18:3n3, C18:4n3, and C20:5n3; n6, which included C18:2n6, C18:2n6 trans, C18:3n6, C20:2n6, C20:3n6, C20:4n6, and C22:2n6; CLA, which included geometric isomers of C18:2n6; SFA, which included C4:0, C6:0, C7:0, C8:0, C10:0, C11:0, C12:0, C13:0, and C13:0 iso form, C14:0 and C14:0 iso form, C15:0, and C15:0 iso and anteiso forms, C16:0 and C16:0 iso form, C17:0, and C17:0 iso and anteiso forms, C18:0, and C18:0 iso and anteiso forms, C19:0, C20:0, C21:0, C22:0, C23:0, and C24:0; monounsaturated FA (MUFA), which included C12:1, C14:1n7, C15:1, C16:1n9, C16:1n7, C16:1, C17:1n7, C18:1n9, C18:1n7 C20:1n9, and C22:1n9; PUFA, which included C18:2n6, C18:2n6 trans, C18:2 (and isomers), C18:3n6, C18:3n3, C18:4n3, C20:2n6, C20:3n6, C20:4n6, C20:5n3, and C22:2n6; UFA, which was the sum of MUFA and PUFA; CLA, which included geometric isomers of C18:2n6; SCFA, which included C4:0, C6:0, C8:0, and C10:0; MCFA, which included C11:0, C12:0, C12:1cis, C12:1trans, C13:0, and C13:0 iso form, C14:0 and C14:0 iso form, C14:1n7, C15:0, and C15:0 iso and anteiso forms, C15:1trans, C16:0, and C16:0 iso form, C16:1n7, C16:1n9, and C16:1 trans; LCFA, which included C17:0, and C17:0 iso and anteiso forms, C17:1, C18:0, and C18:0 iso form, C18:1n9, C18:1n7cis, C18:2n6, C18:2n6 trans, C18:3n6, C19:0, C18:3n3, C18:4n3, C20:2n6, C20:3n6, C20:4n6, C20:5n3, and C22:2n6; desaturation index of C16:0 (DI C16:0), calculated as (C16:1n7 + C16:1n9 + C16:1trans)/(C16:0 + C16:0:iso + C16:1n7 + C16:1n9 + C16:1t) × 100; desaturation index of C18:0 (DI C18:0), calculated as (C18:1n9 + C18:1n7cis)/(C18:0 + C18:0:iso + C18:1n9 + C18:1n7cis) × 100; atherogenicity index (AI),

calculated as (C12:0 × (4 × C14:0) + C16:0)/UFA; elongation index (EI), calculated as (C8:0 + C10:0 + C12:0 + C14:0)/(C4:0 + C6:0); and thrombogenic index (TI), calculated as (C14:0 + C16:0 + C18:0)/[(0.5MUFA) + (0.5 n6) + (3 × n3) + (n3/n6)].

Statistical Analysis

According to fat to protein ratio (F/P), which is considered an indicator of metabolic status of goats (Čejna and Chládek, 2005; Paura et al., 2012), milk samples with F/P < 0.94 were discarded prior to statistical analysis. Normal distribution of the residuals for each trait was assessed. The complete record from samples presenting outliers for major FA and groups of FA were deleted, whereas outliers for minor FA were treated as missing values. Sources of variation of milk yield, FCM 3.5%, SCS, milk composition and individual, groups, and indices of FA were investigated using the MIXED procedure of SAS v9.4 (SAS Inst. Inc., Cary, NC, USA) with repeated measures according to the following mixed linear model:

$$y_{ijk} = \mu + Breed_i + Week_j + (Breed \times Week)_{ij} + Goat_k(Breed_i) + \varepsilon_{ijk} \qquad (3)$$

where y_{ijk} is the dependent variable (milk yield, FCM 3.5%, SCS, fat percentage, protein percentage, lactose percentage or each individual, group, or index of FA); μ is the overall intercept of the model; $Breed_i$ is the fixed effect of the *i*th breed (*i* = GA, GI, JO, MA, MR, SA); $Week_j$ is the fixed effect of the *j*th week of lactation (*j* = 1 to 6, corresponding to every 4-week sampling); $(Breed \times Week)_{ij}$ is the fixed interaction effect between breed and week of lactation; $Goat_k(Breed_i)$ is the random effect of the *k*th goat nested within the *i*th breed ~$N(0, \sigma^2_{Goat\ (Breed)})$; and ε_{ijk} is the random residual ~$N(0, \sigma^2\varepsilon)$. In a preliminary analysis, the interactions Parity ×

Week of lactation and Breed × Parity were not significant and thus they were removed from the final model. Multiple comparisons of least squares means (LSM) were performed for the main effects of breed and week of lactation using Tukey's test adjustment. Significance was set at $p < 0.05$.

RESULTS

Breed Effect

The analysis of variance indicated that breed affected ($p < 0.05$) all studied traits except for fat percentage (Table 2). The greatest milk yield (1.73 kg/day) and FCM 3.5% (1.67 kg/day) were observed for SA, and the lowest for GI and MR breeds; in particular, GI and MR produced 0.67 and 0.60 kg/day less milk than SA, respectively ($p < 0.05$). Milk of GA had greater protein percentage than milk of GI and MA (+0.50%; $p < 0.05$). The greatest F/P was calculated for GI (1.37) and the lowest for GA (1.15) and SA (1.14). In terms of lactose percentage, GA and SA exhibited lower values than JO, MA, and MR ($p < 0.05$). Finally, SA had greater SCS (6.79 units) than GI, MA, and MR ($p < 0.05$; Table 2).

The LSM of individual, groups, and indices of milk FA profile for the different breeds are reported in Table 3. Breed affected C4:0, C14:0, C15:0, C15:0 iso and anteiso forms, C16:0, C16:1, C17:0, C17:0 iso and anteiso forms, C18:0, DI C16:0, and AI ($p < 0.05$) and the main differences were detected between SA and local breeds. Specifically, milk of SA had 0.28 g/100 g FAs more C4:0 than milk of GA; 1.56 and 3.37 g/100 g FAs more C14:0 and C16:0, respectively, and 0.50 greater AI than milk of GI; and 3.71 g/100 g FAs more C16:0 than milk of JO ($p < 0.05$). On the other hand, milk of SA had 0.28, 0.30, and 3.75 g/100 g FAs less C15:0, C17:0, and C18:0 than milk of GI, respectively; 0.23 g/100 g FAs less C17:0 than milk of JO;

and 0.27, 0.28, and 2.44 g/100 g FAs less C15:0, C17:0, and C18:0 than milk of MA, respectively ($p < 0.05$). In addition, SA milk showed lower contents of iso and anteiso forms for C15:0 and C17:0 than local breeds. In detail, milk of SA had less contents of C15:0 and C17:0 iso forms than local breeds (-0.11 and -0.09 g/100 g of FAs, respectively; $p < 0.01$). Whereas, according to C15:0 and C17:0 anteiso forms SA milk produced 0.12 and 0.51 g/100 g of FAs than JO and GI, JO and Ma breed, respectively ($p < 0.05$). Among local breeds, very few differences were detected in FA profile; MR produced 0.30 and 0.27 g/100 g FAs less C16:1 than milk of GA and GI, respectively ($p < 0.05$), and 1.27, 1.36, and 1.11 lower DI C16:0 than milk of GA, GI, and JO, respectively ($p < 0.05$; Table 3).

Effect of Stage of Lactation

Variations of milk yield, and fat, protein, and lactose percentages throughout lactation are depicted in Figure 1. The greatest daily milk production was obtained in the 4th week of lactation (peak of lactation) followed by a decrease of 0.39 kg (22%) until the 8th week of lactation ($p < 0.001$). An overall milk yield reduction of 0.80 kg (46%) was observed between the first and the last sampling week ($p < 0.001$). Protein and fat percentages showed a similar pattern throughout lactation. The lowest values of fat (3.46%) and protein (2.86%) were observed at the peak of lactation, whereas the greatest values were detected at the end of lactation (4.53% and 3.34%, respectively; $p < 0.001$). Regarding lactose percentage, the greatest (4.83%) and lowest (4.22%) values were obtained at the peak and the end of lactation, respectively. Fat to protein ratio ranged from 1.18 (4th week of lactation) to 1.34 (24th week of lactation; $p < 0.05$) and SCS was quite stable between the 4th (5.44 units) and 20th (5.37 units) week of lactation and increased to 6.34 units at the end of lactation (data not shown).

Week of lactation affected all individual, groups, and indices of FA ($p < 0.001$; Table 4). However, several fluctuations were observed throughout lactation. In particular, the greatest values of C8:0, C10:0, C12:0, C14:0, SFA, SCFA, MCFA, SFA/UFA, AI, EI, and TI were observed in the 4th, 8th, and 20th week of lactation, which differed significantly from the lowest contents in the 16th and 24th week of lactation ($p < 0.001$). In particular, the difference between the maximum and the minimum value ranged from 23% to 27% for C8:0, C14:0, SCFA, EI, and TI, from 33% to 36% for C10:0, C12:0, and SFA/UFA, and it was 8%, 15%, and 44% for SFA, MCFA, and AI, respectively. Moreover, C14:0 and MCFA contents in the 4th, 8th, and 20th week of lactation differed significantly from the content in the 12th week of lactation (Table 4). The odd FA (C15:0 and C17:0) showed the greatest amount in the 8th week of lactation and the lowest in the 12th, 20th, and 24th week of lactation; moreover, for C15:0, also the 4th week of lactation showed the lowest content. The difference between the maximum and minimum value for C15:0 and C17:0 was 31% and 24%, respectively. A peculiar pattern was detected for the n6 to n3 ratio (n6/n3) and LCFA; specifically, n6/n3 increased by 22% from 4th to 8th week of lactation, decreased by 37% from 8th to 12th week of lactation, increased by 25% from 12th to 16th week of lactation, and remained quite stable thereafter. The LCFA increased by 20% between 4th and 16th week of lactation, decreased by 16% between 16th and 20th week of lactation and increased by 15% between 20th and 24th week of lactation ($p < 0.001$).

Regarding C18:1, n3, n6, CLA, UFA, MUFA, PUFA, and DI C16:0, they were generally lower in early than late lactation ($p < 0.001$). In particular, C18:1, UFA, and MUFA contents were quite stable from 4th to 12th week of lactation, they increased by 14%, 10%, and 14% from 12th to 16th week of lactation, respectively ($p < 0.001$), decreased by similar percentages from 16th to 20th week of lactation, and peaked in the 24th week of lactation. The CLA content increased by 17% between the 12th and 16th week of lactation and peaked in the 24th week of

lactation. The PUFA and n6 changed moderately between 4th and 16th week of lactation, and they increased by 20% and 21% ($p < 0.001$) from 16th to 24th week of lactation, respectively. The lowest and greatest n3 content was in the 8th and 12th week of lactation, respectively, with a difference of 57% ($p < 0.001$). The DI C18:0 was lower in the 12th and 16th compared with other sampling weeks ($p < 0.001$), and the greatest values were in the 20th and 24th weeks of lactation. The C18:2 fluctuated through the whole lactation, with the lowest and greatest content in the 16th and 24th week of lactation, respectively.

Different patterns to the previous ones were observed for C4:0, C6:0, C16:0, and C18:0. Specifically, C4:0 and C6:0 showed the greatest contents in the 12th week of lactation; nevertheless, they did not differ significantly from the contents in the 20th and 24th week of lactation in the case of C4:0, and from the content in the 4th week of lactation for C6:0. The lowest C4:0 content was obtained in the 8th week of lactation, even if it did not differ from the content in the 4th and 16th week of lactation, and the lowest C6:0 value was observed in the 24th week of lactation, but it did not differ from the content in the 8th, 16th, and 20th week of lactation. The C16:0 decreased from 4th to 16th week of lactation, increased from 16th to 20th week of lactation and decreased again thereafter. Regarding C18:0, this FA exhibited an opposite pattern to that of C16:0 throughout the lactation; in particular, its content increased from 4th to 20th week of lactation, decreased from 16th to 20th week of lactation, and increased again thereafter.

DISCUSSION

Breed Effect

Only few studies have investigated the effect of goat breed on milk FA composition. Moreover, those studies have often dealt with milk fat composition of cosmopolitan breeds, likely because of their major economic interest related to greater milk yield compared with local breeds. Therefore, information on milk composition of native goat breeds is scarce, especially with regard to FA profile. However, many studies found that impact of breed effect on milk FA is lower than of the one of the diet (Roca Fernandez and Gonzalez Rodriguez, 2012; Nantapo et al., 2014). Therefore, breed effect may be considered as the outcome of the adaptation of species to the environment condition (climate, feeding, and water resource) that affect milk yield and composition (Claps et al., 2018).

In the present study, as expected, the greatest milk yield was observed in the cosmopolitan breed, who is the most specialized cosmopolitan dairy breed; however, milk yield of GA, JO, and MA was similar to the one of SA breeds. Moreover, similitudes among those breeds was maintained also when milk production is standardized at 3.5% in title of fat. Fat, protein, and lactose content are in line with those reported by FAO (2013), in goat milk. Fat content among breeds did not differ significantly; however, it is worth mentioning that milk of local breeds had greater fat content than milk of the cosmopolitan breed. Among local breeds, GA milk was the richest one in protein content and differed only to GI and MA breed, whereas SA breed was similar to all local breeds. Those results partially agreed with Tripaldi et al. (1998) who reported lower protein content in SA milk than other local breeds and no difference among GA, MA, and MR breeds. The discrepancies to Tripaldi et al. (1998) are probably due to different management conditions as local and SA breed were reared. The greater lactose content was similar among JO, MA, and MR milk breeds, whereas the lowest one was observed in GA and in SA breed. Furthermore, breeds with greatest lactose content showed lowest SCS in agreement to Sung et al. (1999). Generally, a low lactose content is usually linked to mastitis occurrence (Raynal-Ljutovac et al.,

2007). However, in the present study, the most productive goat breeds (GA, JO, MA, and SA) showed the greatest SCS. However, SCS in goat milk cannot be considered as an indicator of mastitis as in cow milk (Barrón-Bravo et al., 2013), due to different physiological milk secretion of the two species. In fact, milk secretion is of merocrine type in cow and apocrine type in goat (Jiménez-Granado et al., 2014). The high somatic cells in goat milk is due to the great amount of cytoplasmic particles or epithelial cells passed to milk through the apocrine secretion process (Jiménez-Granado et al., 2014). Fat and protein percentages of SA milk were similar to those of local breeds; this agreed with results of a study that compared SA and Portuguese local goat breeds (Trancoso et al., 2010). The F/P for MR was similar to the one reported by Pizzillo et al. (2004) for the same breed (1.23). However, those authors (Pizzillo et al., 2004) reported a lower F/P for GI (1.13) and MA (1.12) breeds. These discrepancies are related to the different fat and protein content in milk considered in Pizzillo et al. (2004) study.

Overall, differences of FA composition were observed between SA and the local breeds. Milk from SA showed greater C4:0, C14:0, C16:0, and AI, and lower C15:0, C15:0 iso and anteiso forms, C17:0, C17:0 iso and anteiso forms, and C18:0 contents than the local breeds. However, the greatest differences among breeds were detected for C18:0, C14:0, and C16:0. Saanen milk was poorer in C15:0, C17:0, and C18:0 and richer in C14:0 and C16:0 compared with GI and MA milk. The odd FA (C15:0 and C17:0) are considered as biomarkers of rumen activity, being ruminal bacteria population affected by diet. Stress stimuli of rumen due to the prevalence of concentrate in the diet could be the cause of an increment of anteiso and linears form of odd FA content in milk (Vlaeminck et al., 2006). Thus, the lower levels of linears and anteiso forms of C15:0 and C17:0 in SA than local breeds suggest that odd FA contents were affected by breed as reported in Hanus et al. (2018) and Bainbridge et al. (2016) studies on cow breeds. The minor

content of odd FA in SA milk might be related to the low adaptability of the cosmopolitan breed to the environmental condition.

The lower content of C18:0 in SA might be due to the greater desaturation activity of C18:0 ($p = 0.07$) in SA mammary gland as the result of the maintenance of balanced condition between saturated and unsaturated forms for milk fluidity control (Gama et al., 2008). The greater AI of SA than GI suggested that milk from the local breed has lower atherogenic impact on human health (Poppitt et al., 2002). In addition, a greater amount of odd FA detected in local breeds than in SA suggested that the consumption of milk from native breeds might help reduce the risk of multiple sclerosis by improving the flexibility of the plasmatic membrane (Jenkins et al., 2015). Yurchenko et al. (2018) indicated that the FA profile differed between SA and Swedish Landrace goat breeds for C4:0 to C16:0, C16:1, C18:1, DI C16:0, and AI. The differences between SA and GA for C4:0 and AI in the present study (0.28 g/100 g FAs and 0.24, respectively) were similar to those between SA and Swedish Landrace breeds in the study of Yurchenko et al. (2018; 0.24 g/100 g FAs and 0.30, respectively). On the contrary, we reported greater C16:0 content in milk of SA than in milk of GI and JO local breeds, whereas Yurchenko et al. (2018) reported lower C16:0 content in the cosmopolitan than in the Swedish Landrace native goat breeds. Finally, n6, n6/n3, and DI C18:0 were not affected by breed in Yurchenko et al. (2018), which is in agreement with our study.

Among local breeds, very few differences were detected in the FA profile. For example, milk from MR had less C16:1 than milk of GA and GI. Moreover, MR had lower DI C16:0 compared with GI, GA, and JO; this suggests that mammary gland Δ-9 desaturase activity of MR was weaker than that of GA and GI. Pizzillo et al. (2004) found significant differences among ricotta cheese from GI, MA, and MR in terms of C10:0, C15:0, C16:0, C18:2, SFA, and PUFA. In particular, ricotta cheese from GI milk had greater concentration of C15:0, C18:2, and PUFA, but

poorer C10:0 content than milk of MA and C16:0 content than MR and MA milk (Pizzillo et al., 2004). Talpur et al. (2009) studied the effect of Kamori and Pateri breeds (Pakistan local goat breeds) reared in the same farm and under the same feeding conditions on milk FA profile; those authors observed differences between those two breeds in terms of C6:0 to C18:0, C16:1, C18:1, SFA, MUFA, and CLA, whereas they did not report differences in terms of C4:0, C17:0, and PUFA.

Effect of Stage of Lactation

The pattern of milk yield and gross composition through lactation are in agreement with several studies on goat milk (Strzalkowska et al., 2009; Currò et al., 2019a). Milk yield decreased through lactation due to the reduction of efficiency in the mammary gland synthesis (Albenzio et al., 2016) and the deterioration of the quality of the pasture during summer season (Sitzia et al., 2015). The lowest concentration of milk protein and fat in the 4th week of lactation, and the greatest at the end of lactation have been already associated to a dilution and a concentration effect, respectively (Goetsch et al., 2011). Moreover, Kawas et al. (1991) demonstrated that a greater forage to concentrate ratio in the ration affects milk fat, i.e., greater milk fat was observed when the ration included 75% instead of 45% of forage. The average F/P and its range were in agreement with other studies on goat milk (Pizzillo et al., 2004; Desnoyers et al., 2008). The average F/P, which is an indicator of the metabolic status of the animal, was quite constant during lactation, suggesting the absence of metabolic disorders during the study (Vlček et al., 2016).

The effect of week of lactation on goat milk FA composition has been reported by several authors; however, the pattern described for FA across lactation is controversial (Craninx et al., 2008). In the present study, week of lactation highly affected all individual, groups, and indices

of FA, similarly to Kuchtik et al. (2015), who studied the FA profile in milk of Brown Short-haired goat breed from 62 to 258 days in milk (from April to October). On the other hand, StrzałKowska et al. (2009) did not observe an effect of stage of lactation on C4:0, C14:0, C16:0, C17:0, and MCFA. The greatest content of C8:0 to C16:0, and consequently of total SFA, in early than late lactation could be related to greater administration of supplementary concentrate in early lactation, which is responsible for an increase of de novo FA synthesis (Nantapo et al., 2014). The greater SCFA content in early lactation suggested a positive energy balance, whereas the low amount of SCFA in late lactation was probably due to the greater content of LCFA, which have an important inhibitory effect on the synthesis of SCFA (Chilliard et al., 2000). The greater content of LCFA in late lactation could be a consequence of (i) lower amount of concentrates administered compared with early lactation, (ii) mobilization of LCFA from adipose tissues (mainly C16:0 and C18:0) due to negative energy balance, or (iii) a combination of the two previous conditions (Chilliard et al., 2000). Moreover, in late lactation, due to lower energy requirements for milk production, the LCFA with long carbon chain undergo an hydrogenation process in the mammary gland (Tsiplakou and Zervas, 2008). In fact, in the present study, the greater value of DI C16:0 and DI C18:0 in late lactation suggests that Δ-9 desaturase activity of mammary gland was higher at the end of lactation.

The C4:0 and C6:0 showed different patterns to other SCFA, which could be explained by the way they are synthesized. Milk C4:0 derives from malonyl-CoA (by condensation of acetyl units) but it is also uptaken by the blood as preformed C4:0 (Palmquist et al., 1993). Milk C6:0 derives from the addition of only one unit of acetyl via malonyl-CoA, thus, it is less influenced by acetyl-CoA availability (Palmquist et al., 1993). The odd FA amount oscillated through weeks of lactation, which is probably related to the amount of bacteria that leave the rumen being affect by the feeding ratio. A correct feeding regime based on animal requirements promotes the bacterial

(containing odd FA in the lipid membrane) numerical growth in the rumen. In fact, a consequence of excessive bacterial growth causes a greater bacteria escaping from the rumen which increases the content of those FA in milk (Craninx et al., 2008). The greater content of n3, n6, CLA, MUFA, and PUFA in late lactation could be due to the greater intake of pasture than in early lactation where the forage to concentrate ratio was lower. Although n6/n3 fluctuated through lactation, it was usually maintained in the recommended ratio (4:1) for the prevention of cardiovascular diseases (Cossignani et al., 2015). The greatest SFA/UFA in early lactation and in the 12th and 20th week of lactation is supported by the lower activity of desaturation of mammary gland detected in the same weeks of lactation. The AI and TI are considered as nutritional indices of lipids and their estimation allows to evaluate the effect of each food on the risk of developing coronary heart disease; in detail, the AI refers to the FA aggregation forming a plaque in the arteries, whereas TI refers to the tendency to form clots in the blood vessels (Ghaeni and Ghahfarokhi, 2015). In general, milk richer in UFA shows a lower AI and TI suggesting that milk could be less harmful to human health. In this study, the greater forage intake in mid and late than early lactation reduced the AI and TI improving the milk FA quality (Ulbricht and Southgate, 1991).

Some discrepancies were detected comparing results of the present study with findings of previous research (Strzalkowska et al., 2009; Kuchtik et al., 2015). In detail, discrepancies to Kuchtík et al. (2015) could be related to the different feeding strategy used in their study; indeed, feed ratio included besides pasture, meadow hay, and mineral lick *ad libitum*, an administration of organic rolled oats and organic feed mixture (0.5 kg/day per doe, respectively). On the other hand, similitudes to StrzaŁkowska et al. (2009) could be related to the feeding strategies formulated according to animal requirements similarly to the present research. However in the StrzaŁkowska et al. (2009) study, besides the concentrate, from October to May (Strzalkowska et

al., 2009), the feeding ratio was integrated with corn silage, hay, carrot, whereas from June to September hay was substituted with fresh grass. In detail, the C4:0 pattern in our study differed to that reported by Kuchtík et al. (2015), who detected the greatest and lowest amount in early and late lactation, respectively. However, the amounts of C4:0 in our study were greater than those observed by Kuchtík et al. (2015; 1.43 g/100 g FAs) and StrzałKowska et al. (2009; 1.27 g/100 FAs) across lactation. Moreover, the pattern of C6:0 to C10:0 and CLA through lactation resembled that of Kuchtík et al. (2015) and StrzałKowska et al. (2009). The C6:0 to C10:0 are responsible for goat flavor of dairy products (Salari et al., 2016; Clark and Garcia, 2017) and thus the greater content of C6:0 to C10:0 in milk in the first than the second half of lactation might be responsible for stronger goaty flavor in dairy products derived from milk collected in early lactation (Fekadu et al., 2005). In addition, the trend for C16:0 and C16:1 in the present study was different than that reported by Kuchtík et al. (2015). In particular, Kuchtík et al. (2015) found a stable content until the 13th week of lactation, followed by an increment in the 18th week of lactation and a decrease in the 23rd week of lactation. However, considering the whole lactation period (62 to 258 days in milk), Kuchtík et al. (2015) observed the greatest content of C16:0 and C16:1 in late lactation, as reported in our study; this similarity could suggesting the effect of stage of herbage maturity on milk FA. On the other hand, the pattern of C16:1 observed in our study was similar to that of StrzałKowska et al. (2009), who found a lower amount in early than late lactation probably due to the inclusion of fresh grass from June to September. However, Kuchtík et al. (2015) found a different trend than that reported in the current study for C12:0 and C14:0; those authors detected the lowest values between 9th and 13th and in the 23rd week of lactation, and the greatest content in the 18th week of lactation. Considering the odd FA, C15:0 pattern differed to that of Kuchtík et al. (2015), who found a lower content in the 9th week of lactation and greater content between 13th and 18th week of lactation, and reporting the lowest

content in the 23rd week of lactation. The C18:0 and C18:1 patterns differed to those of Kuchtík et al. (2015), who observed greater amount in the 9th, 13th, and 23rd week of lactation, and lowest content in the 18th week of lactation. On the contrary, StrzaŁkowska et al. (2009) reported greatest values of C18:0 and C18:1 in early and mid than late lactation. Those discrepancies may depend by the different feeding strategy adopted in each study. The trend of C18:2 was similar to the one reported by StrzaŁkowska et al. (2009), who found lower content in early than mid and late lactation. An opposite situation was described by Kuchtík et al. (2015), who showed the greatest content in the 9th week of lactation and the lowest at the end of lactation. The SCFA trend was similar to Strzałkowska et al. (2009), however, they reported a greater amount of SCFA in early (29.22 g/100 g FAs) and in late (24.38 g/100 g FAs) lactation compared with the present study. Strzałkowska et al. (2009) reported a similar trend for LCFA but with lower content than the present study (30.18 to 33.60 g/100 g FAs). The MCFA showed the greatest content in early lactation and in the 20th week of lactation, contrary to Strzałkowska et al. (2009), who reported similar amount across lactation.

CONCLUSIONS

Results of the present study showed that goat milk FA profile was affected by breed and stage of lactation. Breeds differed for C4:0, C14:0, C15:0, C15:0 iso and anteiso forms, C16:0, C16:1, C17:0, C17:0 iso and anteiso forms, C18:0, DI C16:0, and AI. The main differences of FA composition were detected between SA and some local breeds. Saanen milk was richer in C4:0, C14:0, and C16:0 than milk of GA, GI, and GI and JO, respectively. In addition, the greater AI in SA milk than GI milk breed and the lower contents of odd FA in SA milk than local breeds, may suggest less adaptability of the cosmopolitan breed to the environmental condition than of its

origins. Thus, milk of local breeds may have a greater potential benefit on human health. Very few differences were detected within local breeds. Week of lactation affected significantly all individual, groups, and indices of FA. However, the pasture composition and stage of herbage maturity could affect FA content showing peculiar patterns through lactation. In particular, individual SCFA and some MCFA were more abundant in early than late lactation probably due to the greater amount of concentrate administered in the onset of lactation than in later stages. The greatest desaturation activity of mammary gland was observed at the end of lactation, as suggested by DI C16:0 and DI C18:0. Contrary to SFA, the amount of n3, n6, CLA, UFA, MUFA, and PUFA were greater at the end than at the beginning of lactation. These differences are linked to the increase of forage intake in mid and toward the end of lactation. In fact, late lactation is characterized by low concentrate administration in favor of the pasture. In conclusion, this study offers a contribute to the characterization and comparison of FA profile of milk from local breeds and a cosmopolitan breed under the same farming conditions, evidencing that milk from local breeds could be valorized for its better FA profile may have a greater potential benefit on human health compared to milk of the cosmopolitan breed.

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Table 1. Chemical composition and energy content of the hay and the concentrate administered to the goats during the trial.

Trait [1]	**Hay**	**Commercial Concentrate**
DM (%)	89.1	88.2
CP (% of DM)	15.1	21.7
Fat (% of DM)	1.9	3.5
NSC (% of DM)	20.7	42.7
Cellulose (% of DM)	29.5	10.5
Ash (% of DM)	9.5	9.1
NDF (% of DM)	52.6	23.0
ADF (% of DM)	36.6	9.0
ADL (% of DM)	3.9	3.3
NEL (Mcal/kg DM)	1.10	1.77

[1] DM, dry matter; CP, crude protein; NSC, nonstructural carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NEL, net energy of lactation.

Table 2. Least squares means of milk yield and composition of 6 goat breeds [1].

	Breed						Overall		
Traits	**GA**	**GI**	**JO**	**MA**	**MR**	**SA**	**Mean**	**SEM**	***p***
Milk yield (kg/day)	1.26^{ab}	1.06^{b}	1.48^{ab}	1.39^{ab}	1.13^{b}	1.73^{a}	1.34	0.11	<0.001
FCM3.5% (kg/day)	1.32^{ab}	1.14^{b}	1.45^{ab}	1.43^{ab}	1.16^{b}	1.67^{a}	1.36	0.10	<0.001
Fat (%)	4.15	4.20	4.01	3.92	3.92	3.61	3.97	0.18	0.310
Protein (%)	3.52^{a}	3.02^{b}	3.20^{ab}	3.02^{b}	3.27^{ab}	3.20^{ab}	3.20	0.10	0.012
Lactose (%)	4.31^{b}	4.55^{ab}	4.58^{a}	4.59^{a}	4.69^{a}	4.34^{b}	4.51	0.05	<0.001
F/P	1.15^{b}	1.37^{a}	1.25^{ab}	1.30^{ab}	1.21^{ab}	1.14^{b}	1.22	0.03	0.005
SCS (units)	5.77^{a}	4.20^{b}	5.34^{ab}	4.93^{b}	5.10^{b}	6.79^{a}	5.35	0.34	<0.001

Abbreviations are as follows: GA, Garganica; GI, Girgentana; JO, Jonica; MA, Maltese; MR, Mediterranean Red; SA, Saanen; FCM3.5%, fat-corrected milk at 3.5%; F/P, fat to protein ratio; SCS, somatic cell score; SEM, standard error of the mean. [1] Least squares means with different superscript letters within a row are significantly different ($p < 0.05$).

Table 3. Least squares means of individual, groups and indices of milk fatty acid profile (g/100 g of total identified fatty acids) of 6 goat breeds [1]. In bold, p values lower than 0.05.

	Breed						Overall		
Fatty acid	**GA**	**GI**	**JO**	**MA**	**MR**	**SA**	**Mean**	**SEM**	***p***
C4:0	1.89^{b}	2.03^{ab}	2.01^{ab}	2.00^{ab}	2.09^{ab}	2.17^{a}	2.03	0.06	**0.043**
C6:0	2.19	2.22	2.35	2.24	2.37	2.26	2.27	0.07	0.330
C8:0	2.65	2.58	2.90	2.66	2.79	2.58	2.69	0.10	0.189
C10:0	8.44	7.84	9.01	8.24	8.73	8.22	8.41	0.34	0.186
C12:0	3.43	3.14	3.85	3.44	3.46	3.31	3.44	0.16	0.065
C14:0	8.57^{ab}	7.69^{b}	8.47^{ab}	8.50^{ab}	8.67^{ab}	9.25^{a}	8.52	0.30	**0.029**
C15:0	1.72^{ab}	1.86^{a}	1.82^{ab}	1.85^{a}	1.73^{ab}	1.58^{b}	1.76	0.05	**0.013**
C15:0 iso	0.42^{a}	0.40^{a}	0.41^{a}	0.43^{a}	0.42^{a}	0.31^{b}	0.40	0.02	**0.009**
C15:0 anteiso	0.35^{ab}	0.35^{ab}	0.40^{a}	0.37^{ab}	0.35^{ab}	0.28^{b}	0.35	0.02	**0.018**
C16:0	23.74^{ab}	22.48^{b}	22.14^{b}	23.46^{ab}	24.19^{ab}	25.85^{a}	23.54	0.71	**0.014**
C16:1	1.01^{a}	0.98^{a}	0.90^{ab}	0.86^{ab}	0.71^{b}	0.87^{ab}	0.89	0.05	**0.002**
C17:0	1.87^{ab}	2.02^{a}	1.95^{a}	2.00^{a}	1.89^{ab}	1.72^{b}	1.91	0.04	**0.002**
C17:0 iso	0.63^{a}	0.68^{a}	0.67^{a}	0.66^{a}	0.63^{a}	0.56^{b}	0.64	0.01	**<0.001**
C17:0 anteiso	0.49^{ab}	0.52^{a}	0.50^{a}	0.51^{a}	0.48^{ab}	0.43^{b}	0.49	0.01	**0.003**
C18:0	13.85^{ab}	15.60^{a}	14.16^{ab}	14.29^{a}	13.65^{ab}	11.85^{b}	13.90	0.54	**0.002**
C18:1	23.32	24.59	22.95	23.06	22.68	22.78	23.23	0.69	0.359
C18:1trans-11	1.84	2.09	2.03	2.00	1.92	2.03	1.98	0.10	0.593
CLAc9 trans-11	0.73	0.73	0.73	0.73	0.72	0.85	0.75	0.03	0.119
C18:2	3.00	3.17	3.20	3.18	2.87	3.31	3.12	0.10	0.083

n3	0.86	1.00	0.96	0.99	0.86	0.92	0.93	0.04	0.069
n6	4.30	4.36	4.37	4.41	4.07	4.72	4.37	0.13	0.063
CLA	0.91	0.88	0.88	0.91	0.88	1.05	0.92	0.04	0.068
SFA	70.23	69.14	70.46	71.00	70.91	70.35	70.35	0.65	0.346
UFA	30.16	31.59	29.86	29.97	28.97	29.93	30.08	0.77	0.280
MUFA	24.84	25.53	24.21	23.56	24.11	24.03	24.38	0.55	0.147
PUFA	5.15	5.36	5.33	5.40	4.97	5.65	5.31	0.15	0.102
SCFA	15.28	14.79	16.39	15.25	16.12	15.34	15.53	0.50	0.194
MCFA	38.96	36.34	37.67	38.52	38.87	40.34	38.45	0.87	0.059
LCFA	45.77	48.64	45.84	46.13	44.64	43.36	45.73	1.14	0.057
n6/n3	5.20	4.56	4.67	4.63	4.93	5.36	4.89	0.19	0.070
SFA/UFA	2.36	2.21	2.39	2.40	2.51	2.43	2.38	0.08	0.207
DI C16:0	4.09^{a}	4.18^{a}	3.93^{a}	3.51^{ab}	2.82^{b}	3.32^{ab}	3.64	0.22	**<0.001**
DI C18:0	62.93	61.41	62.10	61.83	62.51	65.73	62.75	1.00	0.073
AI	2.08^{ab}	1.82^{b}	2.05^{ab}	2.10^{ab}	2.22^{ab}	2.32^{a}	2.10	0.10	**0.035**
EI	5.63	5.00	5.59	5.37	5.29	5.30	5.36	0.22	0.342
TI	2.71	2.55	2.62	2.73	2.80	2.80	2.71	0.09	0.258

Abbreviations are as follows: GA, Garganica; GI, Girgentana; JO, Jonica; MA, Maltese; MR, Mediterranean Red; SA, Saanen; CLA, conjugated linoleic acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCFA, short chain fatty acids; MCFA, medium chain fatty acids; LCFA, long chain fatty acids; DI C16:0, desaturation index of C16:0; DI C18:0, desaturation index of C18:0; AI, atherogenicity index; EI, elongation index; TI, thrombogenic index; SEM, standard error of the

mean. 1 Least squares means with different superscript letters within a row are significantly different ($p < 0.05$).

Table 4. Least squares means of individual, groups (g/100 g of total identified fatty acids) and indices of goat milk fatty acids throughout lactation [1] ($p < 0.001$).

	Week of lactation						Overall	
Fatty acid	**4**	**8**	**12**	**16**	**20**	**24**	**Mean**	**SEM**
C4:0	1.96^{bc}	1.92^{c}	2.16^{a}	2.01^{bc}	2.09^{ab}	2.05^{ab}	2.03	0.04
C6:0	2.38^{ab}	2.24^{bc}	2.44^{a}	2.14^{c}	2.33^{bc}	2.09^{c}	2.27	0.05
C8:0	2.93^{a}	2.73^{ab}	2.92^{a}	2.48^{c}	2.79^{ab}	2.31^{c}	2.69	0.07
C10:0	9.47^{a}	8.89^{a}	8.81^{a}	7.37^{b}	8.96^{a}	6.98^{b}	8.41	0.26
C12:0	3.75^{a}	3.73^{a}	3.46^{ab}	3.11^{bc}	3.76^{a}	2.83^{c}	3.44	0.12
C14:0	9.51^{a}	9.31^{a}	8.11^{b}	7.52^{c}	8.81^{a}	7.88^{bc}	8.52	0.19
C15:0	1.69^{c}	2.10^{a}	1.60^{c}	1.90^{b}	1.61^{c}	1.64^{c}	1.76	0.04
C15:0 iso	0.37^{b}	0.50^{a}	0.38^{b}	0.45^{a}	0.33^{b}	0.36^{b}	0.40	0.02
C15:0 anteiso	0.32^{b}	0.44^{a}	0.33^{b}	0.40^{a}	0.28^{b}	0.33^{b}	0.35	0.01
C16:0	24.63^{ab}	23.75^{b}	22.48^{c}	21.52^{c}	25.61^{a}	23.88^{b}	23.60	0.44
C16:1	0.82^{b}	0.90^{ab}	0.77^{b}	0.88^{ab}	0.92^{ab}	1.04^{a}	0.89	0.04
C17:0	1.95^{bc}	2.14^{a}	1.80^{cd}	2.00^{ab}	1.72^{d}	1.84^{cd}	1.91	0.04
C17:0 iso	0.59^{b}	0.67^{a}	0.63^{b}	0.67^{a}	0.62^{b}	0.64^{b}	0.64	0.01
C17:0 anteiso	0.51^{b}	0.59^{a}	0.46^{bc}	0.52^{b}	0.41^{c}	0.44^{c}	0.49	0.01
C18:0	12.43^{cd}	13.05^{cd}	15.75^{b}	17.13^{a}	11.75^{d}	13.28^{c}	13.90	0.35
C18:1	21.61^{c}	21.98^{c}	22.38^{b}	25.50^{a}	22.00^{b}	25.91^{a}	23.23	0.52
C18:1trans-11	1.75^{c}	1.61^{c}	2.03^{b}	2.28^{a}	1.94^{b}	2.29^{a}	1.98	0.07
CLAc9trans-11	0.57^{c}	0.52^{c}	0.67^{c}	0.83^{b}	0.86^{ab}	1.06^{a}	0.75	0.03
C18:2	2.94^{bc}	3.20^{ab}	3.04^{bc}	2.88^{c}	3.21^{ab}	3.46^{a}	3.12	0.08

n3	0.82^{b}	0.70^{c}	1.10^{a}	0.88^{b}	1.06^{a}	1.04^{a}	0.93	0.03
n6	4.02^{c}	4.25^{bc}	4.16^{c}	4.19^{c}	4.55^{b}	5.06^{a}	4.37	0.09
CLA	0.74^{cd}	0.68^{d}	0.83^{c}	1.00^{b}	1.03^{b}	1.23^{a}	0.92	0.03
SFA	72.79^{a}	71.64^{a}	71.30^{a}	68.17^{b}	71.01^{a}	67.17^{b}	70.35	0.49
UFA	27.95^{b}	28.55^{b}	29.03^{b}	32.02^{a}	29.19^{b}	33.74^{a}	30.08	0.60
MUFA	22.43^{b}	23.41^{b}	23.43^{b}	26.67^{a}	23.38^{b}	26.97^{a}	24.38	0.43
PUFA	4.86^{c}	4.96^{c}	5.26^{bc}	5.07^{c}	5.61^{ab}	6.10^{a}	5.31	0.11
SCFA	16.86^{a}	15.90^{a}	16.44^{a}	14.13^{b}	16.33^{a}	13.52^{b}	15.53	0.37
MCFA	40.44^{a}	40.27^{a}	36.91^{b}	35.10^{c}	40.56^{a}	37.43^{b}	38.45	0.54
LCFA	42.26^{c}	43.85^{c}	46.63^{b}	50.51^{a}	42.36^{c}	48.76^{ab}	45.73	0.82
n6/n3	5.04^{bc}	6.16^{a}	3.86^{d}	4.81^{bc}	4.49^{c}	4.99^{bc}	4.89	0.14
SFA/UFA	2.67^{a}	2.54^{a}	2.48^{a}	2.15^{b}	2.47^{a}	1.99^{b}	2.38	0.07
DI C16:0	3.27^{c}	3.65^{bc}	3.27^{c}	3.96^{ab}	3.49^{bc}	4.21^{a}	3.64	0.18
DI C18:0	63.45^{bc}	62.79^{c}	58.72^{d}	60.06^{d}	65.28^{ab}	66.20^{a}	62.75	0.65
AI	2.48^{a}	2.30^{a}	2.06^{b}	1.72^{c}	2.26^{ab}	1.75^{c}	2.10	0.07
EI	5.95^{a}	5.90^{a}	5.04^{b}	4.90^{b}	5.55^{a}	4.83^{b}	5.36	0.14
TI	3.02^{a}	2.88^{ab}	2.71^{bc}	2.53^{cd}	2.71^{bc}	2.38^{d}	2.71	0.06

Abbreviations are as follows: CLA, conjugated linoleic acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCFA, short chain fatty acids; MCFA, medium chain fatty acids; LCFA, long chain fatty acids; DI C16:0, desaturation index of C16:0; DI C18:0, desaturation index of C18:0; AI, atherogenicity index; EI, elongation index; TI, thrombogenic index; SEM, standard error of the mean. 1 Least squares means with different superscript letters within a row are significantly different ($p < 0.05$).

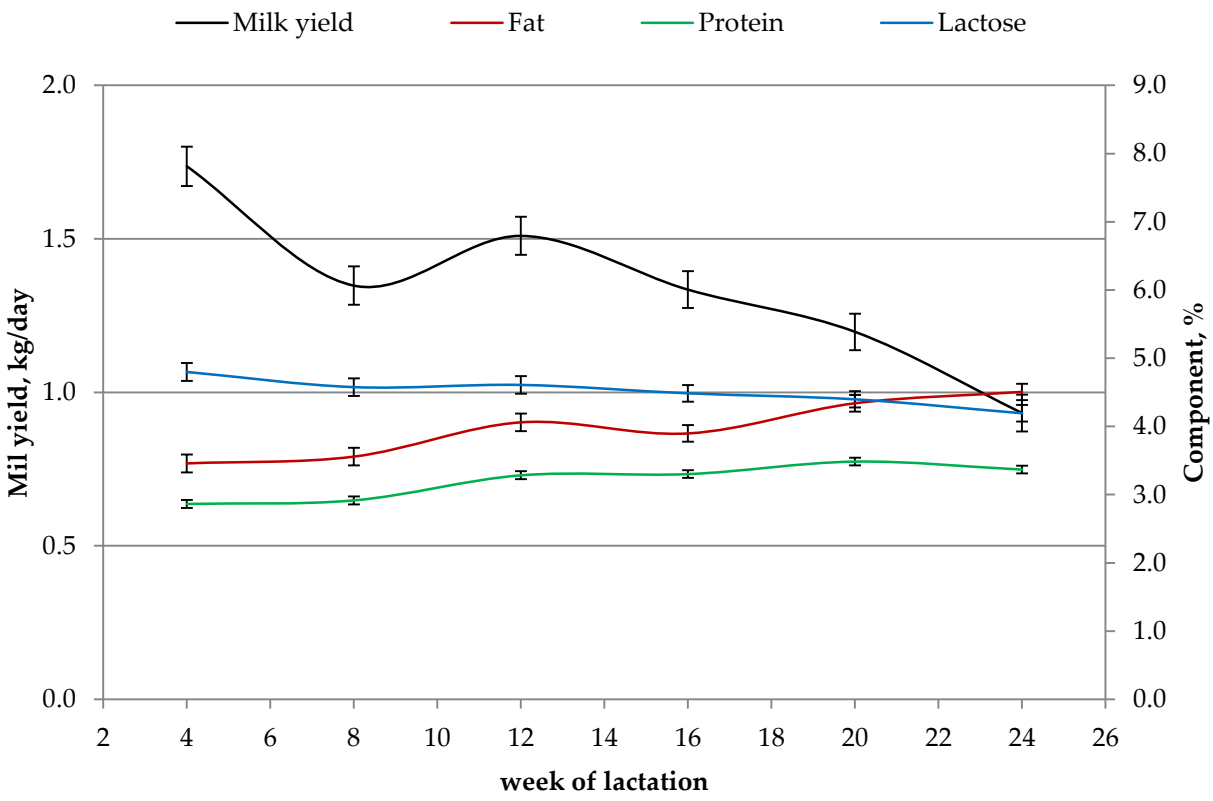

Figure 1. Least squares means (with standard error) of goat milk yield (black line), and fat (red line), protein (green line) and lactose (blue line) percentages throughout lactation.

Chapter 6

Italian local goat breeds have better milk coagulation properties than cosmopolitan breed

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ABSTRACT

Native goat breeds play an important role both in the safeguarding of biodiversity and the development of local economy. This study aimed to evaluate milk coagulation properties of local (Garganica, Girgentana, Maltese, Mediterranean Red) and cosmopolitan (Saanen) goat breeds. From May to August, 46 multiparous goats were sampled monthly in an experimental farm; milk samples were analysed for fat, protein and lactose percentages, pH, SCS and milk coagulation properties. Data were analysed through a mixed linear model with repeated measures, including breed, month of lactation and parity as fixed effects. Month of lactation affected all studied traits, and breed affected milk coagulation properties, protein percentage, lactose percentage and pH. Milk composition and coagulation properties were better in local breeds than Saanen. In particular, milk of local breeds was richer in protein percentage, and coagulated and reached 20 mm of curd firmness 3.14 min and 2.56 min earlier than Saanen, respectively. Moreover, the firmest curd was observed in Girgentana (31.44 mm) and the weakest in Garganica (21.72 mm). Rennet coagulation time and curd firmness decreased toward the end of lactation, differing significantly between May (10.62 min and 31.69 mm, respectively) and August (8.33 min and 20.69 mm, respectively), whereas curd-firming time only differed between May and June-July. In conclusion, milk of local breeds showed better milk coagulation ability than the cosmopolitan breed.

Key words: curd firmness; doe; goat milk; native breed; rennet coagulation time

INTRODUCTION

Goat cheese is the primary market of European goat milk production (Boyazoglu and Morand-Fehr, 2001; Sandrucci et al., 2019) and in the last decade the Italian goat cheese production has increased by 32% (FAOSTAT, 2019). The growing consumer's demand for goat cheeses originated by their peculiar sensory properties as flavour and consistency (Ribeiro and Ribeiro, 2010). Moreover, the interest in goat milk products has increased due to their nutritional and health benefits (Haenlein, 2004; Ribeiro and Ribeiro, 2010; Ranadheera et al., 2019).

The efficiency of milk manufacturing into cheese is generally related to many intrinsic milk characteristics and cheese-making technologies; one of the crucial points is the coagulation process which is related to the interaction between milk and rennet. This process is well described by the milk coagulation properties (MCP), namely rennet coagulation time (RCT, min), curd-firming time (k_{20}, min) and curd firmness 30 min after rennet addition to milk (a_{30}, mm; Mara et al., 1999). In general, milk with shorter RCT and k_{20} and greater a_{30} is desirable to produce cheese (Ikonen et al., 2004). Composition of milk affects cheese yield, nutritional composition and sensory properties (Pizzillo et al., 2004; Pretto et al., 2016; Vacca et al., 2018). Moreover, MCP are affected by species, breed, parity and stage of lactation (De Marchi et al., 2007; Poulsen et al., 2013; Vacca et al., 2018). In general, the majority of the studies on bovine milk and some on goat milk have reported worse milk coagulation ability in breeds selected for greater milk yield (De Marchi et al., 2007; Poulsen et al., 2013; Vacca et al., 2018).

Some recent studies have reported differences in goat milk composition between Italian local breeds (Girgentana, Garganica, Jonica, Maltese and Mediterranean Red) and Saanen (Currò et al., 2019a; b) which might result in differences for MCP. Conversely, Clark and Sherbon (2000) in the comparison of MCP in Nubian, LaMancha, Saanen, Alpine, Oberhasli and Toggenburg goat breeds did not highlight any difference between Saanen and other cosmopolitan breeds. However, Clark and Sherbon (2000) observed differences between LaMancha and Alpine

breeds for RCT; between Nubian and Alpine and LaMancha breeds for k_{20}; and between Alpine and Toggenburg breeds for a_{30}. On the other hand, Vacca et al. (2018) reported longer (but not significant) RCT for Saanen than Mediterranean breeds (Maltese, Murciano-Granadina, Sarda and Sarda Primitiva), and longer k_{20} and smaller a_{30} than Sarda and Sarda Primitiva breeds.

Few researches have investigated MCP in endangered Italian local breeds (Todaro et al., 2005; Zullo et al., 2005). The characterisation of technological milk traits may contribute to valorise native breeds and mitigate the loss of genetic variability. Therefore, the aim of the present study was to compare MCP of local goat breeds (Garganica, Girgentana, Maltese and Mediterranean Red) with MCP of Saanen.

MATERIALS AND METHODS

Animals and management conditions

The study was conducted from May to August 2018 in the experimental farm of the Council for Agricultural Research and Economics, Research Unit for the Extensive Animal Husbandry (CREA-ZOE, Potenza, Italy). A general description of the breeds considered in the present study is reported in Currò et al. (2019a). Experimental procedures and animal care conditions followed the recommendations of European Union directive 86/609/EEC and were approved by the Institutional Animal Care and Use Committee of the Department of Veterinary Medicine of University of Naples Federico II.

Forty-six dairy goats of 5 breeds (Garganica, GA, n=10; Girgentana, GI, n=9; Maltese, MA, n=10; Mediterranean Red, MR, n=8; and Saanen, SA, n=9) of parities 1 to 5 were enrolled in the study. All does, under the same managerial conditions, had single birthing in February and each kid was kept with the mother until 40 days of age.

Does grazed together in a natural pasture per 8 h/day and were supplemented with hay *ad libitum* in the shelter, composed by 60 to 65% of grasses (mainly *Avena sativa* L.) and 35 to 40% of legumes (mainly *Vicia sativa L*.) with the following chemical composition: 89.10% of dry matter (DM), 15.10% of crude protein on DM, 52.60% of neutral detergent fibre on DM and 1.10 Mcal/kg of net energy of lactation on DM. Does body weight, body condition score and concentrate administration during the study was comparable with those reported by Currò et al. (2019a).

Milking and sample collection

Does were milked twice a day (morning and evening) in a double 24-stall herringbone low-line milk pipeline milking parlour (Alfa Laval Agri; Monza, Italy) equipped with recording jars and electronic pulsators at a vacuum of 38 kPa, 90 pulses/min and 60% pulsation ratio. The pre-milking phase consisted of forestripping without any preparation of udder and teats. None of the goats was affected by mastitis throughout the study. Individual milk samples (50 mL) were collected monthly from May to August. In August 6 does (2 does per GA, MR and SA breeds) entered in the dry period, thus the total number of milk samples collected was 178.

Chemical analysis and MCP determination

Milk samples were stored at 4°C, transferred to the milk laboratory of the Breeders Association of Veneto Region (Padova, Italy) and analysed within 24 h from sampling. Fat, protein and lactose percentages were determined using MilkoScan FT6000 (Foss Electric, Hillerød, Denmark), and pH was measured by a digital pH meter (Crison pH-Burette 24, Crison Instruments SA, Barcelona, Spain). Somatic cell count (SCC, cells/mL) was determined using

Fossomatic FC (Foss Electric, Hillerod, Denmark) and transformed to somatic cell score (SCS) through the formula of Wiggans and Shook (1987):

$$SCS = 3 + \log_2(SCC/100{,}000).$$

Milk coagulation properties were assessed using Formagraph (Foss Electric A/S, Hillerød, Denmark). Briefly, milk samples were put in a water bath to reach 35°C and then 200 μL of calf rennet (Hansen Naturen HA-LA-215; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.2% (wt/wt) in distilled water was added to 10 mL of each milk sample. The analysis lasted 30 min from the addition of rennet solution to milk. Milk coagulation properties recorded were: RCT, the time interval between the addition of rennet to milk and the beginning of coagulation; k_{20}, the time interval between the beginning of coagulation and the attainment of a coagulum of 20 mm; and a_{30}, the consistency of the coagulum 30 min after the addition of rennet solution to milk (Mara et al., 1999).

Statistical analysis

From the initial dataset, 5% of samples (9 observations) did not coagulate, and 14 observations did not have information on k_{20}. Inconsistent values for each studied trait were defined as values that deviated more than 2 standard deviations from their respective mean, and they were treated as missing information. Sources of variation of MCP, milk composition, pH and SCS were investigated using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC) with repeated measures, according to the following mixed linear model:

$$y_{ijklm} = \mu + Breed_i + Parity_j + Month_k + Goat_l(Breed_i) + \varepsilon_{ijklm},$$

where y_{ijklm} is the dependent variable (RCT, k_{20}, a_{30}, fat percentage, protein percentage, lactose percentage, pH or SCS); μ is the overall intercept of the model; $Breed_i$ is the fixed effect of the

*i*th goat breed (*i* = GA, GI, MA, MR, SA); $Parity_j$ is the fixed effect of the *j*th parity (*j* = primiparous or multiparous goats); $Month_k$ is the fixed effect of the *k*th month of lactation (*k* = May, June, July, August); $Goat_l(Breed_i)$ is the random effect of the *k*th goat nested within the *i*th breed ~N(0, $\sigma^2_{Goat(Breed)}$); and ε_{ijklm} is the random residual ~N(0, $\sigma^2\varepsilon$). In a preliminary analysis, the interactions Breed × Parity, Parity × Month and Breed × Month were not significant and, consequently, they were removed from the final model. Multiple comparisons of least squares means were performed for the main effects of breed, month of lactation and parity using Bonferroni's test adjustment. Significance was declared at $p < 0.05$. In addition, Pearson correlation coefficients between the residuals of the studied traits were computed.

RESULTS

Descriptive statistics

Descriptive statistics of MCP, milk composition, pH and SCS are summarised in Table 1. The RCT, k_{20} and a_{30} averaged 9.36 min, 3.68 min and 25.56 mm, respectively. Fat content, protein content, lactose content, pH and SCS averaged 3.60%, 3.42%, 4.41%, 6.56 and 5.72, respectively. The greatest coefficient of variation (CV) was observed for k_{20} (51.42%), a_{30} (37.84%) and SCS (30.13%), and the lowest was observed for pH (1.70%) and lactose content (5.09%).

Breed effect

Breed affected RCT, k_{20}, a_{30}, protein content, lactose content and pH ($p < 0.05$). Least squares means of MCP and milk composition for the breed effect are reported in Table 2. Saanen milk had the longest RCT (12.05 min; $p < 0.05$) and k_{20} (6.00 min; $p < 0.05$). The greatest a_{30} was

observed in milk of GI breed, being 9.72 and 8.80 mm firmer than a_{30} of GA and SA, respectively ($p < 0.05$).

Protein content was lower in milk of SA compared with GA (-0.63%; $p < 0.05$), MA (-0.45%; $p < 0.05$) and GI breed (-0.43%; $p < 0.05$). Moreover, lactose content was lower in milk of SA compared with MR (-0.25%; $p < 0.05$). Milk pH from SA was significantly higher than milk pH of GA, GI and MR breeds. Breeds did not differ significantly in terms of fat content; however, it is worth mentioning that milk of local breeds had greater fat content than milk of the cosmopolitan breed. Within local breeds, lactose content was 0.21% higher in milk of MR than MA ($p < 0.05$). Local breeds did not differ for RCT, k_{20}, protein content and pH.

Month of lactation and parity effects

Month of lactation affected significantly MCP, milk composition, pH and SCS (Figure 1). The RCT decreased by 22% toward the end of lactation (May, 10.62 ± 0.35 min; August, 8.33 ± 0.35 min; $p < 0.05$), a_{30} decreased by 35% toward the end of lactation (May, 31.68 ± 1.37 mm; August 20.69 ± 1.35 mm; $p < 0.05$), and k_{20} was shorter in June (3.78 ± 0.29 min) and July (3.53 ± 0.28 min) than May (4.72 ± 0.30 min; $p < 0.05$).

Different patterns were observed for milk composition, pH and SCS (Figure 2). Fat content increased ($p < 0.05$) by 15% from May (3.38 ± 0.11%) to August (3.89 ± 0.11%). Protein content increased by approximately 1% between May (3.39 ± 0.05%) and August (3.43 ± 0.05%); however, protein content was lower in June (3.30 ± 0.05%) than July and August ($p = 0.05$). Lactose and pH decreased progressively toward the end of lactation; in particular, lactose content decreased by 9% and pH by 2% between May and August. Conversely, SCS showed a

progressive increment toward the end of lactation with a variation of +35% ($p < 0.05$) between May (4.78 ± 0.38) and August (6.44 ± 0.28).

Parity effect showed a tendency for protein percentage ($p = 0.05$) and SCS ($p = 0.06$). Among MCP, parity affected significantly only a_{30} ($p = 0.05$). In particular, primiparous does showed greater a_{30} (27.39 ± 0.98 mm) than multiparous (23.64 ± 1.03 mm).

Correlations

Pearson's correlations between the investigated traits are reported in Table 3. A weak positive correlation was observed between protein and fat percentage (0.26; $p < 0.001$). Lactose percentage was weakly and positively correlated to fat percentage (0.20; $p < 0.05$), pH (0.16; $p < 0.05$) and protein percentage (0.19; $p < 0.05$), and it was moderately and negatively correlated to SCS (-0.37; $p < 0.001$). A strong positive correlation was estimated between RCT and k_{20} (0.62; $p < 0.001$), and a moderate negative correlation was assessed between k_{20} and a_{30} (-0.41; $p < 0.001$) and between RCT and a_{30} (-0.32; $p < 0.001$). Negative correlations were observed between protein percentage and k_{20} (-0.46; $p < 0.001$), and between pH and a_{30} (-0.18; $p < 0.001$). The RCT was not correlated to protein, but it was positively correlated to pH (0.42; $p < 0.001$). Moreover, a positive correlation was estimated between pH and k_{20} (0.29; $p < 0.001$), between a_{30} and lactose (0.26; $p < 0.001$), and between a_{30} and protein percentage (0.29; $p < 0.001$).

DISCUSSION

Means and variation of MCP and milk composition

Overall, means and CV of MCP, milk composition, pH and SCS were lower than those reported by Vacca et al. (2018), who observed longer RCT (13.2 min) and k_{20} (4.5 min) and greater a_{30} (36

mm) than the present study. Moreover, Vacca et al. (2018) reported greater contents of protein (3.60%), fat and lactose (4.60%), SCS (5.79) and pH (6.72) compared with our results. Among studied traits, the greater CV was observed for k_{20}, similarly to Vacca et al. (2018; CV = 53%). Conversely, in the present study a slightly greater CV for a_{30} than that reported by Vacca et al. (2018; CV = 34%) was observed. Moreover, Vacca et al. (2018) reported greater CV for fat (CV = 31%), SCS (CV = 35%) and protein (CV = 15%). The discrepancies observed comparing the present study to Vacca et al. (2018) might be related to the inclusion of Sarda and Sarda Primitiva breeds in their study, which produce milk richer in fat, protein and lactose than other studied breeds. Moreover, discrepancies between the studied traits (except for lactose and pH) might also be related to the longer lactation period and several parities (1 to 15) included in Vacca et al. (2018). On the other hand, CV of lactose and pH observed in the present study was similar to those reported by Vacca et al. (2018), which might be due to the low variability of those traits during the lactation (Niero et al., 2018).

Breed effect

In the present study breed affected MCP, protein content, lactose content and pH. These results partially agree with Vacca et al. (2018) who reported significant differences between Alpine (SA and Camosciata delle Alpi) and Mediterranean breeds (Maltese, Murciano-Granadina, Sarda and Sarda Primitiva) for k_{20}, a_{30}, fat content, protein content, lactose content and pH. In our study, the main differences of MCP were observed between local and cosmopolitan breeds. In detail, the shorter RCT and k_{20} detected in local breeds compared with SA may suggest that milk of local breeds is more suitable for cheese manufacturing than milk of SA (Ikonen et al., 2004; Varotto et al., 2015; Niero et al., 2016). However, differences of a_{30} among breeds were less evident.

Conversely, no significant differences among local breeds were observed for the studied traits. Vacca et al. (2018) did not report significant differences for RCT between Alpine and Mediterranean breeds; however, they reported a slightly longer RCT for SA (13.1 min) than MA breed (11.9 min). The same authors observed similar a_{30} between SA and MA; however, they reported greater a_{30} (33.5 mm) in SA milk than the present study. On the other hand, results for k_{20} in the present study disagree to those of Vacca et al. (2018), who reported similar results for SA, Murciano-Granadina and MA breeds (5.00 min), and also significant differences within Mediterranean breeds. These discrepancies between our study and Vacca et al. (2018) might be related to the greater number of days in milk (10 to 374) considered in their study.

Because GA milk had short RCT and k_{20}, we expected greater a_{30} than the other breeds, but this was not the case; this result may be partly the consequence of the relatively low association between RCT and a_{30}. In particular, this could be due to a low content of αs_1-casein that promotes short RCT and k_{20} and causes weak a_{30} (Clark and Sherbon, 2000; Zullo et al., 2005). Moreover, the lower fat, protein and lactose content of SA than local milk breeds could be the cause of the delay in RCT and a_{30}, as reported in Ambrosoli et al. (1988). Currò et al. (2019b) and Vacca et al. (2018) also highlighted poorer fat, protein and lactose content in milk of SA than local (GA, GI, Jonica, MA and MR) and Sarda and Sarda Primitiva goat breeds, respectively. The low milk composition of SA breed might be due to the intensive selection for milk yield, which has likely caused a dilution of milk components (Serradilla, 2001). In general, milk of specialized dairy goat breeds showed reduced coagulation ability (Poulsen et al., 2013; Penasa et al., 2014; Vacca et al., 2018).

In the present study, GI showed shorter RCT, longer k_{20} and stronger a_{30} than those reported in Todaro et al. (16.96 and 2.01 min, and 31.44 mm, respectively; 2005). The delay of

RCT and k_{20} in Todaro et al. (2005) compared with our results could be explained considering the different origin of rennet used in the studies; indeed, to assess MCP Todaro et al. (2005) used rennet from lamb which contains less pepsine and has lower proteolytic activity than calf rennet used in the present study (Anifantakis and Green, 1980). Furthermore, similar protein content was detected in both studies; however, Todaro et al. (2005) reported milk richer in fat (3.93%) and lactose (4.55%) and with higher pH (6.59) than the present study. Those discrepancies might be due to the whole lactation considered in the study of Todaro et al. (2005); in fact, the inclusion of early lactation (characterised by milk poorer in fat and protein percentage than late lactation) in their study could be the cause of the delay in RCT and k_{20} in early than late lactation.

Month and parity effects

Generally, parity order affect MCP (Todaro et al., 2005; Visentin et al., 2017b; Vacca et al., 2018); however, in the current study parity affected only a_{30}, with decreasing estimates across parities, in agreement with results of Vacca et al. (2018), who reported that only a_{30} was influenced by parity.

Rennet coagulation time and a_{30} decreased from May to August. Considering that in the present study the protein content was similar between May and August, the shorter RCT toward the end of lactation could be due to the variation of the casein fractions (Brown et al., 1995; Díaz et al., 1999). Indeed, Brown et al. (1995) observed an increment of total casein, in particular of αs_1-casein and κ-casein, and a decrease of αs_2-casein and β-casein toward the end of lactation in SA goat breed. Díaz et al. (1999) reported an increment of αs_1-casein and a decrease of κ-casein and β-casein contents from spring to summer. On the other hand, Clark and Sherbon (2000) reported that milk without αs_1-casein was characterised by short RCT and $k_{20,}$ and weak a_{30};

however, those results did not differ significantly to those of milk characterised by high level of αs_1-casein which showed longer RCT and greater a_{30} at the end of analysis.

According to the positive correlation of a_{30} with lactose and protein contents, the smaller a_{30} at the end of lactation could depend on the reduction of lactose and casein fractions content (αs_2-casein and β-casein) through lactation. In general, the trends of RCT, k_{20} and a_{30} reported in this study followed the trend reported by Salari et al. (2016), who found shorter RCT and weaker a_{30} in summer than spring, without highlighting significant variation of protein content.

Generally, an increase of milk components in late lactation due to lower milk yield and thus a concentration of components is expected (Goetsch et al., 2011; Mestawet et al., 2012). Indeed, in the present study it was observed a slight increment of protein and SCS toward the end of lactation. Conversely, the fat content showed fluctuation through the period considered, and in July an inversion of fat to protein ratio was observed. The greater protein than fat content could suggests the presence of metabolic disorder status known as milk fat depression caused by nutrition (Koch and Lascano, 2018). Lactose affects the volume of milk showing similar trends through lactation and reporting both the lowest content and volume at the end of lactation (Costa et al., 2019a). A decrease of lactose in goat milk and an increment of SCC and SCS was observed in Sung et al. (1999) and in Currò et al. (2019a), respectively. However, the greater SCS (6.44) with lower lactose (4.26%) associated with lower pH (6.52) in August than in previous months suggested the absence of udder diseases. Indeed, Hassan (2013) studied the variation of milk composition in different species (cow, ewe and goat) affected by sub-clinical mastitis and found that goats with infected udder produced milk poor in lactose and with high pH. In general, goat milk showed greater SCS than cow milk due to the apocrine system of goat milk secretion

(Raynal-Ljutovac et al., 2007) which excretes a great amount of cytoplasmic particles or epithelial cells into the milk causing the increment of somatic cells.

Correlations

Rennet coagulation time was negatively correlated to protein content, though this correlation was not significant, similarly to Visentin et al. (2017a), who studied the correlation between milk composition and MCP in dairy cows. Opposite to cow milk, the correlation between protein and RCT of goat milk is generally positive (Clark and Sherbon, 2000; Vacca et al., 2018); this is mainly due to the greater β-casein and αs_2-casein and lower or null αs_1-casein content in goat than cow milk (Albenzio et al., 2012). Indeed, αs_1-casein is mainly bound to Ca^{2+} ions reducing the availability for the binding with κ-casein after rennet addition resulting in a delay of RCT (Ambrosoli et al., 1988). The absence of clear correlation in this study could depend on caseins composition of each breed (Damián et al., 2009) and also on casein genotypes (Wedholm et al., 2006; Caravaca et al., 2011). Indeed, Chessa and Caroli (2014) reported that goat species has 23 variants of αs_1-casein, 7 variants of αs_2-casein, 16 variants of κ-casein and 9 variants of β-casein. This makes difficult to evaluate the single or combined effect of casein polymorphisms on MCP. Nevertheless, protein was correlated negatively to k_{20} and positively to a_{30}. According to Visentin et al. (2017a) pH correlated positively with RCT and k_{20}, and negatively with a_{30}; indeed, milk with higher pH had a delay of RCT and k_{20} and weaker a_{30} than milk with low pH. Moreover, we observed a positive correlation between lactose and a_{30}, which was in accordance with previous studies (Todaro et al., 2005; Salari et al., 2016; Costa et al., 2019b).

CONCLUSIONS

In the present study, local goat breeds showed better milk composition than Saanen, thus reflecting in better MCP than the cosmopolitan breed. However, a_{30} of Saanen was similar to a_{30} of local breeds. Significant differences were detected through month of lactation, observing shorter RCT and weaker a_{30} toward the end of lactation. Results of the present study are relevant to better understand the variation of MCP in goat local breeds and provide useful information to safeguard and valorise local genetic resources which have economic relevance for the sustainability of marginal areas.

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Table 1. Descriptive statistics of milk coagulation properties, composition traits, pH and SCS of goat milk.

Trait[1]	N	Mean	SD	CV[2], %	Minimum	Maximum
Milk coagulation properties						
RCT, min	154	9.36	2.52	26.99	4.00	16.15
k_{20}, min	144	3.68	1.89	51.42	1.30	10.30
a_{30}, mm	160	25.56	9.67	37.84	6.00	46.00
Milk composition						
Fat, %	159	3.60	0.77	21.37	1.91	5.38
Protein, %	161	3.42	0.39	11.33	2.48	4.39
Lactose, %	157	4.41	0.22	5.09	4.00	4.86
pH	160	6.56	0.11	1.70	6.31	6.80
SCS, units	135	5.72	1.72	30.13	0.75	9.99

[1]RCT: rennet coagulation time; k_{20}: curd-firming time; a_{30}: curd firmness 30 min after rennet addition to milk; SCS: somatic cell score.

[2]CV: coefficient of variation.

Table 2. Least squares means of milk coagulation properties, milk composition, pH and SCS for goat breeds.

	Breed				
Trait[1]	Garganica	Girgentana	Maltese	Med. Red	Saanen
Milk coagulation properties					
RCT, min	9.16 ± 0.50^{b}	9.69 ± 0.50^{b}	9.12 ± 0.48^{b}	7.66 ± 0.55^{b}	12.05 ± 0.60^{a}
k_{20}, min	3.31 ± 0.46^{b}	3.70 ± 0.48^{b}	3.62 ± 0.45^{b}	3.09 ± 0.52^{b}	6.00 ± 0.58^{a}
a_{30}, mm	21.72 ± 1.54^{b}	31.44 ± 1.53^{a}	25.25 ± 1.49^{ab}	26.52 ± 1.68^{ab}	22.64 ± 1.66^{b}
Milk composition					
Fat, %	3.82 ± 0.16	3.42 ± 0.16	3.68 ± 0.15	3.83 ± 0.17	3.17 ± 0.17
Protein, %	3.68 ± 0.09^{a}	3.48 ± 0.09^{a}	3.50 ± 0.08^{a}	3.30 ± 0.10^{ab}	3.05 ± 0.09^{b}
Lactose, %	4.47 ± 0.04^{ab}	4.42 ± 0.04^{ab}	4.35 ± 0.04^{b}	4.56 ± 0.05^{a}	4.31 ± 0.04^{b}
pH	6.51 ± 0.02^{b}	6.53 ± 0.02^{b}	6.57 ± 0.02^{ab}	6.53 ± 0.02^{b}	6.65 ± 0.02^{a}
SCS, units	5.84 ± 0.40	5.63 ± 0.35	4.85 ± 0.38	6.06 ± 0.39	5.98 ± 0.39

Least squares means with different superscript letters within a row are significantly different ($p < 0.05$).

[1]RCT: rennet coagulation time; k_{20}: curd-firming time; a_{30}: curd firmness 30 min after rennet addition to milk; SCS: somatic cell score.

Table 3. Pearson's correlation coefficients between residuals of milk composition, pH, SCS and milk coagulation properties.

	Fat	Lactose	pH	SCS	RCT	k_{20}	a_{30}
Protein	0.26***	0.19*	0.04	0.11	-0.12	-0.46***	0.29***
Fat		0.20*	0.04	0.05	-0.12	-0.06	0.02
Lactose			0.16*	-0.37***	-0.10	-0.09	0.26***
pH				-0.12	0.42***	0.29***	-0.18**
SCS					-0.03	-0.14	-0.15
RCT						0.62***	-0.32***
k_{20}							-0.41***

RCT: rennet coagulation time; k_{20}: curd-firming time; a_{30}: curd firmness 30 min after rennet addition to milk; SCS: somatic cell score.

*** $p < 0.001$; ** $p < 0.01$; $p < 0.05$

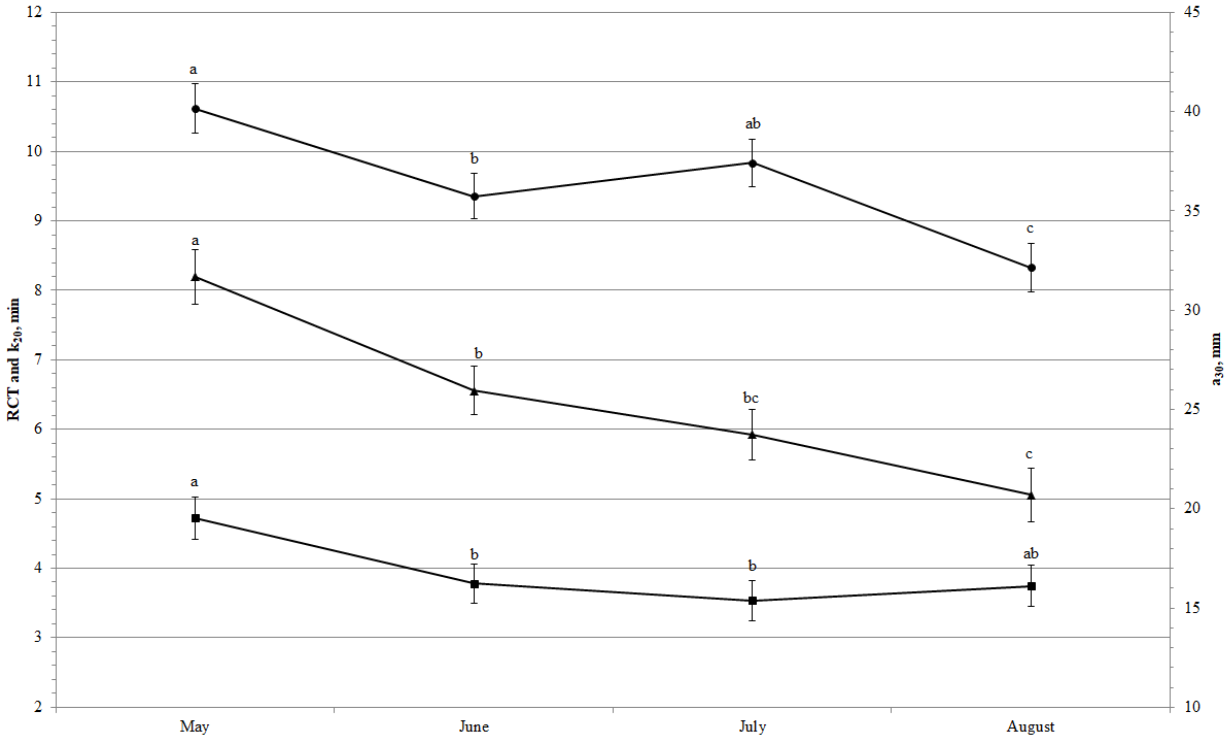

Figure 1. Least squares means (with standard errors) of rennet coagulation time (RCT, min; ●), curd-firming time (k_{20}, min; ▲), and curd firmness 30 min after rennet addition to milk (a_{30}, mm; ■) across month of lactation.

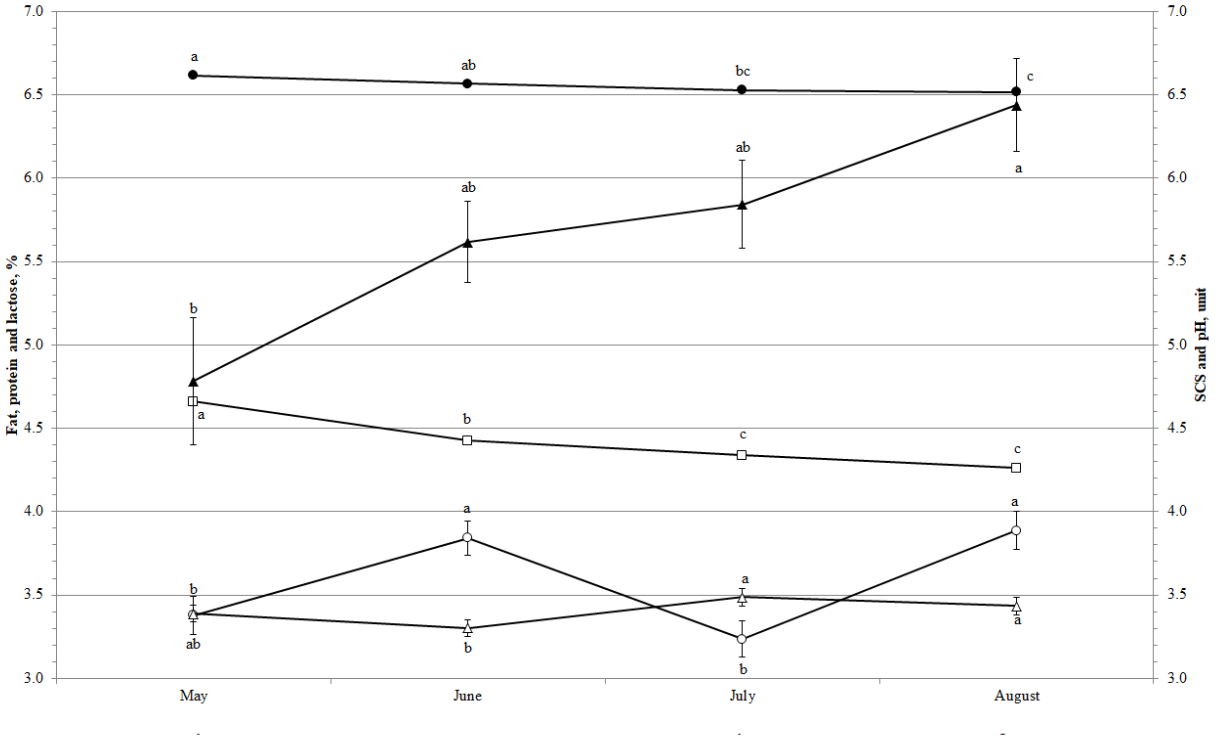

Figure 2. Least squares means (with standard errors) of fat percentage (○), protein percentage (△), lactose percentage (□), pH (●) and SCS (▲) across month of lactation.

Chapter 7

General conclusions

According to the results obtained by different studies, the following main conclusions can be reported:

i. Breed and stage of lactation highly affected milk yield and composition. In general, local breeds yield less milk than cosmopolitan breed. However, milk of local breeds showed better milk quality in fat and lactose percentage than cosmopolitan breed;

ii. Breed affects only P, Mg and Zn. Few differences in minerals profile was detected between locals and cosmopolitan breed. Stage of lactation affects all major and trace minerals (except B), observing their greater content in late than early lactation.

iii. Milk fatty acids composition was affected by breed and stage of lactation. According to other studies, breed affected C4:0, C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, and desaturation and atherogenetic indexes. Fatty acids profile of local milk breed suggests that it could be less harmful to human health than Saanen breed. Differences of fatty acids between early and late lactation could be related to feed variation. In fact, in early lactation feeding is characterised by greater amount of concentrate than late, affecting the greater content of some short and medium fatty acids in early than late lactation. Whereas n3, n6, CLA, UFA, MUFA and PUFA were greater at the end of lactation probably due to the greater pasture grazing.

iv. Local goat breeds yielded milk with better composition and milk coagulation properties than Saanen breed. However, Saanen milk showed a curd firmness similar to that one of local breeds. Stage of lactation affected milk coagulation properties; in

detail, shorter rennet coagulation time and weaker curd firmness was observed toward the end of lactation. The better milk coagulation properties of local breeds could provide practical issue to safeguard and valorise local genetic resources which have economic relevance for the sustainability of marginal areas.

List of Publications

Journal Publications

Niero, G., M. Penasa, **S. Currò**, A. Masi, A.R. Trentin, M. Cassandro, and M. De Marchi. 2016. Development and validation of a near infrared spectrophotometric method to determine total antioxidant activity of milk. Food Chemistry 220:371-376.

Manuelian, C.L., **S. Currò**, M. Penasa, M. Cassandro, and M. De Marchi. 2017. Prediction of minerals, fatty acid composition and cholesterol content of commercial cheeses by near infrared transmittance spectroscopy. International Dairy Journal 71:107-113.

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Currò, S., M. De Marchi, S. Claps, A. Salzano, P. De Palo, C.L. Manuelian and G. Neglia. 2019. Differences in the Detailed Milk Mineral Composition of Italian Local and Saanen Goat Breeds. Animals. 9:1-11.

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Currò, S., C.L. Manuelian, M. De Marchi, P. De Palo, S. Claps, A. Maggiolino, G. Campanile, D. Rufrano, A. Fontana, G. Pedota and G. Neglia. 2019. Autochthonous dairy goat breeds showed better milk quality than Saanen under the same environmental conditions. Archives Animal Breeding. 62: 83-89.

Niero, G., M. Penasa, A.Costa, **S.Currò**, G. Visentin, M.Cassandro and M. De Marchi. 2019. Total antioxidant activity of bovine milk: Phenotypic variation and predictive ability of mid-infrared spectroscopy. International Dairy Journal. 89:105-110.

Congresses

Currò, S., Manuelian C.L., Penasa M., Cassandro M., De Marchi M. 2017. Use of mid-infrared spectroscopy to predict coagulation properties of buffalo milk. Agriculturae Conspectus Scientificus . Vol. 82 (2017) No. 2 (171-174). ASD congress, Brandlucken, Austria.

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