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Genetic and phenotypic investigation of milk composition and technological properties in dairy sheep

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CHAPTER 1

Introduction

1.1. General introduction

1.1.1. Trends in milk and cheese production

The livestock dairy industry contributes to the economies of several countries. According to Food and Agriculture Organization of the United Nations (FAOSTAT, 2014), the gross production value of raw milk across the world was about 330 billion US$ in 2014, that represent 26% of the value of all livestock productions. In the last decade, the quantity of milk produced on a global scale, has shown an increasing trend. Southern Asia and European Union are the most important world dairy producers, accounting for 45 percent of global milk production. In 2014, sheep milk worldwide production was estimated at about 10,4 million tonnes, of which about 3 million tonnes were produced in Europe by about 130 million sheep. Greece holds the largest production with 772 thousand tonnes of sheep milk. In Italy, 372,526 tonnes of sheep milk were produced by 7.166.020 of sheep. About 44% of the world's sheep milk production and more than 14% of goat milk production is concentrated in the Mediterranean basin, as compared to about 11% of cow milk and only 3% of buffalo milk (FAOSTAT, 2014).

In Europe, in particular, in the Mediterranean area, the sheep milk produced is rarely used as milk for drinking, it is mainly destined to cheese production. Italy is the fifth country in the world and the third in Europe for the sheep cheese production. In addition, Italy has a good number of locally-made cheeses obtained from sheep milk. Among these, from Sarda sheep milk are currently manufactured three different Protected Designation of Origin (PDO) cheeses: Pecorino Romano, Pecorino Sardo, and Fiore Sardo. In 2015, an overall quantity of 17.066 tonnes of Pecorino and Fiore Sardo was exported, mainly in the United States (10.809 tonnes) (CLAL, 2015).

1.1.2. Dairy sheep

Probably, the domestication of sheep (*Ovis aries*) occurred in 9000 B.C. in the present-day countries of Iran and Iraq. Initially, the first domesticated animals were goats and later sheep (Zygoyiannis, 2006). Sheep have an extraordinary ability to adapt to diverse environmental conditions, even if adverse. Nowadays, sheep farming is widespread in several countries, among which developing countries where represents an important economic resource for household livelihoods (Pollott and Wilson, 2009). Sheep breeds are currently classified according to their primary production purpose:

- meat, such as Appeninica, Barbaresca, Biellese and Bergamasca, among Italian breed and Ile de France, Berricchon du Cher, Dorsetdown and Suffolk as foreign breeds;
- dairy, such as Sarda, Comisana, Massese and Valle del Belice in Italy, and East Friesian, Awassi, Lacaune, Churra and Manchega in Europe;
- wool, such as Gentile di Puglia and Sopravvissana among Italian breed and a French breed Merino Rambouillet.

1.1.3. Sarda dairy sheep

The Sarda is the most important Italian dairy sheep breed. Autochthonous of the Island of Sardinia it is widely spread also in some areas of central Italy, in particular Tuscany and Latium. The Sarda population consists of about 3 million sheep, but only about 8% are officially recorded in the Flock book (Carta et al., 2009). The breeding programme in Sarda dairy sheep started in 1927 when the SardinianFlock book was established (Sanna et al., 1997). Currently about 220,268 ewes (ICAR, 2014) are involved in pedigree and milk recording practices. The farming system is characterized by the seasonality of lambings. Multiparous ewes lamb in autumn (with mating season in late-spring, early summer) whereas primiparous ewes lamb in spring (with mating season in autumn). Lactations generally start in autumn-winter and spring and lasts until June-July. As reported in Table 1 Sarda dairy ewes produce about 140 l, 200 and 210 l per lactation in primiparous, secondiparous and multiparous (≥3 lactations), respectively (ASSONAPA, 2017). The livestock systems of Sarda breed varies from extensive, based on natural grassland and strongly affected by seasonal variation of feed availability, to intensive, based on forage crops, agriculture by-products and concentrates (Carta et al., 2009).

1.2. Milk composition

Sheep milk is characterized by higher fat and protein contents compared to cow milk. Therefore, it is most suitable for cheese production. Sheep milk composition varies according to several factors, such as diet, breed, parity, season, feeding, management, stage of lactation, and health status of the udder (Voutzourakis et al., 2014; Summer et al., 2012; Morand-Fehr et al., 2007; Park et al., 2007; Pulina et al., 2006; Bencini and Pulina, 1997).

During the early post-partum period, the chemical composition of sheep colostrum is different from milk in other lactation stages. Is richer than cow colostrum: fat 13.0% and 5.1%, protein 11.8% and 7.1%, lactose 3.3% and 3.6%, minerals 0.9% and 0.9%, total solids 28.9% and 15.6%, respectively (Anifantakis, 1986).

The predominant component of milk is the water, but principal solid constituents are fat, protein and lactose. The average milk composition in sheep and, in particular, in Sarda dairy sheep are shown in Table 2 and 3, respectively.

1.2.1. Milk fat

Fat is one of the most important component of milk, either in quantitative and qualitative terms. Fat is the major energy component in milk, it provides essential fatty acids and carries and dissolve fat-soluble vitamins. Moreover, it strongly affects organoleptic properties of dairy products. Milk fat content and composition is affected by several factors, including species, breed, feeding, stage of lactation and parity.

In milk, lipids are made up of triacylglycerols for about 98%; the remaning part includes, diacylglycerols, monoacylglycerols, free fatty acids, cholesterol, phospholipids and fat-soluble vitamins. The amount of components in milk fat varies according to species, as shown in Table 4.

Lipids are present in milk as globules emulsified in the aqueous phase. The diameter of the globules ranges from <1 to about 18 μm; the average fat globule diameters is largest in buffalo milk (5.92 μm), followed by cow, goat and sheep milk (4.55, 3.49, 3.30 μm, respectively) (Park et al., 2007). Globule diameter decreases as lactation proceeds, and it increases during milking (Alais, 2000). Fat globules consist of two sections: the hydrophobic lipid core (composed mainly by triacylglycerol) and the hydrophilic membrane (mainly phospholipids and glycoprotein).

Fatty acids (FA) profile is different between milk phospholipids and the triacylglycerol fraction. The former contains more polyunsaturated (PUFA) and less saturated (SFA)

FA than the latter (Gordon, 2013). About 95% of the total sterol in sheep milk is cholesterol, which comes mainly from exchange with plasma, and for a little part from mammary synthesis (Goudjil et al., 2003).

1.2.2. Milk fatty acids

Triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids and phospholipids are made up of FA. Fatty acids have a hydrocarbon chain and a carboxyl group (COOH) at the end of the molecule. About 400 different FA have been identified in milk fat. According to the length of hydrocarbon chain, fatty acids can be classified as: i) short-chain fatty acids (SCFA), that have less than 12 carbon atoms ii) medium-chain fatty acids (MCFA), that have between 12 and 18 carbons; iii) and long-chain fatty acids (LCFA), that have 18 or more carbons.

Another system of FA classification is based on their unsaturation degree. Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids have zero, one, or more than one double bonds, respectively. Table 5 shows the concentration of SFA, MUFA and PUFA in milk fat on four dairy sheep breed.

Milk fatty acids derive from two different pathways: *de novo* synthesis in the mammary gland (40%); uptake from blood lipoproteins (60%). *De novo* FA (C4:0-14:0 and half of C16:0) are synthesized starting from two volatile FA: acetate and butyrate. The latter is converted into β-idroxybutyrate in the rumen epithelium. Acetic and butyric acids originate from degradation and fermentation of feed carbohydrates, operated by rumen microbial population. In the mammary gland, acetate and β-idroxybutyrate are the carbon source for the synthesis of fatty acids operated by acetyl-CoA carboxylase (ACC) and fatty acids synthase (FAS) (Barber et al., 1997).

Long-chain FA and half of C16:0 in milk come from blood lipoproteins. They derived from dietary lipids and, in condition of negative energy balance, also from lipolysis of adipose tissue (i.e. NEFA). When feed components enter the rumen, dietary lipids are hydrolyzed by microbial lipases to release FA (lipolysis). Unsaturated FA in the rumen are rapidly converted to saturated FA in a process called biohydrogenation. This process is realized because of toxic effects of polyunsaturated fatty acids on ruminal microbes (Maia et al., 2007). The biohydrogenation occurs in two steps. The first is the isomerization that turns *cis* bond of unsaturated acids into a *trans* bond; the second is the hydrogenation that consist in hydrogen addition to remove double bonds in a fatty acids chain, converting it from unsaturated to saturated (Jenkins et al., 1993). During

rumen digestion, some dietary fatty acids and some intermediate products of biohydrogenation could avoid the rumen fermentation and reach the bloodstream without further modifications. Biohydrogenation of α-linolenic (LNA, C18:3 *n*-3), linoleic (LA, C18:2 *n*-6) and oleic (OA, *cis*-9 C18:1) acids leads to the production of conjugated linoleic acid (CLA, *cis*-9, *trans*-11 C18:2) and vaccenic acid (VA, *trans*-11 C18:1) as intermediate products, and stearic acid (C18:0) as final product (Figure 1).

VA and C18:0 can be converted in the mammary gland into CLA and OA, respectively. This process is performed by the stearoyl-CoA desaturase enzyme (SCD), also known as Δ^9-desaturase. This enzyme catalyzes the insertion of a double bond between carbon atoms 9 and 10 of a fatty acids chain (Pereira et al., 2003).

Milk fat contains a great amount of SFA, that are a risk factor of cardiovascular disease (CVD) (Caggiula et al., 1997; Nicolosi et al.,1997). However, recent studies showed that there is no significant evidence of positive association between dietary saturated fat and an increased risk of CVD (Siri-Tarino et al., 2010); furthermore, a moderate intake of SFA may have a positive effect on human health (Dabadie et al., 2005). However milk and dairy products contain also beneficial FA such as polyunsaturated fatty acids. VA is converted into *cis*-9, *trans*-11 CLA, a FA that has anticarcinogenic activity (Banni et al., 2002). Moreover, *cis*-9, *trans*-11 CLA showed anti-diabetic effects in animal and human (Khanal, 2004) and inhibitory effect on atherosclerosis (Kritchevsky et al., 2002). Linoleic acid (LA, C18:2 *n*-6) and α-linolenic (LNA, C18:3 *n*-3) acids are precursors of PUFA that are not synthesized by the human body and should be taken with diet. Omega 3-PUFA play an important role in the prevention and treatment of the heart diseases, hypertension, diabetes, arthritis, osteoporosis, other inflammatory and autoimmune disorders (Simopoulos, 2002).

The milk FA content is affected by several environmental and animal factors. One of the main factor is the species (Table 6). Some short chain fatty acids (SCFA) such as caproic (C6:0), caprylic (C8:0), capric (C10:0) and lauric (C12:0) acids, that influence cheese flavor, exhibit an higher content in sheep and goat milk compared to cow milk. The most represented FA in ruminant milk fat are usually myristic (C14:0), palmitic (C16:0), oleic (*cis*-9 C18:1), and stearic (C18:0). Cow milk contains less *trans*-11 C18:1 (VA), *cis*-9 *trans*-11 CLA (RA), and C18:3 *n*-3 (LNA) than sheep and goat milk.

1.2.3. Protein

Milk proteins have several biological functions. They are a source of amino acids, provide calcium phosphate, essential for bone growth, and they could product, through gastrointestinal processes, a bioactive peptides. Milk protein include caseins, whey (serum) proteins and minor proteins (Table 7). Non protein nitrogen (NPN) in sheep milk accounts for about 5% of total nitrogen in sheep milk.

Milk proteins are made up of two different forms: unstable micellar and soluble phase. The suspended micelles include casein, calcium phosphate with a little amount of other minerals, their structure are responsible of milk color. Soluble phase consist largely of whey proteins. Caseins represent the main portion of milk protein (76-83%) and at low pH (≤4.6), or after rennet addition, precipitate. They are synthesized in the mammary gland in response to lactogenic hormones. Casein and fat both affected significantly the cheese yield. There are four type of caseins: α_{s1}, α_{s2}, β (α_{s1}-CN, α_{s2}-CN and β-CN, respectively) which are calcium sensitive, and κ casein (κ-CN) that is calcium-insensitive. The first (α_{s1}-CN, α_{s2}-CN and β-CN) are in the centre of micelle, whereas κ-CN in the surface.

Sheep caseins are characterized by great variability among individuals and breed, mainly because of genetic polymorphism. Genetic polymorphism is the effect of changes in amino acid sequence in casein due to mutations in nucleotide sequence in genes that control the protein synthesis (Amigo et al., 2000). In sheep, the α_{s1}-Casein variants are eight (A, B, C, D, E, F, H, I), seven and five variants have been found for α_{s2}-Casein (A, B, C, D, E, F, G) and β-CN (A, B, C, X, Y), respectively, whereas κ-Casein is considered monomorphic (Selvaggi et al., 2014).

The main serum proteins are α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) synthesized in the mammary gland (Alais, 2000). The serum proteins are in the soluble phase of milk and in the whey after casein precipitation. The (α-LA is essential in the lactose synthesis (Ley et al., 1970) and (β-LG is determining for fatty acids adsorption and the transport of retinol in serum blood (Kontopidis et al., 2004). Two and three protein patterns were made evident in sheep milk for α-LA (A, B) and (β-LG (A, B and C) (Erhardt, 1989; Schmidt and Ebner, 1972). In milk there are also numerous minor protein, which include serum albumin, immunoglobulins, lactoferrin, lysozyme and lactoperoxidase (De la Fuente et al., 2013). Non-protein nitrogen consist of urea, ammonia, free amino acid, creatinine, creatin and other minor nitrogen compounds.

1.2.4. Lactose

Lactose is the main source of energy for young animal and promotes intestinal absorption of some minerals as calcium, magnesium and phosphorus. It is the main milk carbohydrates, but milk sugar include also a small amount of other compounds such as glycopeptides, glycoproteins, oligosaccharides and nucleotide sugars. Lactose regulates the osmotic equilibrium between blood stream and the mammary cells. Lactose consists of two monosaccharides, glucose and galactose. The synthesis of lactose takes place in mammary gland from hematic glucose that originates from volatile fatty acids of rumen fermentation, in particular propionate (Akers , 2002).

1.2.5. Somatic cell count (SCC)

Somatic cell in milk include two classes of cells: cells of immune system (75%) originated from blood, and epithelial cells (25%) that derive from the physiological involution of the mammary epithelium (Sharma et al., 2011). The white blood cells include neutrophils, macrophages and lymphocytes. In the uninfected mammary gland, macrophages represent an important protective mechanisms toward infection, but in infected udders the predominant cells are polymorphonuclear neutrophils (PMN) leucocytes (Harmon, 1994). The typical infection of udder is mastitis, which occurs after bacterial contamination. The increase of PMN leads to an increase of milk somatic cell count (SCC), and for this reason this parameter has been used to evaluate the health of the udder. Several methods are used for SCC determination, e.g. fluoro-optical electronic counter (Fossomatic) and Wisconsin Mastitis Test; California Mastitis Test as indirect determination of SCC of milk, has also been used. In sheep and goat milk is difficult to identify the threshold of level for SCC that separate healthy from infected mammary glands (Rosati et al., 2005). Pirisi et al. (2000), in Sarda dairy sheep, distinguished three different SCC classes: low (<500,000 cells/ml), medium (500,000-1,000,000 cells/ml) and high (1,000,000-2,000,000 cells/ml). These three classes have different percentage of infected ewes (30, 40 and 45%, respectively) (Pirisi et al., 2000). Rosati et al. (2005) found, on Sarda and Comisana ewes, a geometric average of SCC of about 1.100.000 cells/ml. Somatic cell level affect milk composition. Some minerals, such as chloride and sodium, exhibit higher contents in blood than in milk. However, they increase when SCC increases in milk (Batavani et al., 2007). It has been showed that also lactose is significantly lower in class with higher SCC, especially in mid and late lactation (Nudda et al., 2003a). In regard to effect of an increase of SCC on milk

protein, there are conflicting results. Some authors found high values of total milk proteins when SCC values are high (Albenzio et al., 2004; Bianchi et al., 2004). On the other hand, Jaeggi et al. (2003) reported in ewes group with >1,000,000 cells/ml, a lower value of total protein (5.02%) than those found in the group with <100,000 cells /ml (5.23%). High SCC in sheep milk during mastitis, could be associated with higher serum protein content (Nudda et al., 2003a). Concerning fat, milk from ewes with high SCC shows low milk fat content (Jaeggi et al., 2003). This result could be explained by a smaller synthetic and secretory capacity of infected udders. Moreover, PMN which reach mammary gland during infection, release numerous lipolytic enzymes that inflict harm on milk fat globule membrane and increase the level of free fatty acids (Azzara and Dimick, 1985). Great amount of free fatty acids could be responsible of off flavour in milk (Duncan et al., 1991).

1.2.6. Minerals

Milk minerals are classified as macroelements (Ca, P, Mg, Na, K, Cl) and trace elements (Fe, Cu, Zn, Se, Mn, I, Cr, Pb, Cd, Co, Ni). As reported in Table 8, Ca, K, P, Cl, Na and Mg are the most abundant minerals in sheep milk. The ash percentage and its composition is not constant in sheep milk because of several physiological or environmental factors such as stage of lactation, nutrition or possible environmental contamination (Rincon et al., 1994). Milk contains only 0.9% of minerals on total milk composition, despite the use of dairy product represent an important source of Ca and P. Some of milk minerals are in the soluble phase (Na, K and Cl) and during the cheesemaking process are lost in the whey. Others (Ca, Mn and P) are associated with micellar phase and are partially retained in the curd. With regard to the Ca bioavailability, it is not affected by cheese making process and therefore it does not differ between milk and cheese (Ramos and Juarez, 2011). Minerals are of nutritional and health interest. Ca is important for bone constitution, muscle contraction, it is a cofactor of enzymatic systems and plays a positive role in colorectal and prostate cancer prevention, and weight management and obesity (Kato et al., 2002; Black et al., 2002; Zemel et al., 2004). The cheese making process is affected by milk mineral content. In particular, an increase of Ca can indirectly increase the rate of enzymatic reaction, because it lowers milk pH (Lucey and Fox, 1993); moreover, the proportion of colloidal calcium phosphate influenced with pH the structure and texture of cheese (Lawrence et

al., 1987). A linear increase of rennet clotting time with increasing concentrations of NaCl has been observed (Famelart et al., 1999).

1.3. Cheese making properties

Due to its high fat and protein contents, ewe milk is mainly proceesed into commercial or handmade quality cheeses, fermented milk and ricotta. Many world-famous cheeses are produced from sheep milk, particularly in Mediterranean area. Most popular examples are Roquefort, Feta, Pecorino Romano and Manchego, produced in France, Greece, Italy and Spain, respectively. For this reason, the attention of researchers has been focused in studying strategies to improve not only nutritional traits, but also technological properties of milk. An improvement of milk coagulation properties (MCP) and cheese yield can be used as a tool to increase dairy industry efficiency. Milk coagulation properties have been extensively studied in cow (Ikonen et al. 2004; Cassandro et al. 2008; Bittante et al. 2015), whereas literature on small ruminant is quite limited (Park, 2007; Jaramillo et al. 2008; Pazzola et al. 2014). The major aim of the studies investigating MCP and cheese yield is to find the existence of a genetic basis (Othmane et al., 2002c; Puledda et al., 2016), in order to include these traits in the breeding programs.

1.3.1. Coagulation

Coagulation of milk consists in the precipitation of casein. At normal pH, any aggregation occurs because of the repulsion casein micelles that have a negative charge and the stabilizer function of k-Casein on their surface.

Milk coagulation occurs with the addition of rennet or when milk acidity value reaches the isoelectric point of the casein. Acid coagulation is obtained by means of microbial starter addition, which converts lactose in lactic acid, with consequent decrease of pH until isoelectric point of the casein. In this acid conditions, micelles lose their repulsion force and make a coagulum. Presamic coagulation is a process that uses rennet to coagulate milk. Rennet is an extract obtained from the fourth stomach of calves, kids or lambs. This compound contains the chimosin enzyme. To produce particular cheeses, extracts of some vegetable species (*Ficus*, *Cynara*) are used as a substitute for traditional animal rennet (De Sa et al., 1972; Gupta et al., 1977). Chimosin hydrolyzes the Phe_{105}-Met_{106} peptide bond of κ-Casein. From this process originates two portions: para-k-Casein (residues 1-105) and casein-macropeptide (106-169). The casein core is hydrophobic, thus when rennet modifies the κ-Casein on micelles surface, caseins begin to aggregate and make a coagulum. The consistence of curd obtained from acid

coagulation is weaker than that obtained from presamic coagulation. Cheese is obtained from drying of curd.

1.3.2. Measurements of Milk Coagulation Properties

Generally, milk coagulation properties consists in two main parameters: rennet clotting time (RCT, min) which is the interval in minute from the addition of the rennet to the gelation of the milk; the curd-firmness (CF) which is the consistence of curd measured at known time after rennet addition, for example a_{30} (mm) is the curd-firmness at thirty minutes. Various techniques and different devices have been used to monitor the coagulum formation in cheesemaking. All of these alternatives can be sorted in optical, thermal, mechanical and vibrational methods, and they have been reviewed in detail by O'Callaghan et al. (2002). MCP measures have been reported in a large number of studies. However the results are sometimes difficult to compare because of the large variability among different analytical conditions. Among commercially available instruments, the most frequently used in studies on MCP are: 1) computerized renneting meter (CRM, Polo Trade, Monselice, Italy); 2) Formagraph (FORM, Foss Electric, Hillerød, Denmark); 3) Optigraph (OPT, Ysebaert, Frepillon, France); and 4) mid-infrared reflectance spectroscopy (MIRS). CRM and FORM are mechanical instruments and measure the same parameters, RCT and CF. Mechanical measures are based on continuous recording of the movements of small loop pendulums (made of stainless steel), that are immersed in oscillating milk samples. Movements of pendulum, recorded by the device, represent the drag force of milk as a consequences of coagulation (McMahon and Brown, 1982). Moreover, FORM measures an additional parameter, curd firming time (CFT) which is the time needed until the curd is firm enough to be cut, which correspond to a width of 20 mm of diagram (k_{20}) (Zannoni and Annibaldi, 1981) . The result of Formagraph analysis is a diagram as shown in Figure 2.

OPT and MIRS belong to optical instruments that are used for the assessment of MCP. Measurements of coagulation time and curd firmness determined with the Optigraph are based on measurement of the absorbance of a near infrared radiation, which passes through the coagulating milk samples. During coagulation, induced by rennet addition, the absorbance varies depending on changes in the structure of milk, i.e. micellar structure of casein. To determine the coagulation parameters the optical information is converted using a calibration equation (Kübarsepp et al., 2005). MIR spectroscopy technique is a indirect methods to predict MCP on raw untreated milk by means of

calibration equations, without rennet addition. MIRS could be used to provide instantaneous measurements of MCP, in large scale milk sampling program, with nondestructive methods and reducing the cost of analysis (Dal Zotto et al, 2008; De Marchi et al. 2009).

1.3.3. Cheese yield

Cheese yield is defined as the amount of cheese obtained from a given quantity of milk, usually expressed as percentage. The direct measure of cheese yield is obtained by making cheese recording the weight of curd after the draining at the end of the process. The cheese yield obtained in this way is expressed as kilograms of cheese per 100 kilograms of milk or as percentage. The studies that used the cheese-making procedures to assess cheese yield have often used bulk milk (Cologna et al., 2009; Mercanti et al., 2008). The use of cheese yield for breeding purpose needs measurements on a large number of individual samples not applicable in cheese-making plants and for routine application. Such individual measurements are time consuming and required trained personnel. For this reason an inexpensive and reliable cheese micro-manufacturing methods has been developed, which used small quantities of milk in a laboratory scale (Melilli et al., 2002; Othmane et al., 2002a). In recent times, researchers have assessed the possibility of using Fourier-transform medium infrared (FT-MIR) spectroscopy to predict the final cheese yield on raw milk from dairy cows (Ferragina et al., 2013). FTIR is a very rapid biochemical and nondestructive technique. Compared with other techniques, mid-infrared reflectance spectroscopy (MIRS) is simple to use, with high sensitivity and low operational costs (Caredda, 2014).

An indirect way for obtaining cheese yield is based on the use of specific equations based on milk composition (Table 9). Predictors are usually fat and protein contents that have a major effect on cheese yield (Pirisi et al. 1994; Zeng et al., 2007). To obtain this formulas the characteristic cheese-making process on industrial scale have been assessed and statistically analyzed.

1.4. Factors affecting milk quality

1.4.1. Factors affecting milk composition

The composition of ewe milk is affected by several genetic, physiological and environmental factors. Milk composition traits and the factors affecting them in dairy sheep are well reviewed (Othmane et al., 2002b; Ploumi et al., 1998; Bencini and Pulina, 1997). Some factors could be controlled by the farmer, such as feeding or milking technique, others are specific of the animal (i.e., breed, age and stage of lactation).

The first difference can be observed between breeds specialized for milk production and other specialized for meat and wool production, respectively, as shown in Table 10. Bencini and Pulina (1997) reported variation range in the concentration of main milk components among some dairy breeds. Protein concentration varied from 4.5 in Targhee sheep to 7.3 in Comisana ewes, respectively. Fat concentration exhibited a larger variation from 5.3 in the new Zealand Romney to 9.1 in Comisana ewes, respectively.

Genetic factors affecting milk composition include genetic polymorphisms of casein (Corral et al., 2010; Moioli et al., 2007; Pirisi et al., 1999). The C variant of α_{s1}-Casein has been associated with higher total protein and casein content and smaller micelle diameters, whereas D variant showed negative effects on milk composition in Sarda dairy ewes (Pirisi et al., 1999). Corral et al. (2010) found that GG genotype of β-Casein was associated with an increase in milk production. On the other hand, the AA genotype was associated with an increase in fat and protein percentage. In dairy sheep, no association has been reported between κ-Casein (considered monomorphic) and milk yield (Staiger et al., 2010).

Among environmental factors, a major factor that impacts milk composition is the diet. There is a positive correlation between neutral detergent fiber (NDF) in the diet and milk fat concentration. A higher milk fat content might derive from a reduction of milk production caused by a decrease of dry matter intake and digestibility that occur with a high NDF content in the ration (Nudda et al., 2004). Milk fat content can be modified through addition of fat supplements to the diet. This supplements can be protected against rumen microbial activity, such as calcium soaps of fatty acids, or unprotected, such as oilseed. Differences in doses and physical form of fat sources have caused contrasting results on milk yield, fat and protein concentrations, in the studies with fat supplementation (Pulina et al., 2006). Milk protein content is harder to modify by

nutrition than the fat content. Generally, high energy diets in early lactation increase the percentage of milk protein because this type of diet helps ewes in negative energy balance and increases rumen propionate production, increasing amminoacid availability for protein synthesis (Susin et al., 1995). In contrast, when ewes are in positive energy balance in mid lactation, no effect of high energy diets on milk protein has been observed (Cannas et al., 1998). With regard to milk mineral content, macroelements (Ca, P, Mg, Na, Cl) are not affected by nutrition, whereas microelement content (Mn, Co, I, Se) can be improved by feeding (Pulina et al., 2006).

A further factors affecting milk composition is parity. A progressive increase of milk protein and fat contents with increasing number of lactations up to age 3-4 years has been reported by Sevi et al. (2000) and Othmane et al. (2002b). An increase of casein and whey protein with age has been reported for the Sarda breed (Nudda et al., 2003a).

Stage of lactation is an important source of variation of milk components. The concentration of protein (both casein and whey) and fat, total solid and somatic cell count of milk gradually increased, while the lactose content decreased progressively with the advancement of lactation (Bencini and Pulina, 1997; Cappio Borlino et al., 1997; Aganga et al. 2002). Mineral contents of milk are also affected by stage of lactation, chloride and magnesium tend to increase and potassium to decrease during lactation, respectively (Bencini and Pulina, 1997).

Usually, primiparous ewes lamb in spring and multiparous in autumn-winter, for this reason lambing season is often influenced by age of ewes. Previous research has shown that lambing season affected significantly milk yield and milk composition (Gabiña et al. 1993; Cappio Borlino et al., 1997; Sevi et al., 2004; Allah et al., 2011). Cappio Borlino et al. (1997) found that Valle del Belice ewes lambing in conventional period (from august to November) produced more milk than ewes lambed in the later season (from March to April). By contrast, in Allah et al., 2011 milk yield was higher in ewes lambed in February to March season. These different results may be due to different climatic conditions that affected the pasture availability. Milk yielded by ewes lambed in autumn in early lactation is richer in fat, protein and lactose content than ewes lambed in winter (Sevi et al., 2004).

Number of lambs born or weaned influence mainly milk yield. Previous studies have showed higher milk yields for ewes with multiple births (Snowder et al., 1990; Cappio-Borlino et al., 1995). Birth type effect is also significant on milk composition (Snowder et al., 1991; Fuerst-Waltl et al., 2005). There are contrasting literature reports on the

effect of type of lambing on fat and protein percentage. Fuerst-Waltl et al. (2005) reported low fat and high protein percentage in milk from ewes with twins, whereas Snowder et al. (1990) found an opposite effect.

1.4.2. Factors affecting milk fatty acids profile

Milk fat and milk fatty acids profile are characterized by a large variability, either in quantitative and qualitative terms. Several environmental and physiological factors affect milk fatty acids profile, in particular the diet (De La Fuente et al., 2009; Nudda et al., 2014).

The milk concentration of some useful fatty acids for human health, in particular, C18:3 *n*-3 and C18:2 *n*-6, depends on their concentration in the diet of the animal. The effect of nutrition on milk fatty acids is related to forage quality, forage/concentrates ratio and the diet supplementation with fat (Tripathi, 2014; Ashes et al., 1997). Both pasture and fat supplementation influence the process of biohydrogenation of dietary unsaturated fatty acids and improve the quality of milk fatty acids profile (Nudda et al., 2003b; Zhang et al., 2006). Another source of variation of milk fatty acids is the altitude of location of farms. Previous studies have reported a higher content of CLA and *n*-3 fatty acids in milk from mountains than milk from plain, this finding may be due to differences in feeding management and botanical composition of the pasture (Collomb et al., 2006; Cividini e Simčič, 2015).

Effect of parity on fatty acids profile has been scarcely studied in ewes. Some authors did not find any significant difference between primiparous and multiparous dairy sheep (Barbosa et al., 2003; Tsiplakou et al., 2006), whereas Mierlita et al. (2011) has found that α-linolenic (LNA, C18:3 *n*-3), linoleic (LA, C18:2) and vaccenic acid (VA, *trans*-11 C18:1) increase as lactation number increases.

Stage of lactation has a significant effect on most fatty acids. Polyunsaturated fatty acid, especially CLA, increase during the lactation (Signorelli et al. 2008; De La Fuente et al., 2009). these variations could be explained by dietary factor and pasture availability.

1.4.3. Factors affecting cheese making properties

Factors influencing milk coagulation properties are well documented in cattle (Ikonen et al. 2004; Bittante et al., 2012), ewes (Bencini, 2002; Pazzola et al. 2014) and goats (Park, 2007). Several studies showed the relationship between physic-chemical traits and the clotting properties of milk (Bencini, 2002; Ikonen et al., 2004; Park, 2007). Casein micelle size affects coagulation parameters: milk with small casein micelles has

shorter rennet clotting time (RCT) and higher curd firmness than milk with large casein micelle (Logan et al. 2014). Coagulation properties are also affected by pH and temperature. Low pH and high temperature of coagulation cause a decrease of RCT (Bencini, 2002). Cheese yield is positively and strongly correlated with milk fat and protein content and negatively with milk yield (Verdier-Metz et al., 2001; Bousselmi and Othmane, 2015). Cheese making properties are also influenced by somatic cell count. Some authors have reported that milk with low values of somatic cells presented shorter coagulation time and higher curd firmness than milk with high somatic cells count (Nudda et al., 2001; Albenzio et al., 2004; Caballero-Villalobos et al., 2015). Genetic polymorphisms of milk protein are mainly related to milk protein content, therefore they can affect the technological properties of milk (Moioli et al. 1998; Selvaggi et al., 2014). Sheep milk with CC α_{s1}-casein genotype is characterized by an higher casein content, and has better technological properties than milk with DD genotype (Pirisi et al., 1999). Positive, negative and no effect of AA β-Lactoglobulin variants on cheesemaking properties have been observed in previous studies (Pilla et al., 1995; Nudda et al., 2000; Gutiérrez-Gil et al., 2001). Concerning cheese yield, milk with CC α_{s1}-casein and AA β-lactoglobulin variants provided higher yield in cheese and Ricotta (Di Stasio et al., 1997; Lopez-Galvaz et al., 1993; Pirisi et al., 1999). Milk coagulation properties are also affected by animal-specific aspects such as breed. Duranti et al., (2003) compared three dairy sheep breeds (Comisana, Massese, Sarda). Massese ewe milk showed higher rennet clotting time, but lower rate of firming and stronger curd than Comisana and Sarda breed. No significant differences in coagulation parameters and cheese yield, between Guirra and Manchega dairy sheep, were found by Jaramillo et al. (2008).

Results about the effect of altitude on milk coagulation properties is limited in sheep, and no difference was found between milk of sheep located in lowland and hill (Martini et al., 2008).

Usually, sheep milk production is seasonal, lambings are concentrated in autumn-winter and early spring. Lambing and lactation season influence milk yield, composition and consequently the cheese making efficiency, due to differences in climate and nutrition management, during pregnancy and lactation (Cappio-Borlino et al., 1997; Sevi et al. 2004; Allah et al., 2011). Pugliese et al., (2000) reported better lactodynamographic parameters in ewes with a short lactation that started in spring than ewes that lambed in

autumn. Martini et al. (2008) did not find significant differences in cheese yield between the two lambing season of ewes.

Several studies investigated the effect of stage of lactation on milk coagulation properties (Sevi et al., 2004; Albenzio et al., 2004; Jaramillo et al., 2008; Pazzola et al., 2014). A worsening of cheese making properties of milk with the advance of lactation was observed in Italian (Sevi et al., 2004) and Spanish (Jaramillo et al., 2008) dairy sheep breeds. Milk coagulation is related with milk physicochemical composition. In late lactation, renneting parameters are characterized by a longer coagulation time and a weak curd, because of the increase of pH and somatic cells count (Pugliese et al., 2000; Sevi et al., 2004). Sarda dairy sheep showed a better coagulation parameters in mid lactation (Pazzola et al., 2014). Cheese yield is also correlated with milk composition. Milk fat and protein content are the main source of variation of cheese yield (Pirisi et al. 1994; Zeng et al., 2007). In late lactation, when milk yield decreases, fat and protein percentage increase for concentration effect and consequently cheese yield increases (Othmane et al., 2002b; Bousselmi and Othmane, 2015).

Parity is a significant source of variation of cheese-making aptitude. An increase of cheese yield in ewes with more than two lactations was reported in Spanish dairy sheep (Othmane et al., 2002b; Jaramillo et al., 2008). Positive (Sevi et al., 2000) and no effect (Jaramillo et al., 2008) of parity, on all or few clotting parameters were found in dairy sheep. In Sarda ewes, RCT, k20 and a30 were affected by parity, with significant difference only for orthogonal contrast between primaparous and multiparous ewes (1^{st} parity vs 2^{nd}+3^{rd}+4^{th}+5^{th} parities). A better renneting behavior in oldest sheep (Sevi et al., 2000; Pugliese et al., 2000) could be explained with a selection effect which occurs in the farms on the best sheep.

1.5. Genetic parameters

The main selection goal for dairy ewes has been always limited to the total milk yield per lactation. Nowadays, because of increasing global demand for dairy products and the growing interest in milk components, in particular those with potential benefits for human health, breeding strategies have moved towards new goals regarding milk quality. In order to obtain the best milk features for consumers and dairy industry, in terms of nutritional value and cheesemaking properties, sheep milk composition, fatty acids profile, and technological properties have been recently investigated in literature (Carta et al., 2008; Pazzola et al., 2014). The inclusion of these new traits in the breeding program needs to record the traits in easier and cheaper way than the current one (Cipolat-Gotet et al., 2012; Ferrand-Calmels et l., 2014; Caredda et al., 2016). But the most important step of breeding program is the genetic characterization of the traits by estimating its fundamental genetic parameters, i.e. heritability and genetic correlations. The heritability is the proportion of the phenotypic variance that is ascribable to additive genetic variance. Traits with high heritability may be easily selected, whereas lower heritability values prove that environmental factors greatly influence the phenotypic differences among animals. The genetic correlation between two traits shows the extent to which these traits are independent or not, and the proportion of their common genetic variance. High values of genetic correlation could be caused by genes at the same locus or closely linked genes which affect both traits.

1.5.1. Technological properties

As reviewed by Bittante et al. (2012), several studies were carried out on genetic parameters of cheesemaking aptitude in bovine milk, but the literature is limited for small ruminants. Generally, the conditions of analytical procedures to measure MCP vary markedly, for this reason is hardly to compare the genetic parameters estimated in different studies (Bittante et al., 2012). The genetic additive variance for RCT found in 15 previous studies, was moderate (0.26 ± 0.06); similar heritability values (0.27 ± 0.11) were reported for a_{30} (Bittante et al., 2012). With regard to $k_{20,}$ different values of heritability were found in literature. Tervala et al. (1985) found a low value of heritability corresponding to 0.02. In recent times, Cecchinato et al. (2013), estimate a moderate heritability for this trait (0.21). In general, RCT of bovine milk shows a negative genetic correlation with a_{30}, and positive with k_{20} (Cassandro et al., 2008; Cecchinato et al., 2011). In fact, high value of RCT, typical of slowly coagulating milk

samples, is correlated with a low curd firmness due to a high degree of moisture. Similarly, low values of RCT are associated with high values of a_{30} (Cecchinato et al., 2013; Cassandro et al., 2008).

As far as cheese yield is concerned, a previous study on Churra dairy sheep (Othmane et al., 2002c) reported a low value of heritability for individual cheese yield (0.08). Cheese yield had also a positive genetic correlation with the major milk components (fat, protein and casein) and was negatively correlated with milk yield (Othmane et al., 2002c, Puledda et al., 2016).

1.5.2. Milk fatty acids

Very few studies have estimated genetic parameters for milk fatty acids (FA). The limited number of studies on these traits is likely related to the high analytical costs associated to gas-chromatography (GC) analysis (Mele, 2009). The advantage of using GC to measure these traits lies in a great measurement accuracy, also with low concentrations. Recently, to obtain data with less expensive methods, several authors have tested the potentiality of mid-infrared spectroscopy (MIRs) in predicting the fatty acids composition of milk fat (Gottardo et al., 2013; Ferrand-Calmels et al., 2014; Caredda et al., 2016). Because of this differences in methodology of analysis and in the predictive statistic model used (multi-trait or single-trait models), a large variability of heritability estimates of fatty acids profile was obtained (Mele et al., 2009). Generally, previous studies have reported genetic parameters on a limited number of fatty acids both in cows (Soyeurt et al., 2007; Bastin et al.,2011) and in sheep (Sanchez et al., 2010), whereas a few authors (Bilal et al., 2014; Pegolo et al., 2016) have focused on a large number of individual and groups of fatty acids.

Milk fatty acids can originate from two different pathways: i) *de novo* synthesis in the mammary gland from acetate and 3-hydroxybutyrate; ii) the mammary gland uptake of preformed fatty acids from bloodstream, deriving from mobilization of adipose reserves or digestion of dietary feed (Chilliard et al., 2000). As reported in literature, the heritability of individual fatty acids does not exceed values of 0.55 in the three main dairy species, cow (Pegolo et al., 2016), goat (Maroteau et al., 2014) and sheep (Boichard et al., 2014).

Generally, among individual fatty acids, *de novo* individual fatty acids show the highest heritabilities estimates, ranging from 0.31 for C4:0 to 0.54 for C10:0 (Stoop et al., 2008; Bastin et al., 2011).

Heritability estimated α-linolenic, linoleic, oleic, conjugated linoleic acid (CLA, *cis*-9, *trans*-11 C18:2) and vaccenic acid, is smaller than that observed for de novo fatty acids, ranging from 0.00 for *trans*-11 C18:1 (Garnsworthy et al., 2010) to 0.42 for CLA (Stoop et al., 2008). These findings can be explained with the different origin of fatty acids. The synthesis of de novo is related to the activity of some enzyme (acetyl-CoA carboxylase and fatty acids synthase) that seem to be under genetic control. Long chain unsaturated fatty acids, in particular LNA and LA, are originated from bloodstream and are strongly influenced by diet composition and for this reason are less heritable (Bastin et al., 2011).

1.6. References

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1.7. Tables and Figures

Table 1. Milk yield (litres) in Sarda dairy ewes from 2004 to 2016

Year	Primiparous		Secondiparous		≥3 Lactations	
	n[1]	Milk yield	n[1]	Milk yield	n[1]	Milk yield
2004	33,030	141	51,684	212	121,605	222
2005	26,376	134	50,072	197	118,474	204
2006	27,610	134	49,485	198	125,293	206
2007	24,642	136	44,895	204	143,251	214
2008	27,476	135	36,635	198	156,397	203
2009	25,108	133	38,792	191	148,419	197
2010	25,595	136	49,052	190	142,892	208
2011	22,061	141	45,584	203	128,439	208
2012	22,257	141	44,318	202	126,442	216
2013	20,584	141	39,765	195	125,892	213
2014	17,777	141	34,363	203	124,770	210
2015	20,419	148	39,438	212	114,529	222
2016	24,816	150	44,328	220	112,794	228
Mean	24,442	139	43,724	202	129,938	212

Source: ASSONAPA
[1]n=number of lactations recorded

Table 2. Composition of sheep milk

Component	Average content (%)	Range (%)
Water	82.50	79.3 – 83.3
Total solids	17.50	16.2 – 20.7
Fat	6.50	5.1 – 8.7
TN[1]	5.70	4.8 – 6.6
Casein	4.50	-
Serum protein	1.00	-
Lactose	4.80	4.1 – 5.0
Ash	0.92	0.7 – 1.1

adapted from De la Fuente et al., 2013, Recio et al., 2009, Pulina, 2004
[1]TN = total nitrose (N×6.38)

Table 3. Composition of milk in Sarda dairy sheep

Traits	Mean
Fat (%)	6.55
Protein (%)	5.59
Lactose (%)	4.79
Casein (%)	4.34
Somatic cells count (cells/µl of milk)	1010
Total bacterial count (cfu/ µl of milk)	230
Freezing point (°C)	- 0.58
pH	6.72
Milk Urea Nitrogen (mg/100ml)	36.50

Source: Sardinia Breeders Association (ARAS, 2016)

Table 4. Milk fat composition (main lipid classes %) in the main three ruminant dairy species.

Lipid classes (%)	Cow	Goat	Ewe
Triacylglycerol	97.75	97.32	98.11
Diacylglycerol+Cholesterol+ Free Fatty Acid	1.81	1.89	1.45
Monoacylglycerol	0.04	0.10	0.03
Phospholipids	0.36	0.65	0.38

Adapted from Rodríguez-Alcalá and Fontecha (2010)

Table 5. Fatty acids group in four dairy sheep breed.

Fatty acids group	Awassi[1]	Massese[3]	Sarda[2]	Suffolk × East Friesian[4]
SFA	77.90	76.49	75.07	67.00
MUFA	16.90	18.05	19.20	18.60
PUFA	5.13	5.43	5.73	8.40

[1]Abbeddou et al. (2011) (% of the total fatty acids methyl esters)
[2]Correddu et al. (2016) (g/ 100 g lipids)
[3]Adapted from Mele et al. (2007) (g/100 g of fatty acids methyl esters)
[4]Zhang et al. (2006) (g/100 g of fatty acids)

Table 6. Fatty acids composition of sheep (Sarda), goat (Saanen) and cow (Holstein-Friesian) milk.

Fatty acids	Sheep[1]	Goat[2]	Cow[3]
C4:0	2.58	2.55	2.63
C6:0	2.10	1.94	1.76
C8:0	2.20	1.86	1.14
C10:0	8.98	6.04	2.86
C12:0	6.18	2.60	3.78
C14:0	13.35	8.12	11.40
cis-9 C14:1	0.33	0.10	1.07
C15:0	1.26	0.96	1.14
C16:0 iso	0.29	0.26	0.20
C16:0	29.97	23.40	31.40
cis-9 C16:1	1.18	0.52	1.32
C17:0	0.65	0.78	0.54
cis-9 C17:1	0.25	0.28	0.16
C18:0	5.43	13.50	9.76
cis-9 C18:1 (OA)	13.29	24.80	18.8
trans-11 C18:1 (VA)	1.03	1.49	0.99
CLA total	1.02	1.28	0.58
cis-9 *trans*-11 CLA (RA)	0.69	0.85	0.44
C18:3 *n*-3 (LNA)	0.74	0.74	0.59
C18:3 *n*-6	0.10	0.01	0.03

[1]Adapted from Correddu et al., 2016 (g/100 g of FAME)
[2]Adapted from Nudda et al., 2013 (mg/100 mg total FAME)
[3]Adapted from Kliem et al., 2016 and Kelsey et al., 2003 (g/100 g fatty acids)

Table 7. Protein composition in sheep milk.

Protein	Amount
Total protein (g kg^{-1})	45–66
Total casein (g kg^{-1})	42–52
αs1-Casein (% of total casein)	6.66
α_{s2}-Casein (% of total casein)	22.84
β -Casein (% of total casein)	61.60
κ -Casein (% of total casein)	8.90
Whey proteins (g kg^{-1})	10–13
α-Lactalbumin (% of total whey protein)	13.50
β -Lactoglobulin (% of total whey protein)	46.70
Minor whey proteins (% of total whey protein)	39.80

Adapted from Selvaggi et al. (2014)

Table 8. Mineral content (mg/100 g) in sheep milk

	Range
- Macroelements	
Ca	195-200
K	136-140
P	124-158
Cl	110-112
Na	44-58
Mg	18-21
- Microelements	
Fe	0.072-0.122
Cu	0.040-0.068
Zn	0.520-0.750
I	0.014
Mn	0.0053-0.009
Cr	0.004-0.040
Cd	0.003-0.006
Ni	0.001-0.040
Se	0.0031
Co	0.0004-0.009
Pb	0.0003-0.0009

Adapted from: Raynal-Ljutovac et al. (2008), Haenlein and Wendorff (2006)

Table 9. Equations estimating cheese yield (CY) (g of cheese/100 g of milk) as a function of fat and protein concentration in ewe's milk

Equation	Cheese
CY= 1.747*protein (g/100ml)+1.272*fat (g/100ml)	Pecorino Romano
CY= 1.733*protein (g/100ml)+1.257*fat (g/100ml)	Pecorino Sardo
CY=0.32 *protein(g/l)+ 0.06*fat(g/l)+1.81	Roquefort

Adapted from Pulina et al. (2006)

Table 10. Average daily milk yield, fat and percentage of milk in different meat, wool and dairy sheep breed.

	Daily* milk yield	Fat (%)*	Protein (%)*	References
Meat and wool breed				
Romney	1.281[1]	8.70	4.70	Geenty et al., (1979) [1]kg/d
Pool Dorset	0.92[2]	8.82	6.15	Knight et al., (1995) [2]L/d
Austalian Merino	1.23[3]	8.48	4.85	Bencini and Purvis, (1990) [3]L/d
Dairy breed				
Awassi	0.66-2.36[4]	6.10-8.30	4.50-5.70	Galal et al., (2008) [4]kg/d
East Friesian	2.10[5]	4.80	5.21	McKusick et al., (2001) Haenlein, (2007) [5]kg/d
Lacaune	1.63[6]	7.40	–	Haenlein, (2007) [6]kg/d

*calculated by author

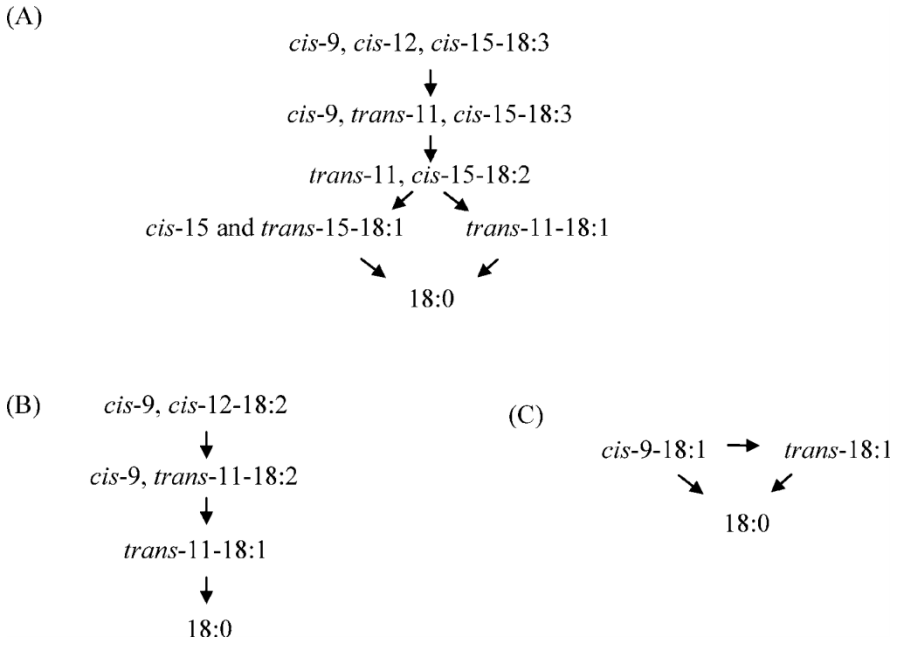

Figure 1. Biohydrogenation process of (A) α-linolenic, (B) linoleic, and (C) oleic acids. Adapted from Jenkins et al. (2008).

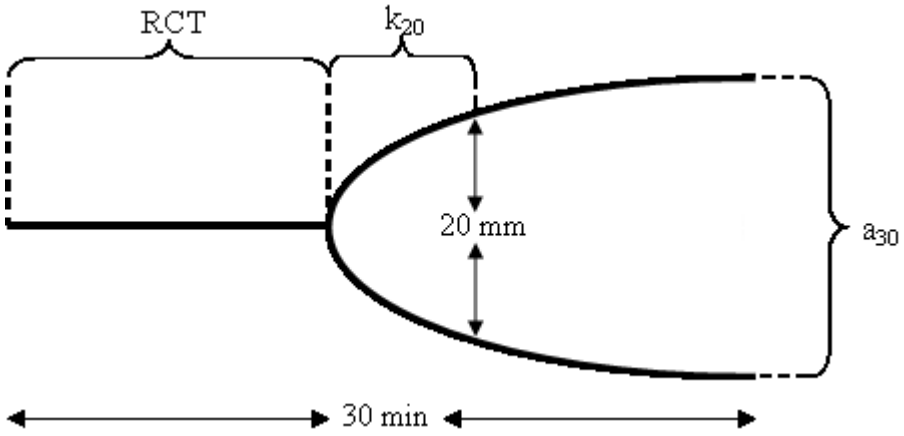

Figure 2. Diagram of coagulation and curd firmness as a function of time as recorded with the Formagraph. Adapted from McMahon and Brown, 1982.

CHAPTER 2

Derivation of multivariate indices of milk composition, coagulation properties and individual cheese yield in dairy sheep

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Abstract

Milk composition and its technological properties are traits of great interest for the dairy sheep industry, since almost all the milk produced is processed into cheese. However, several variables contribute to define milk technological properties and a complex correlation pattern exists among them. In the present work, milk composition, coagulation properties, and individual cheese yield were measured in a sample of 991 Sarda breed ewes farmed in 47 flocks. The work was aimed at studying the correlation pattern among measured variables and at obtaining new synthetic indicators of milk composition and cheese-making properties. Multivariate factor analysis was carried out on individual measures of milk coagulation parameters, cheese yield, fat, protein, and lactose percentage, somatic cell score, casein percentage, NaCl content, pH, and cryoscopy index. Four factors able to explain about 76% of the original variance were extracted. They were clearly interpretable: the first was associated with composition and cheese yield, the second with the udder health status, the third with coagulation, and the fourth with curd characteristics, respectively. Factor scores were then analyzed with a mixed linear model that included the fixed effect of parity, lambing month and lactation stage, and the random effect of flock test date. The patterns of factor scores along lactation stages were coherent with their technical meaning. A relevant effect of the flock-test date was detected, especially on the two factors related to milk coagulation properties. Results of the present study suggest the hypothesis of the existence of a simpler latent structure that regulates relationships between variables defining milk composition and coagulation properties in sheep. Heritability estimates for the four extracted factors were from low to moderate, suggesting a possible use of these new variables as breeding goals.

2.1. Introduction

Dairy sheep farming is widespread in many regions of Europe, especially in the Mediterranean area where about 63% of the entire world's sheep milk is produced (FAOSTAT, 2014). The most important producers are Greece (699,500 t/yr), Romania (651,912 t/yr), Spain (552,517 t/yr) and Italy (406,177 t/yr) (FAOSTAT, 2014). The Italian dairy sheep stock consists of about 5,500,000 heads (FAO, 2014), 60% of which are of Sarda breed. The average gross milk production of the Sarda dairy industry is about 330,000 tonnes per year (Osservatorio Regionale per l'Agricoltura, 2012). All milk is destined to cheese production. In particular, three Protected Designation of Origin (PDO) cheeses from Sarda sheep milk are currently manufactured in Italy: the Pecorino Romano (Reg. CE n.1107/96), that is largely exported in the USA (about 10,000 tonnes/ year, ISMEA), the Pecorino Sardo (Reg. CE n.1263/96) and the Fiore Sardo (Reg. CE n.1107/96).

The breeding program of the Sarda breed involves 220,268 ewes (ICAR, 2014). Current selection goals are lactation milk yield and scrapie resistance. Fat and protein percentages, and udder morphology are measured routinely only on first lactating ewes, mainly for reducing costs of phenotype recording. However there is a relevant interest in milk quality, especially on novel phenotypes related to nutritional characteristics, as milk fatty acids composition (Carta et al., 2009), and cheese making suitability.

Several variables contribute to the definition of technological properties of milk, and different phenotypes have been proposed to characterize its cheese making ability in a reliable manner. Micro-manufacturing experiments, have been suggested as a tool to quantify the individual cheese yield in sheep (Othmane et al. 2002). This variable is of great interest even though its use in routine phenotype recording programs appears rather problematic. Milk coagulation properties (MCP) are assessed measures of milk cheese making aptitude (Bittante et al., 2012; Pretto et al., 2013). They have been suggested as breeding goals for dairy cattle (Ikonen et al., 2004; Bittante et al., 2012; Chessa et al., 2013). MCP are usually defined by three variables: rennet coagulation time (RCT, min), curd firming time (k20, min) and curd firmness (a30, mm). They are measured directly by mechanical and optical techniques (Bittante, 2011). Moreover, they can be estimated by medium infrared reflectance spectroscopy (MIRS) of fresh milk by using appropriate calibration algorithms (Dal Zotto et al., 2008; Bittante et al., 2011).

Although most of literature on MCP deals with dairy cattle, some researches have been also carried out on dairy sheep. In particular, effects of fat and protein contents, temperature, pH (Park, 2007), SCC (Nudda et al., 2001), milk protein polymorphism (Piredda et al., 1993), and parity (Pazzola et al., 2014) on MCP were investigated. Moreover, environmental factors such as stage of lactation (Jaramillo et al., 2008), flock-test date (Pazzola et al., 2014) and lambing season (Martini et al., 2008), have been found to affect sheep milk renneting properties.

Some authors have pointed out that a complex correlation pattern exists between traits that contribute to define the technological properties of milk, resulting in difficulties in the elucidation of causal relationships among variables (Ikonen et al, 2004; Cecchinato et al., 2011; Bland et al., 2014). In the specific case of MCP, the high degree of correlation that exists among RCT, k20, and a30, represents a limit for the use of this variables in the interpretation of the milk coagulation pattern (Bittante et al., 2015).

Multivariate statistics offers a set of methodologies for studying and dissecting complex correlation patterns. In particular, factor analysis (FA) was used to study the correlation structure among milk compositional variables and MCP in Italian Brown Swiss cattle, allowing for the extraction of latent factors that were interpreted as indicators of milk composition, coagulation, acidity and mammary gland health (Macciotta et al., 2012). Todaro et al. (2001) used FA to study milk composition of Valle del Belice breed ewes and found three latent factors from 14 original variables, related to cheese yield, mastitis infection and aptitude of milk for cheese making. In a further work on Girgentana breed goats, the same authors extracted three factors related to coagulation time, milk yield and curd firmness (Todaro et al., 2005). Factor analysis was also used by Abilleira et al. (2010), to study relationships between milk composition, coagulation properties and season of production in Latxa breed sheep.

Aim of the present work was the study of the relationship pattern between milk composition, MCP and experimental cheese yield in Sarda breed ewes by using multivariate factor analysis. This approach was expected to generate new variables with a possible technical meaning that could be used for management and breeding purposes.

2.2. Materials and Methods

2.2.1. Animals and milk sampling

The study was carried out on 991 Sarda ewes farmed in 47 flocks (about 18 ewes per herd) located in the four historical provinces of Sardinia, Italy. All the ewes involved in the experiment were officially recorded in the Herd book of Sarda breed. The individual milk samples (1 per animal), from morning milking, were collected between April and July 2014. All milk samples were added of preservative, stored at room temperature and processed 24 h after collection. Chemical composition of milk (fat percentage, protein percentage, lactose percentage, pH, urea, NaCl) and cryoscopy index were determined by Fourier transform mid infrared spectroscopy equipment (FT-MIR) (MilkoScanTM, FOSS Electric, Denmark). Calibration algorithms were developed according to FIL-IDF rules (ISO 9622:2013; IDF 141:2013). Somatic cell count values (SCC) (FossomaticTM, FOSS Electric, Denmark) were also measured. SCC were converted by logarithm transformation into somatic cell score (SCS) as proposed by Ali and Shook (1980).

2.2.2. Analysis of milk coagulation properties

The MCP were measured using a Formagraph (Foss Electric A/S, Hillerød, Denmark). Briefly, 10 mL of each individual sample were heated to 35° C before the addition of 200 µL of rennet solution (Hansen Naturen 215, with 80 ± 5% and chymosin and 20 ± 5 % pepsin, Pacovis Amrein AG, Bern, Switzerland) diluted to 0.8% in distilled water, resulting in a final dosage of 0.034 IMCU/mL. This analysis ended within 30 min after rennet addition and produced a diagram as reported by Bittante et al. (2012). The following coagulation traits were recorded: RCT (min, that describes the time from the addition of the enzyme to the beginning of the coagulation process), k20 (min, describes the time needed to obtain a curd firmness of 20 mm) and a30 (mm, describes the width of the diagram at 30 min after the rennet addition).

2.2.3. Individual laboratory cheese yield analysis

The individual laboratory cheese yield (ILCY) was measured according to the method proposed by Othmane et al. (2002) with some modifications. Raw milk samples were heated at 40°C and gently mixed by shaking, then 10 g were exactly weighed into 15mm i.d. test tubes and kept 10 min to equilibrate at 36°C in a water bath. The employed equipment allowed to process 12 tubes at the same session. Immediately

before the coagulation, a 15 IMCU/mL rennet work solution was prepared by diluting with ultra-pure water a 1000 IMCU/mL 100% chymosin solution (CHY-MAX ® M 1000 Hansen A/S, Denmark).

A volume of 40 µL of the work solution was added to the tubes in order to reach a final dose of 0.060 IMCU/g. Then the tubes were closed and rapidly inverted, in order to ensure uniform distribution of the rennet, and left undisturbed at 36° C in a water bath for 1 h. The coagulum was then cut in the form of a cross and underwent centrifugation at 4000 rpm for 15 min at 36° C to allow whey separation. The residual whey was removed after draining for 45 min with the test-tube facing downwards. ILCY was defined as the relative weight of the centrifuge residue on the original weighed milk and was expressed in % (w/w).

2.2.4. Statistical analysis

The basic theoretical assumption of the factor model is that the total variance of a multivariate system could be partitioned into two components: one that is shared by all the variables and that is called communality; the other, that is peculiar of each variable, is named uniqueness. As a consequence, each of the n original variables could be modeled as a linear combination of p common factors that generates the communality between variables plus a residual specific (Morrison, 1976)

Multivariate factor analysis was carried out on the whole data set. Twelve variables were considered: RCT, k20, a30, individual cheese yield (ILCY), fat percentage (FP), protein percentage (PP), lactose percentage (LAC), somatic cell score (SCS), casein percentage (CAS), NaCl content (NaCl), pH, and cryoscopy index. Analysis were carried out with the PROC FACTOR of SAS (SAS Institute, 2008). A VARIMAX orthogonal rotation was performed in order to improve the readability of factors in terms of biological and technical meaning, and of relationships with the original variables.

The number of factors to be retained was assessed by examining the amount of variance explained by the extracted factors and their readability (Morrison, 1976). Factor meaning was assessed by visual inspection of patterns: a variable was considered to be significantly associated with a factor when its loading (i.e. the correlation coefficient between the variable and the extracted factor) was equal or higher 0.60 (Macciotta et al., 2012).

Individual factor scores calculated for each ewe were used as new phenotypes and analyzed with the following mixed linear model:

$$y_{ijklm} = FTD_i + LMONTH_j + DIM_k + PAR_l + e_{ijklm} \quad [1]$$

where:

y is the score of the p-th factor of the m-th ewe;

FTD is the random effect of the i-th flock- test date (66 levels) ~N(0, σ^2_{FTD});

LMONTH is the fixed effect of the j-th month of lambing (four levels: January, February, November, December);

DIM is the fixed effect of lactation stage k (8 intervals of DIM of 30 d, starting from parturition);

PAR is the fixed effect of the l-th parity (8 levels: 1 to7, and >7);

e is the random residual, ~N(0, σ^2_e).

Average lactation curves corrected for other effects included in the model were obtained by plotting least squares means of the DIM effect against days in milk (Stanton et al., 1992).

In order to evaluate the possibility of using these new extracted variables as breeding goals for the genetic improvement of milk quality and cheese making ability, their genetic background was investigated. Factor scores were analyzed with the following animal model

$$y_{ijklmn} = FTD_i + LMONTH_j + DIM_k + PAR_l + a_m + e_{ijklmn} \quad [2]$$

that had the same structure of model [1] plus the inclusion of the random additive effect (a) of the m-th animal N(0,A σ^2_A), where σ^2_A is the additive genetic variance. (Co)variance components were estimated using the VCE software (Groeneveld et al., 2010). A total of 5,671 known ancestors without records were tracked from the pedigree, up to four generation. Thus 6,551 animals were included in the numerator relationship matrix. Heritability was calculated as $\sigma^2_a / (\sigma^2_e + \sigma^2_a + \sigma^2_{FTD})$,where σ^2_e and σ^2_{FTD} are the residual and test date-variance, respectively.

2.3. Results

2.3.1. Factor Analysis

A total of 111 milk samples did not coagulate within the time period of the 30 minutes and were not considered in the analysis. The considered sample size was therefore of 880 ewes. A value of about 10% of non-coagulating milk has been reported as a common event in Finnish Ayrshire cattle (Ikonen et al., 2004). Average chemical composition, milk coagulation properties and individual cheese yield are reported in Table 1. Values observed for fat, protein and lactose percentage were in agreement with previous report for the Sarda breed either for individual (Pazzola et al., 2014; Nudda et al., 2015) and bulk tank milk data (Pirisi et al., 2000). The average of somatic cell score was similar to values previously observed in Sarda breed by Mele et al. (2006), but higher than previous reports by Nudda et al. (2003; 2015).

As far as MCP are concerned, RCT average was similar to values obtained for Sarda (Pirisi et al., 1999; Mele et al., 2006) and Spanish (Abilleira et al., 2010; Jaramillo et al., 2008; Rovai et al., 2015) sheep breeds, whereas it was markedly higher than a recent report on Sarda ewes (Pazzola et al., 2014). k_{20} and a_{30} averages were similar to previous values observed in dairy sheep (Bittante et al., 2014; Pazzola et al., 2014). The mean for ILCY was larger than values reported by Othmane et al. (2002) for Churra ewes, but similar to those found by Corral et al. (2009) for Merino ewes.

A preliminary step in factor analysis is the assessment of the suitability of the data set to this statistical approach. The evaluation is made by comparing the Pearson (r_PEA) and partial (r_PAR) correlations between the observed variables (Table 2). A marked decrease of r_PAR in comparison with r_PEA supports the factor hypothesis of an underlying latent structure that regulates correlations of the multivariate system under study. In the present study, such a decrease was observed for several pairs of variables (Table 2). Exceptions were some correlations involving lactose content, freezing point and the Sodium Chloride content: r_PEA and r_PAR tended to increase, sometimes also changing in sign. A measure of the difference between values of r_PEA and r_PAR is the Kaiser measure of sampling adequacy (MSA) (Cerny and Kaiser, 1977). The value of MSA in this study was 0.57, similar to the those reported in previous studies for dairy cattle (Macciotta et al., 2012) and goats (Todaro et al., 2005).

Four factors were retained for further analysis according to the amount of the variance explained, and to their interpretation in terms of biological meaning and relationships

with the original variables (Morrison, 1976). The four factors were able to explain about 76% of the original variance (Table 3). Their structure, after the VARIMAX rotation, was quite simple to read. Each factor exhibited few large and many small loadings, respectively. On the other hand, each variable had a large loading only in one factor and small in the others, respectively. An exception was the somatic cell score that did not reach the threshold of significance in any of the extracted factors, even though it exhibited similar moderate correlations (even though with opposite sign) with the second and third factor, respectively.

The first factor (that explained 25% of the original variance) was correlated mainly with fat, protein, and casein contents, and with ILCY. The milk of animals with higher scores for this factor is therefore richer in main components and it is able to give higher cheese yields, as expected. This factor has been considered as a COMPOSITION_YIELD index. The second factor (about 20% of the original variance explained) was positively associated with lactose percentage and freezing point, and negatively with Sodium Chloride. Lactose and NaCl contents are two indicators of the integrity of mammary gland cells and tend to decrease and increase, respectively, in ewes affected by clinical mastitis (Martì De Olives et al., 2013; Pulina and Nudda, 2004). Freezing point is an indicator of the osmotic equilibrium in milk and blood (Henno et al., 2008) Larger scores of this factor were therefore associated with higher lactose and lower NaCl contents, respectively, i.e. with a better health status of the mammary gland. It was named as UDDER HEALTH index. It is interesting to notice that SCS showed its second largest loading in this factor, even though it was below the threshold of 0.6.

The third factor presented large (>0.60) and positive loadings with pH and RCT. An increase of scores for this factor indicates a worsening of milk renneting performances. It has been therefore defined as a COAGULATION index. Also in this case it is interesting to notice that SCS was close to the threshold of significance in this factor. Finally, the fourth factor was correlated positively with a_{30} and negatively with k_{20}. So it was considered as an index of CURD characteristics.

As expected, variables expressed different communalities (Table 3). Largest values were for lactose, protein, and casein percentages; lowest for somatic cell score and freezing point. These findings are in agreement with a previous report for dairy cattle (Macciotta et al., 2012). However, values of communality for MCP were markedly larger than those reported in dairy goats (Todaro et al., 2005).

2.3.2. Mixed model analysis

The COMPOSITION factor was affected only by stage of lactation (P<0.001). Its pattern mimicked the curve of fat and protein contents (Figure 1a). An increase of ILCY across lactation stages was observed by Othmane et al. (2002). The contribution of the FTD to the phenotypic variance, calculated as $\sigma^2_{FTD} / (\sigma^2_{FTD} + \sigma^2_e)$, was 0.22.

None of the considered effects in model [1] significantly affected the UDDER HEALTH factor. However, the lactation stage was close to significance (P=0.07). A tendency of UDDER HEALTH scores to decrease, i.e. of a worsening of mammary health status, could be observed as the lactation proceeds (Figure 1b). The pattern is similar to the standard shape of the lactation curve, in agreement with a previous report in cattle for a factor with a similar meaning (Macciotta et al., 2012). The contribution of the FTD on the phenotypic variance was 0.19.

The COAGULATION factor was affected by parity (P=0.009) and stage of lactation (P=0.046). In particular, values tended to increase from first to older parities (Table 4), indicating a worsening of milk coagulation properties with the age of animals. A significant effect of parity on RCT was reported for Sarda sheep (Pazzola et al., 2014). A decrease of COAGULATION scores could be noticed across lactation stages, especially in late lactation (Figure 1c). The contribution of the FTD on the phenotypic variance was 0.25.

Finally, scores of the CURD factor were affected by lambing month (P=0.039) and stage of lactation (P=0.008). In particular, ewes lambing in November had the highest value of LS mean for the scores of this factor (Table 5), i,.e., their milk showed the best curd characteristics. The lactation pattern (Figure 1d) showed a worsening of curd characteristics at the end of lactation. The contribution of the flock-test date factor to the phenotypic variance was 0.46.

2.3.3. Genetic parameters

Heritabilities of the four extracted factors showed low to moderate values (Tables 6). The largest value of h^2 was estimated for the UDDER HEALTH scores. It was markedly larger than a previous estimate for a factor of similar meaning in dairy cattle (Macciotta et al., 2012). However, large heritability values for lactose content, which exhibits a high loading in the UDDER factor of the present study, have been estimated in Danish Holstein and Jerseys cattle (Poulsen et al., 2015). COMPOSITION_YIELD showed an h^2 of about 0.20, larger than the value estimated for ILCY on Churra dairy

ewes, but similar to heritability of protein and casein percentages estimated in the same study (Othmane et al., 2002). Moreover, it was similar to results obtained for individual cheese yield in Italian Simmental Cattle (Cecchinato et al., 2015). The heritability estimated for COAGULATION was very low (about 0.05), smaller than values usually reported for RCT (Bittante, 2012), but close to estimates reported for pH (Cecchinato et al., 2011; Bittante et al., 2012), a variable that is highly associated to this factor. The value of the CURD factor was similar to the h^2 reported for a_{30} in cattle (Cassandro et al, 2008; Cecchinato et al., 2011). The contribution of the Flock-Test-date variance to the total phenotypic variance confirmed its relevant influence on all extracted factors, especially for CURD, with values similar to those obtained with the phenotypic model [1].

2.4. Discussion

2.4.1. Factor analysis

As pointed out by many authors, a large variability of results could be observed between studies that report correlations between milk compositional and rheological traits. Values obtained in the present study (Table 2) in general agree with some previous reports for dairy species. For example, the correlation pattern between MCP is in agreement with studies in sheep (Pazzola et al., 2014) and cattle (Cassandro et al., 2008; Poulsen et al., 2015). On the other hand, low relationships between MCP and milk composition were highlighted. These results confirm previous findings on cattle (Ikonen et al., 2004) and goats (Todaro et al., 2005), whereas they are not in agreement with reports by Audilst et al. (2004) in cattle and by Jaramillo et al. (2008) in sheep, that found moderate correlations (about -0.45) between curd firmness and fat and protein percentages. Such a disagreement between studies could be partly ascribed to sampling effect. In many cases, as the present work, one sample per animal is considered and a great variability in experimental conditions between studies could be observed. On the other hand, a relevant role is also played by the used statistics. Pearson correlations do not represent adequately causal relationships between variables (de la Fuente et al., 2004). Partial correlations, whose calculation is a fundamental preliminary step in factor analysis, helps to elucidate the direct causal relationships between pairs of variables by removing the quota due to other variables. In the case of the present study, for example, the moderate positive correlation between RCT and SCS measured by R_PEA markedly decreased when R_PAR is considered (Table 2). A similar result, even if at genetic level, was observed by Tiezzi et al. (2015) that, in a study aimed at clarifying possible causal relationships between milk composition and MCP did not found any relationships between additive variations of RCT and SCS. All the above mentioned results and considerations seem to validate the suitability of the theoretical assumption of the factor model, i.e., the existence of a simpler structure with few unobservable variables that controls the correlation structure among milk compositional and technological properties.

Multivariate factor analysis confirmed its ability in analyzing complex correlation patterns, as the one existing among variables related to milk composition, coagulation properties, and laboratory cheese yield. In particular, MFA was able to extract from eleven traits four new variables able to represent a relevant quota of the original

variance, with a clear technical meaning and with moderate to high values of heritability. Factor interpretation basically confirmed some previous reports in sheep, cattle and goats (Todaro et al., 2005; Macciotta et al., 2012), even though some differences have been detected.

A factor related to MCP has been obtained by performing MFA on milk composition and coagulation properties in cattle (Macciotta et al., 2012). However, in the present study the two basic components of MCP, the coagulation and the curd characteristics, were represented in different factors (COAGULATION and CURD). A similar result was obtained in a work on bulk milk of Latxa sheep (Abilleira et al., 2010) and on individual data of Girgentana Goats (Todaro et al., 2005). The availability of two different uncorrelated indicators of MCP could represent an advantage in the studies of milk coagulation pattern that are hampered by the high degree of correlation between traditional RCT, a30 and k20 (Bittante et al., 2015). A further peculiarity of the COAGULATION factor was its association with pH, a variable strongly related to the coagulation process (Albenzio et al., 2004; Bonfatti et. al., 2011; Martì De Olives et al., 2015), in agreement with previous reports on dairy sheep (Abilleira et al., 2010) and goats (Todaro et al., 2005). The substantial independence of MCP from composition traits as somatic cell score and casein content was also observed by Tiezzi et al., (2015) in cattle. Authors concluded that selection for RCT should be performed directly on the measured variable and not to the correlated traits as milk components.

An interesting result of the present study is also the association of individual cheese yield to the COMPOSITION_YIELD factor and not to COAGULATION or CURD. These findings seem to suggest a substantial independence of the cheese yield from the coagulation process. Large correlations between individual cheese yield and milk fat content have been reported in dairy sheep (Othmane et al., 2002 GSE; Jaramillo et al., 2008). Results of the present study are in agreement with the work of Bonfatti et al. (2014) that did not find any relationship between variation in MCP and laboratory cheese yield. On the other hand Pretto et al. (2013) found a moderate correlation between MCP and Grana Padano cheese yield measured under field conditions in cheese plants. Differences in the experimental conditions (species, bulk or individual milk, laboratory vs plant cheese making) could be among the main causes for different results. Values of ILCY measured in the present study are markedly larger than industrial yield of Pecorino Romano cheese reported for Sarda ewes, on average 17.30% (Pirisi et al., 2002). Such differences could be explained with the peculiarities of the

micro-manufacturing experiments, i.e. the small amount of milk processed and the use of a forced draining (Othmane et al., 2002). In any case, a correlation 0.44 was found between ILCY values obtained in the present study and the theoretical cheese yields for Pecorino Romano predicted from milk composition using the equation proposed by Pirisi et al. (1994) (Cheese yield = 1.747 * protein content + 1.272*fat content). This equation was estimated using data from 34 industrial cheese making processes (5,000 lt each) of Pecorino Romano and Pecorino Sardo.

If the three factors related to milk composition, coagulation and curd characteristics, may have an interesting role in ranking the animals for management and breeding purposes, the UDDER HEALTH factor is undoubtedly of relevant interest. Also in dairy sheep, as in other dairy species, diseases of the mammary gland, and in particular mastitis, have a large economic impact on farm profitability (Riggio et al., 2009). UDDER_HEALTH factor was associated basically to osmotic components of milk but, quite surprisingly, not to somatic cell score as observed in a factor of similar meaning in dairy cattle (Macciotta et al., 2012). The relationship between SCS and mastitis has been assessed also in dairy sheep (Cuccuru et al., 2011; Rovai et al., 2015). However, it should be pointed out that studies on dairy sheep highlighted also the relevant role of non infectious factors in the variation of SCS (Arias et al., 2012). In the present paper SCS did not reach the threshold of 0.60 for the loading in any of the extracted factors (the largest value, 0.47, was on COAGULATION). Moreover, it did not show a large correlation with any of the considered variables; the largest R_PEA were with RCT and lactose (about 0.40 and -0.40, respectively) but they markedly decrease in absolute value as R_PAR (Table 2). The results here obtained, both for correlations involving SCS and for its absence in factor patterns, are similar to those reported in sheep (Jaramillo et al., 2008) and goats (Todaro et al., 2005).

2.4.2. Mixed model analysis and heritability estimates

Mixed model analysis of individual factor scores highlighted a relevant effect of management and environmental conditions on all the four factors, particularly on those related to MCP. It is worth mentioning that the flock-test date factor accounted for about 45% of the total variance of the CURD factor. Previous reports on Sarda and Spanish sheep breeds have underlined relevant seasonal and management effects on MCP (Abilleira et al. 2010; Pazzola et al., 2014), confirming reports in cattle (De Marchi et al., 2007). The other seasonal effect included in the model, i.e., month of

lambing, showed a significant effect ($P<0.05$) only on the CURD factor. The better values for the scores of this factor for ewes lambing in November compared to December were in agreement with results of Sevi et al. (2004) that in a study carried out on bulk tank milk reported higher a30 and lower k20 for autumn compared to winter lambings, respectively. Authors explained this result with the interaction between seasonal factors and stage of lactation. Similar results were obtained in a study on Massese breed ewes (Martini et al., 2008).

As expected, an effect of lactation stage on both COAGULATION and CURD factors was detected. The lactation pattern for CURD scores (Figure 1d) highlighted a worsening of curd characteristics in late lactation, in agreement with previous reports in sheep (Sevi et al., 2004; Albenzio et al 2004; Jaramillo et al., 2008). In dairy cattle the worst curd characteristics have been frequently observed in midlactation (Ikonen et al., 2004; Cipolat – Gotet et al., 2012; Penasa et al., 2014). The behavior of COAGULATION factor (Figure 1c) was rather unexpected. Its scores tended to decrease along the lactation, indicating a reduction of RCT as lactation proceeds. An increase of RCT with lactation stage has been observed in cattle (Ikonen et al., 2004) and in sheep (Jaramillo et al., 2008). On the other hand, Pazzola et al. (2014) in Sarda sheep reported a less defined lactation pattern of RCT, and found the shortest time for mid-lactating ewes. As previously reported, the strict seasonality of productive cycle for ewes farmed in the Mediterranean countries often results in an interaction between stage of lactation and season of production. Sarda primiparous ewes lamb at the end of the winter, whereas older parities have lambings in fall-early winter. Most of sheep are dried off between June and July (Macciotta et al. 1999), therefore when temperature increases (from May) they are in middle-late lactation. Sevi et al. (2004) reported an increase of RCT along the lactation for winter lambings, but no difference for autumn lambings. Moreover, Sitzia et al. (2015) in their review pointed out that some experiments did not found any difference in MCP between lactation stages. In fact, stage of lactation and diet quality had confounding effects in the farming system of sheep. An improvement of MCP in Sarda sheep in late lactation was obtained when animals were fed a balanced diet instead of the usual grazing on stubbles (Pulina et al., 1993). Moreover, Audilst et al. (2010) reported no negative effects of extended lactations in milk composition and cheese making properties.

The stronger effect of parity on COAGULATION, confirms previous results on Sarda sheep that underlined a lower RCT of first lambing ewes compared to older parities

(Pazzola et al., 2014). Lower RCT for primiparous cattle were reported by several authors (Tyrisevä et al., 2003; Penasa et al., 2014). Other studies carried out on cattle did not found any effect of parity on MCP (Ikonen et al., 2004; Bittante et al., 2015). Sevi et al. (2000) observed an improvement of k20 and a30 as the number of lactations increased in Comisana breed ewes but no significant differences in RCT.

All the extracted factors except COAGULATION exhibited genetic variability. Of particular interest is the moderately high value of heritability obtained for the factor related to UDDER_HEALTH. The use of the extracted factors as selection goals for breeding purposes obviously would increase costs and complexity of the selection program. However, equipments currently used for measuring milk composition are based on FT-MIR technology. The MIR spectrum could be used to predict MCP (Chessa et al., 2013) and ILCY (Manca et al., 2015) with reasonable accuracies. Thus economic efforts needed for enlarging the size of population subjected to milk quality recording could be at least partly balanced by the opportunity of predicting additional traits (MCP and ILCY) that could be used to calculate factor scores.

2.5. Conclusions

Results of the present study confirm the usefulness of the multivariate factor analysis as a tool for dissecting complex correlation patterns as the one existing among variables that contribute to define the quality and the technological properties of sheep milk. Similarly to what observed in cattle, a simple structure seems to be able to explain the correlation pattern that exists between measured variables. The four extracted factors showed a well defined technical meaning. They represent useful indicators of milk characteristics that could be potentially used for management and breeding purposes.

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2.8. Tables and Figures

Table 1. Mean and standard deviation of milk composition, coagulation properties and individual cheese yield

Trait	Mean	SD
Fat percentage	6.00	1.38
Protein percentage	5.41	0.68
Casein percentage	4.20	0.55
Lactose percentage	4.83	0.42
Somatic Cell Score	4.31	2.03
Freezing point	- 0.57	0.04
pH	6.55	0.24
NaCl	139.08	33.19
Individual cheese yield (%)	35.22	8.21
Rennet coagulation time (min.)	13.39	4.45
Curd firming time (min.)	1.75	0.73
Curd firmness (mm)	54.99	12.10

Table 2. Pearson (above the diagonal) and partial (under the diagonal) phenotypic correlations among milk composition traits and milk coagulation properties. Standard errors range were 0,010-0.029 and 0,013-0.029 for Pearson and partial correlations, respectively.

	RCT	a_{30}	k_{20}	ILCY	FP	PP	LAC	SCS	CAS	FRE	Ph	NaCl
RCT	*	-0.40	0.66	0.34	0.11	0.28	-0.27	0.40	0.27	-0.06	0.61	0.29
a_{30}	-0.02	*	-0.70	-0.30	-0.17	0.02	0.25	-0.23	0.03	0.19	-0.15	-0.23
k_{20}	0.47	-0.56	*	0.28	0.09	0.00	-0.31	0.34	-0.01	-0.20	0.36	0.36
ILCY	-0.01	-0.16	0.05	*	0.55	0.42	-0.29	0.30	0.42	0.07	0.21	0.16
FP	-0.08	-0.05	-0.06	0.45	*	0.46	-0.49	0.26	0.48	0.09	-0.06	0.17
PP	-0.08	-0.03	-0.01	0.05	-0.26	*	-0.43	0.25	0.99	0.14	-0.05	0.13
LAC	0.03	0.03	-0.07	0.26	-0.72	-0.02	*	-0.41	-0.39	0.40	0.04	-0.84
SCS	0.07	0.00	0.07	0.01	0.15	0.01	0.12	*	0.24	-0.09	0.26	0.41
CAS	0.13	0.05	-0.02	-0.00	0.21	0.99	-0.08	0.01	*	0.17	-0.06	0.07
FRE	-0.02	0.03	0.02	-0.17	0.57	-0.00	0.77	-0.11	0.08	*	0.03	-0.26
pH	0.55	0.14	-0.03	0.14	0.07	0.02	0.15	0.09	-0.04	-0.08	*	0.09
NaCl	0.08	0.06	-0.01	0.23	-0.58	0.20	-0.92	0.21	-0.30	0.66	0.09	*

Fat percentage (FP); protein percentage (PP); casein percentage (CAS); lactose percentage (LAC); Somatic Cell Score (SCS); Freezing point (FRE); Sodium chloride percentage (NaCl); Individual laboratory cheese yield (ILCY); rennet coagulation time (RCT), curd firming time (k_{20}), and curd firmness (a_{30}).

Table 3. Rotated factor pattern and variable communality

Trait	Factor 1 Composition _yield	Factor 2 Udder_health	Factor 3 Coagulation	Factor4 Curd	Communality
Fat percentage	0.72	-0.12	-0.18	-0.34	0.68
Protein percentage	0.91	-0.09	0.12	0.15	0.88
Casein percentage	0.92	-0.03	0.11	0.14	0.89
Lactose percentage	-0.42	0.87	-0.02	0.14	0.95
Somatic Cell Score	0.29	-0.42	0.47	-0.09	0.49
Freezing point	0.28	0.64	0.08	0.14	0.52
pH	-0.09	0.08	0.86	-0.08	0.77
NaCl	0.10	-0.86	0.19	-0.08	0.79
Individual cheese yield	0.62	0.03	0.17	-0.48	0.64
Rennet coagulation time	0.20	-0.14	0.82	-0.30	0.83
Curd firming time	-0.04	-0.26	0.49	-0.70	0.80
Curd firmness	0.01	0.16	-0.15	0.87	0.82
Variance explained (%)	25	19	17	15	

Table 4. Least squares means and standard errors (within brackets) of the COAGULATION factor scores for different levels of parity estimated in the mixed model analysis.

Parity	n. of ewes	LS mean	SE
First	172	-0,3014[a]	0,1252
Second	113	-0,1747[a]	0,1182
Third	138	0,0506[ab]	0,1140
Fourth	155	0,1010[ab]	0,1161
Fifth	105	0,0363[ab]	0,1263
Sixth	81	0,1816[ab]	0,1335
Seventh	59	0,2988[b]	0,1468
Eighth or greater	57	0,1577[ab]	0,1509

[a, b, c, d]=means within columns with different superscripts differ (Tukey adjusted P<0.05)

Table 5. Least squares means and standard errors of the CURD factor for different months of lambing estimated in the mixed model analysis.

Month of lambing	LS mean	SE
January	-0,2304[ab]	0,1344
February	-0,4704[ab]	0,1878
November	0,09233[a]	0,1244
December	-0,1214[b]	0,1178

[a, b,] =means within columns with different superscripts differ (Tukey adjusted P<0.05)

Table 6. Variance components and heritability estimates (standard errors in brackets) for the four extracted factors

Factor	σ^2_a	σ^2_{FTD}	σ^2_e	r^2_{FTD}	h^2
COMPOSITION_YIELD	0.130	0.142	0.451	0.196 (0.039)	0.180 (0.100)
UDDER_HEALTH	0.363	0.162	0.464	0.168 (0.042)	0.378 (0.119)
COAGULATION	0.042	0.226	0.683	0.247 (0.045)	0.046 (0.070)
CURD	0.138	0.434	0.410	0.442 (0.053)	0.140 (0.081)

σ^2_a = additive genetic variance; σ^2_{FTD} =flock-Test date variance; σ^2_e =residual variance; r^2_{FTD} = contribution of the Flock-test date variance to the total variance ($\sigma^2_{FTD} /(\sigma^2_{FTD} + \sigma^2_a + \sigma^2_e)$

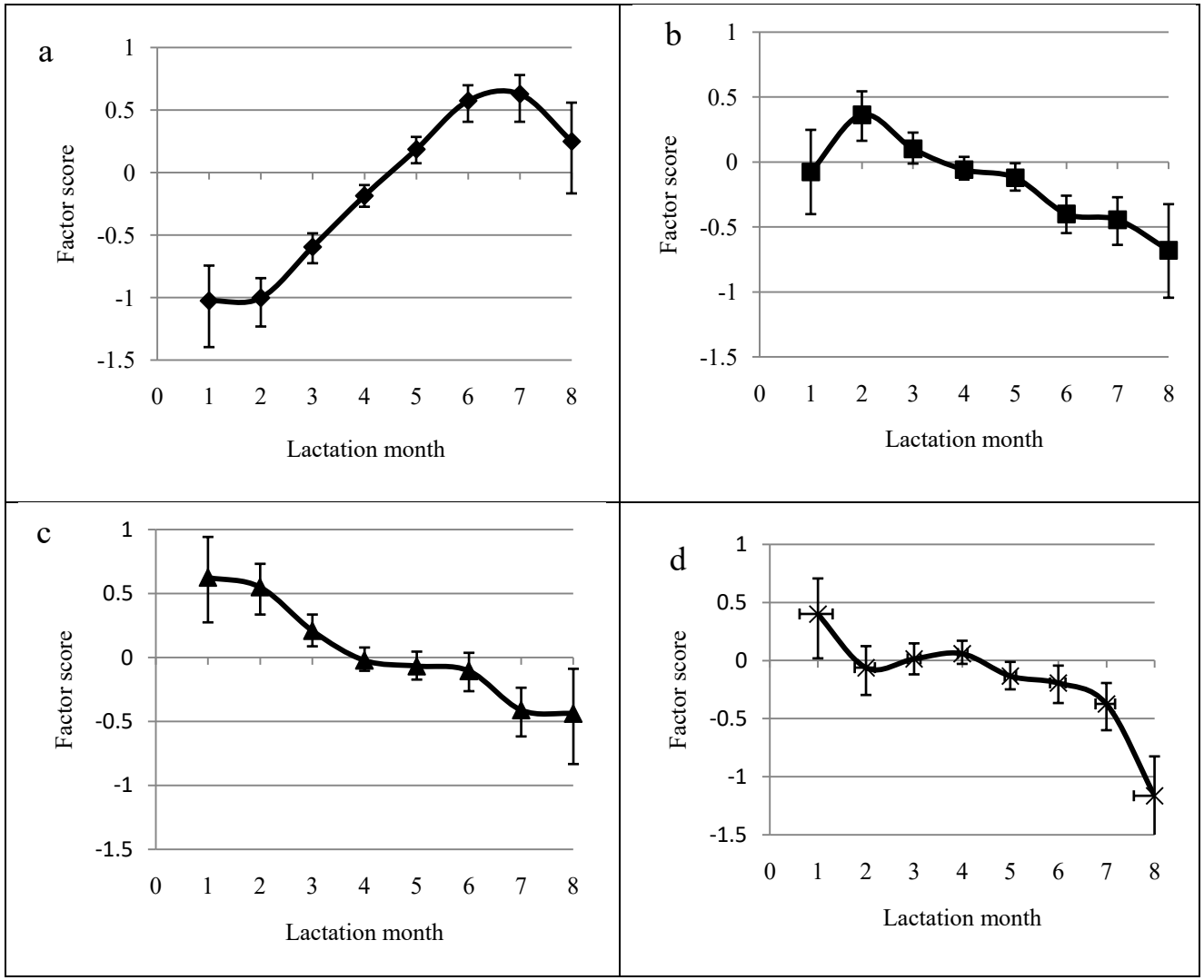

Figure 1. Pattern of the extracted common factor scores along the lactation (a= COMPOSITION; b=UDDER HEALTH; c=COAGULATION; d=CURD)

CHAPTER 3

Factors affecting milk composition, coagulation properties and individual cheese yield in dairy sheep

3.1. Introduction

Sheep milk production represents 1.3% of the total milk produced in the world (FAOSTAT, 2014). About one-third of world sheep milk is produced in Europe, especially in Southern Countries (1,984,852 tonnes). Among them, an important producer is Italy, with 372,526 tonnes of sheep milk produced in 2014 (FAOSTAT, 2014). The Sarda is the most important dairy sheep breed of Italy.

Almost all sheep milk is destined to cheese processing. Ewe's cheeses have higher fat content and better organoleptic characteristics than cheeses made from milk of other dairy species (Pappa et al., 2006). In the last decade, milk technological quality has received a great of attention, because of the worldwide increase of cheese production (FAOSTAT, 2014). The quality of sheep milk is based not only on its composition but also on its cheese making aptitude. Milk coagulation properties (MCP) are commonly defined by three parameters: rennet coagulation time (RCT, min), curd firming time (k_{20}, min), and curd firmness (a_{30}, mm). Another trait used for evaluating milk technological properties is the individual cheese yield, either predicted by milk composition using mathematical equations (Pirisi et al., 2002; Zeng et al., 2007) or measured with cheese making processes performed at laboratory scale (ILCY: Individual Laboratory Cheese Yield) (Othmane et al., 2002a). Several studies showed the relationship between milk technological traits and the main physicochemical variables such as somatic cells count (Caballero Villalobos et al., 2015; Nudda et al., 2001; Pirisi et al., 2000;), pH (Rapaccini et al., 2003), fat, protein and casein contents (Barron et al., 2001; Pellegrini et al., 1997; Bencini, 2002).

Usually, sheep breeding programmes consider as selection goals milk yield and a few milk composition traits (fat and protein contents), but not milk cheesemaking aptitude (Ugarte et al., 2001; Carta et al.2009). Previous studies on cattle and sheep found an exploitable additive genetic variation for MCP (Cassandro et al., 2008; Puledda et al., 2016). Therefore, MCP could represent interesting proxies for milk technological qualities in dairy sheep breeding programmes.

In order to evaluate the feasibility of selecting in favor of milk technological properties, a fundamental step is the identification of environmental factor affecting their variability. Animal diet is the environmental factor that can be more easily modified to improve sheep milk quality (Pulina et al., 2006). FA profile of milk is affected by the composition of dietary fat supplements (Nudda et al., 2014). Moreover, physical-

chemical characteristics of milk and, consequently, its technological properties are affected by several factors such as breed, feeding, management and other environmental factors (Pazzola et al., 2014; Park et al., 2007; Bencini and Pulina, 1997).

Most of previous studies on sheep milk technological quality have been carried out on bulk tank milk (Martini et al., 2008; Sevi et al., 2004) or on limited number of sheep sampled once per lactation. (Jaramillo et al., 2008; Jaeggi et al., 2005). Environmental factors may have effect peculiar to different lactation stages on milk technological properties. Therefore the use of repeated measures of milk technological properties along the lactation together with suitable statistical model for their analysis should be advisable. The lactation curves of milk yield and milk physicochemical composition have been studied in several studies (Nudda et al., 2005; Pugliese et al., 2000; Othmane et al., 2002b; Cappio-Borlino et al., 1997; Carta et al., 1995). On the contrary, the studies on the evolution of MCP and ILCY during lactation in dairy sheep are limited (Pugliese et al., 2000; Othmane et al., 2002b).

Aim of the present study was the investigation of the evolution of milk composition, coagulation properties and cheese yield starting, from individual records along the lactation.

3.2. Materials and Methods

3.2.1. Data collection

Individual milk samples were collected in the period January to July 2015 from 605 multiparous ewes farmed in 34 flocks located in the Island of Sardegna (Italy). Some information about distribution of sampled animals are reported in Table 1. Repeated samples were taken from each ewe approximately at monthly intervals. A total of 2,655 milk samples were collected.

3.2.2. Milk analysis

Milk samples were stored at room temperature and Bronopol preservative was added. Contents of fat, protein, casein, lactose percentage, milk urea nitrogen (MUN), pH, saturated (SFA), polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids, vaccenic acid (VA, *trans*-11 C18:1), α-linolenic acid (LNA, C18:3 *n*-3) and conjugated linoleic acid (CLA, *cis*-9, *trans*-11 C18:2) were determined by Fourier transform mid-infrared (FTMIR) spectroscopy equipment (MilkoScan, Foss Electric, Hillerød, Denmark). Somatic cell count values (SCC) were measured with the Fossomatic equipment (Foss Electric, Hillerød, Denmark). SCC were converted into logarithm to normalize the distribution in order to perform the statistical analysis. All milk samples were warmed up to 36°C before these analyses.

Milk coagulation properties (MCP) were determined with a Formagraph instrument (Foss Electric). Milk samples (10 mL), were coagulated by the addition of 200 µL of rennet solution (Hansen Naturen 215, with 80 ± 5% chymosin and 20 ± 5% pepsin, Pacovis Amrein AG, Bern, Switzerland) diluted to 0.8% in distilled water (Zannoni and Annibaldi, 1981). This analysis continued for 30 min after rennet addition. The following parameters were recorded: RCT=rennet clotting time (min): the time from the addition of rennet to the beginning of coagulation; k_{20}= curd firming time (min): the time from the start of coagulation until an amplitude for curd firmness of 20 mm; a_{30}=curd firmness (mm): measured the curd consistency 30 minutes after the addition of rennet. Individual cheese yield was measured according to the methods proposed by Othmane et al. (2002a), modified as described by Manca et al. (2016).

3.2.3. Statistical analysis

Test day records for milk composition, MCP and cheese yield were analyzed by the following mixed linear model using the PROC MIXED of SAS:

$$[1] \qquad y_{hijklmno} = \mu + DIM_h + PA_i + ML_j + TL_k + AL_l + FTD_m + EWE_n + e_{hijklmno}$$

where:

y is the observed trait; μ is the overall mean;

DIM is the fixed effect of the *h-th* lactation stage (8 intervals of 30 days each starting from lambing);

PA is the fixed effect of the *i-th* parity (1, …, 7 and >7);

ML is the fixed effect of the *j-th* month of lambing (October and November, December, January, February-April);

TL is the fixed effect of the *k-th* type of lambing (single or multiple);

AL is the fixed effect of the *l-th* altitude of location of flocks (plain=<200 mt. above the sea level; hill=>200 and <500 mt a.s.l.; mountain = >500 mt a.s.l.));

FTD is the random effect of the *m-th* flock test-date combination (196 levels);

EWE is the random effect of the *n-th* animal (605 ewes);

e is the random residual.

The random effects of model [1] were assumed to be normally distributed as $\boldsymbol{N}(0,\mathbf{I}\sigma^2_{FTD})$, as $\boldsymbol{N}(0,\mathbf{I}\sigma^2_{EWE})$, and as $\boldsymbol{N}(0,\mathbf{I}\sigma^2_{e})$,where **I** is an identity matrix, σ^2_{FTD}, σ^2_{EWE}, and σ^2_{e}, are flock-test date, ewe, and residual variances, respectively. Pairwise comparisons among different levels of fixed effects considered in model [1] were performed using a Tukey adjusted test. Average lactation curves were constructed by plotting the least squares estimates of the DIM effect against days in milk.

3.3. Results and discussion

3.3.1. Descriptive statistics

Average values for variables that define chemical composition of milk (Table 2) are in agreement with previous reports on Sarda (Nudda et al., 2015; Manca et al., 2016; Pulina et al., 2006) and other (De la fuente et al., 2013; Haenlein et al., 2006) dairy sheep breeds. Milk urea nitrogen (MUN) was rather high, similar to values reported for multiparous Sarda ewes by Buccioni et al. (2015). MUN concentrations higher than 40-50 mg/100 ml are considered as an evidence of to high crude protein concentration in the diet (Cannas, 2004). Values observed in the present study may be due to the feeding regime of Sarda ewes that especially in late winter and spring (i.e. when most of these data have been collected) is based on pasture grass that is characterized by high protein concentration. The comparison with the literature for NaCl milk concentration is difficult because of lack of information about this component. Raynal-Ljutovac et al. (2008) reports a range of 44-58 (mg/100 g) for Na and 110-112 (mg/100 g) for Cl, these concentrations can be increased during mastitis, as found in cow milk (Gaucheron, 2005; Batavani et al. 2007).

Contents of main fatty acids categories (Table 2) were consistent with previous reports on Sarda (Correddu et al., 2016) and other (Biondi et al., 2008; Zhang et al.,2006) dairy sheep breeds. However, SFA content was lower than that obtained by other studies (Nudda et al., 2014; Atti et al., 2006). With regard to values of individual fatty acids, vaccenic acid (VA, *trans*-11 C18:1), α-linolenic acid (LNA, C18:3 *n*-3) and conjugated linoleic acid (CLA, *cis*-9, *trans*-11 C18:2) were close by values obtained for Churra sheep breed (De La Fuente et al., 2009). Our values of VA and CLA were lower than those reported by Addis et al. (2005) for ewes fed with different forage species (*Lolium rigidum* Gaudin*, Hedysarum Coronarium* L. and *Medicago polymorpha* L.) and Nudda et al. (2005) in bulk tank milk of Sarda breed. However, Nudda et al. (2005) reported a value of α-linolenic acid (LNA, C18:3 *n*-3) similar to value observed in the present study. The mean values found in Correddu et al. (2016) for VA, LNA and CLA in control diet were markedly lower than our results.

The mean for individual laboratory cheese yield (ILCY) was 33.34%, it was slightly lower than previous values observed for Sarda (Manca et al., 2016) and Merino sheep breed (Corral et al., 2009), but higher than the average reported in the literature for Churra sheep (Othmane et al., 2003; 2002a,b,c). It has to be noticed that ILCY

measured in the present work exhibited larger values in comparison with previous researches on bulk milk (Martini et al., 2008; Pirisi et al., 1999), in which the gross cheese yield ranged from 17.52% to 19.95%. In Pirisi et al. (1999) the manufacturing process was carried out in a cheesemaking pilot plant, 11 kg of whole bulk milk have been used for each cheesemaking trials and the cheese loaves were salted in saturated brine for 7 h. The recording of cheese yield was made only after 24 h of draining. Salt and a long time of draining have facilitated the decrease of moisture. Higher cheese yield in our research may be due to different laboratory procedure and reduced milk amount used, as highlighted in previous studies (Othmane et al., 2002b).

As regards milk coagulation properties (MCP), the mean value of rennet coagulation time (RCT) was close to previous values reported on Massese (Martini et al., 2008) and Spanish (Abilleira et al., 2010; Jaramillo et al., 2008) dairy sheep. Analogous values were also found in Sarda ewes (Manca et al., 2016; Mele et al., 2006), but they are higher than values found by Pazzola et al. (2014) on the same breed. Average value of curd firming time (k_{20}) was lower and the mean of curd firmness (a_{30}) was higher than those reported in recent studies (Manca et al., 2016; Pazzola et al., 2014), respectively.

3.3.2. Mixed model analysis

The stage of lactation (DIM) affected significantly almost all considered traits (Table 3) (P<0.001), except from k_{20}.This latter result is in contrast with a previous study on sarda sheep (Pazzola et al., 2014).

The altitude of location of flocks did not affect milk composition, fatty acids profile, and MCP. A slight effect of altitude (P=0.0546) was observed only for individual cheese yield (Table 3). These results confirm previous studies on Sarda (Mangia et al., 2007) and Massese (Martini et al., 2008) ewes that did not found significant effect of altitude of location of flocks on milk technological properties and fatty acids profile. On the contrary milk yield was affected (P=0.0122) by level of altitude, in agreement with a previous study on Sarda sheep that reported an higher milk yield for ewes farmed in flock located in plain than for those farmed on mountain flocks, especially in the first phase of lactation (Macciotta et al., 1999).

Type of lambing affected significantly only the vaccenic acid content (Table 3). Some studies on sheep highlighted an effect of type of lambing on milk fat percentage (Snowder and Glimp, 1990; Fuerst-Waltl et al., 2005; Othmane et al., 2002c) but none of them has considered the fatty acids profile.

Parity was an important source of variation for milk yield, and some milk composition traits (fat and lactose percentage, MUN, NaCl, SCC, PUFA) (Table 3). As far as MCP are concerned, parity affected both RCT and curd a_{30}. Statistically significant differences were not found between different parities after Tukey's test for MUN, RCT and a_{30} (data not shown).

Month of lambing influenced several milk composition traits, curd firming time and similarly PUFA and individual milk fatty acids (VA, LNA, CLA) (Table 3). In this study, least squares means of these variables could be affected by the difference in lactation length because ewes that lambed in late winter (after January) had a smaller number of measurements than those lambed in late fall.

The proportion of phenotypic variance explained by FTD was large, especially for milk fatty acids (Table 3). In particular, values greater than 0.50 were observed for PUFA, VA and CLA. Such a relevant weight of FTD variance could be ascribed to management and seasonal effects. It has been widely assessed that milk fatty acids profile changes as a consequence of variations in the chemical and nutritional composition of pastures that occur in the different seasons of the year (Tripathi, 2014; Correddu et al.2016; Nudda et al., 2005).

The lactation curve for milk yield (Figure 1) does not show the standard shape typical of dairy cattle, characterised by a first increasing phase till a peak followed by a decreasing phase (Macciotta et al., 2005). The lactation peak is expected at around 4 weeks in sheep but in the ovine farming system of the Mediterranean area the milk of the first month of lactation is suckled by the lamb (Carta et al., 1995; Cappio Borlino et al., 1997).

The effect of parity on daily milk yield was significant (Table 4). Ewes from third to sixth lactation exhibited higher milk yields than younger or older ones. In this study, a slight increase of milk production with parity was observed up to fourth lactation, in agreement with previous results obtained in Sarda sheep (Macciotta et al., 1999).

Lambing season had a significant effect on milk yield (Table 4). Ewes which had lambed in October and November produced less milk than those with lambings in December and January, probably due to the differences in feeding which was based on hay during the winter months (early lactation), as herbage was not available. Similar findings were reported in Sarda dairy sheep by Fois et al. (1997). Different results were reported by Cappio Borlino et al. (1997), that found that Valle del Belice ewes lambing

in winter (from December to February) had a lower curve for milk yield than ewes that lambed in conventional period (from August to November).

In agreement with Macciotta et al. (1999), who reported an influence of altitude of farm location on milk yield, in this study significant differences were observed between ewes farmed in hill flocks, which showed the higher yield, and those farmed in mountain, with intermediate values for plain farms (Table 4). These results may be due to the differences in nutrition and management system among the different levels of altitude.

As expected, lactation curves for fat, protein and casein content exhibited an opposite shape compared to milk yield (Figure 2), as observed in other studies on dairy sheep (Carta et al., 1995; Cappio-Borlino et al., 1997; Ploumi et al., 1998; Othmane et al., 2002b). A significant effect of parity was observed for fat content (Table 5). High values of fat content in ewes with parity higher than seven may be a consequence of decrease of milk yield. These patterns support the findings of Sevi et al. (2000) and Mierlita et al. (2011) for Comisana and Romanian ewes, respectively. Protein and casein milk content were affected by season of lambing (Table 6). Milk from ewes lambed in October to December months showed a higher protein and casein content higher than ewes lambed from February to April. These results were in agreement with reports of Fois et al. (1997), which observed the highest protein contents in milk from ewes lambed in autumn. On the other hand, results of the present study were in contrast with that observed in Cappio-Borlino et al. (1997), which found the highest protein contents in milk of ewes that lambed in March to April months and the lowest protein contents for winter lambings.

Lactation curves for lactose and pH exhibited a decrease along lactation whereas $\log_{10}$SCC and NaCl content showed an ascending pattern, respectively (Figure 3). These results were in agreement with previous reports in sheep (Ploumi et al., 1998; Sevi et al., 2000). These four variables are considered indicators of the health status of the mammary gland. In milk of animals affected by clinical and subclinical mastitis, SCC and mineral (sodium and chloride) increase (El-said et al., 1998; Gaucheron, 2005; Batavani et al., 2007) and lactose decreases (Leitner et al., 2004), respectively. During the course of inflammation, immune cells move from blood towards the mammary gland leading to an increase of SCC in milk (Pirisi et al., 2000). Moreover, the degradation of tight junctions complex of the epithelial cells allows the diffusion of lactose from milk to blood and of sodium and chloride in the opposite way (Batavani et al., 2007; Bruckmaier et al., 2004). However, occurrence of high values of SCC and low

of lactose contents in sheep are not only due to mastitis, but they could also ascribe to other factors as flock management, age of the animals and lactation stage (Menzies and Ramanoon, 2001). An increase of SCC at the end of lactation could be due to a concentration effect and to physiological changes of mammary gland such as involution of the mammary epithelium (Wiggans and Shook, 1987; Colditz, 1988). Usually, in healthy ewes milk lactose concentration is constant.

Our results showed a decrease of lactose content with increasing number of lactations (Table 7), and it was significantly higher in the second lactation than in the older ewes (>7 lactation). Similar results were reported for sheep in previous studies (Ploumi et al. 1998; Sevi et al., 2000; Leitner et al., 2004). On the other hand, NaCl and SCC, $\log_{10}$ increased with increasing number of lactation (Table 7), with the lowest values in milk from ewes at second lactation. Pulina et al. (1990) explained this pattern in milk component as the consequence of a worsening of udder health status as the number of lactations increased.

Month of lambing had a significant effect on lactose and NaCl content of milk (Table 8). High level of lactose and low value of NaCl found in milk from winter lactation may be due to a better intramammary epithelial integrity. This udder health status could originate from a good nutritional management during the transition period. Pasture availability can take advantage of temperate climate in autumn and early winter typical of Mediterranean area. The highest values of MUN in milk from ewes lambed in October to January months, supports this theory (Table 8). There is a strong correlation between milk urea and protein content of diet (Pulina et al., 2006). Decreasing values of MUN during lactation in the present study (Figure 4) could be justified with seasonal variation in protein content of pasture forage plant, from winter to summer.

As expected, milk fatty acids profile was affected by stage of lactation (De La Fuente et al., 2009). As lactation progressed, LNA, VA and CLA contents decreased (Figure 5). Such patterns, already observed in this breed (Nudda et al., 2005), can be explained with the role of these FA and with the seasonality of Sarda sheep productive cycle. LNA is the precursor of CLA and VA. LNA content is high in pasture grass, which represents the main feeding source of Sarda sheep, especially in spring when plants are in vegetative stage. In this period of the year, most of sheep are in early or middle lactation. This is because the Sarda sheep has two lambing seasons: late fall for multiparous ewes, late winter-early spring for first lambing ewes, respectively. As lactation proceeds, i.e. in late spring, pasture LNA content decrease because grass turns

into reproductive stage. As a consequence, also the milk content of these fatty acids and of his products decreases.

As far as main FA groups are concerned, SFA proportion showed a slight increase in early lactation, followed by a decrease from mid to late lactation (Figure 6). This result is in agreement with those reported by Signorelli et al., (2008). On the other hand, MUFA and PUFA showed an increasing and a slightly decreasing trend, respectively (Figure 7). Higher values of MUFA in late lactation could be explained with a greater Δ-9Desaturase activity in this period, as shown by increasing of desaturation index of CLA *(cis*-9 *trans*-11 CLA / (*trans*-11 C18:1+ *cis*-9 *trans*-11 CLA)) (Figure 8). Probably, in early lactation, the desaturation activity of mammary gland was inhibited by a higher amount of PUFA (Sessler and Ntambi, 1998).

Although data in the literature on the effect of parity on milk fatty acids profile are limited, it is indisputable that this factor affects milk fatty acids composition in cattle (Kelsey et al., 2003) and sheep (De la Fuente et al., 2009; Mierlita et al., 2011). Present study found a marked reduction in PUFA content of milk from ewes with eight or more lactations (Table 9). Decrease of PUFA content in milk with parity or age of ewes has been previously reported (De la Fuente et al., 2009; Mierlita et al., 2011). This may be partially explained by changes in activity of mammary gland and in rumen microflora of later parity sheep.

Ewes that had lambed in early winter had a higher milk fatty acids contents than the ewes which had lambed from later winter to spring (Table 10). Mele et al. (2007) did not find any significant influence of the season lambing effect, so further studies in ewes would be needed to support our results and to determine their physiological or metabolic meaning.

Type of lambing significantly affected VA milk content (Figure 9). The difference is marked in late lactation; the milk of ewes with one lamb had more VA than milk of ewes with twins. Some authors have studied the effect of birth type in milk yield and milk composition (Cappio Borlino et al., 1997; Fuerst-Waltl et al., 2005) but none of them had studied the effect of birth type on milk fatty acids profile to compare our results.

The lactation curve for RCT showed a decrease in the first 140 days of lactation followed by an increase (Figure 10). The curd firmness exhibited an opposite pattern (Figure 11). These results are in agreement with a previous report on Sarda ewes (Pazzola et al., 2014). On the other hand, Sevi et al. (2004) did not find relevant changes

of RCT along the lactation. In Comisana breed ewes that lambed in autumn. Moreover, these authors observed a decrease of a_{30} as lactation progressed. An overall worsening of coagulation properties from mid to late lactation has been reported by several authors (Sevi et al., 2004; Jaramillo et al., 2008). The poor cheese-making aptitude, characterised by long RCT and weak curd consistency, observed at the end of lactation could be explained by physiological changes in ewes that result in a relevant modification of milk composition, such as an increase of SCC, casein and protein content (Pellegrini et al., 1997; Nudda et al., 2001). Curd firming time (k_{20}) was strongly affected by season of lambing (Table 11). K_{20} is the time needed to reach curd firmness to be able to properly cut the curds. This coagulating behavior of milk may be due to effects of climatic and nutritional factors related to lambing season.

Cheese yield was also affected by lactation stage. In particular, it exhibited an increase as lactation proceeds (Figure 12). These results agree with those reported on Guirra, Machenga (Jaramillo et al., 2008) and Churra (Othmane et al., 2002b) dairy breeds. The lactation curve for cheese yield was similar to those of fat and protein contents. It is widely assessed that fat, protein and casein content, represent the main constituents of total solids of milk that contribute to cheese yield (Pulina et al., 2006; Verdier-Metz et al., 2001; Wendorff, 2003). Fat and protein content are often used as predictors of cheese yield (Pirisi et al., 1994; Barillet et al., 1996; Zeng et al., 2007). Some studies observed a reduction of curd draining at the end of lactation due to an increase of total solids (Jaramillo et al., 2008; Pellegrini et al., 1994). The increase of cheese yield in this stage could be also due to a reduction of curd draining capacity.

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3.5. Tables and Figures

Table 1. Distribution of animals (n=605) involved in this study in different level of flocks, parities and provinces.

Item	class	Count
Flock	34	17.79[1]
Number of lactation	2	103
	3	86
	4	108
	5	108
	6	68
	7	67
	>7	65
Province	Cagliari	179
	Nuoro	187
	Oristano	101
	Sassari	138

[1] means of ewes sampled per flock

Table 2. Descriptive statistics for MCP, cheese yield, and milk composition traits

Variables	Min	Max	Mean	SD
Physical-chemical composition[1]				
Fat (%)	2.05	15.88	5.95	1.38
Protein (%)	3.60	9.67	5.29	0.54
Casein (%)	2.63	7.60	4.07	0.45
Lactose (%)	3.65	5.80	4.90	0.29
MUN (mg/100 ml)	9.90	99.90	44.52	13.42
NaCl (mg/100 ml)	57.60	324.70	136.37	34.04
SCC, $\log_{10}$	0.60	4.48	2.43	0.66
pH	4.68	7.03	6.66	0.15
Fatty acids[2]				
SFA	45.61	94.25	64.53	5.24
MUFA	7.61	41.68	22.63	4.97
PUFA	0.77	12.90	5.51	1.35
trans-11 C18:1, VA	0.01	6.54	2.19	1.10
C18:3 n-3, LNA	0.01	7.69	1.04	0.51
cis-9, *trans*-11 CLA, CLA	0.01	3.01	0.94	0.45
Technological properties[3]				
Cheese yield (%)	6.10	70.98	33.34	8.41
RCT (mm:ss)	01:22	30:00	13:10	04:48
k_{20} (mm:ss)	00:30	18:07	02:18	01:08
a_{30} (mm)	0.00	129.36	48.33	12.03

[1]MUN = milk urea nitrogen; SCC = cells/µl of milk;
[2]SFA, UFA, MUFA, PUFA = satured, unsatured, monounsatured and poliunsatured fatty acids (g/100 g of fat); VA = vaccenic acid (g/100 g of FAME); LNA = alfa linolenic acid (g/100 g of FAME); CLA = conjugated linoleic acid (g/100 g of FAME);
[3]RCT= rennet coagulation time, mm:ss = minutes:seconds; k_{20}=curd firming time, mm:ss = minutes:seconds; a_{30}=curd firmness, mm=millimeters;

Table 3. Statistical significance (*P*-value) of the effects of various factors on the test-day variables

	Factors[4]					
Variables	DIM	PA	ML	TL	AL	r^2_{TD}[5]
Milk yield	**<.0001**	**<.0001**	**0.0009**	0.2956	**0.0122**	0.49
Physical-chemical composition[1]						
Fat (%)	**<.0001**	**0.0067**	0.6149	0.5445	0.0877	0.41
Protein (%)	**<.0001**	0.8808	**<.0001**	0.1519	0.0733	0.17
Casein (%)	**<.0001**	0.8847	**<.0001**	0.2330	0.0737	0.17
Lactose (%)	**<.0001**	**0.0213**	**<.0001**	0.1268	0.3648	0.27
MUN (mg/100 ml)	**0.0309**	**0.0043**	**0.0002**	0.6953	0.9532	0.51
NaCl (mg/100 ml)	**<.0001**	**0.0015**	**<.0001**	0.0610	0.1635	0.22
SCC, $\log_{10}$	**<.0001**	**<.0001**	0.1769	0.4035	0.6630	0.07
pH	**0.0008**	0.6968	0.1157	0.4370	0.0656	0.45
Fatty acids[2]						
SFA	**<.0001**	0.3297	0.3950	0.2697	0.3726	0.43
MUFA	**<.0001**	0.2896	0.3587	0.1368	0.5105	0.49
PUFA	**<.0001**	**0.0002**	**0.0003**	0.3216	0.0776	0.59
trans-11 C18:1, VA	**<.0001**	0.4151	**<.0001**	**0.0035**	0.1184	0.69
C18:3 n-3, LNA	**<.0001**	0.1313	**0.0004**	0.0980	0.5012	0.44
cis-9, *trans*-11 C18:2, CLA	**<.0001**	0.1012	**<.0001**	0.1977	0.0839	0.61
Technological properties[3]						
Cheese yield (%)	**0.0099**	0.1270	0.7128	0.3435	0.0546	0.41
RCT (mm:ss)	**0.0008**	**0.0422**	0.9154	0.3036	0.8319	0.22
k_{20} (mm:ss)	0.1593	0.0642	**0.0138**	0.5167	0.0843	0.10
a_{30} (mm)	**0.0013**	**0.0345**	0.8523	0.5182	0.1605	0.16

[1] MUN = milk urea nitrogen; SCC = cells/µl of milk;
[2] SFA, UFA, MUFA, PUFA = satured, unsatured, monounsatured and poliunsatured fatty acids (g/100 g of fat); VA = vaccenic acid (g/100 g of FAME); LNA = alfa linolenic acid (g/100 g of FAME); CLA = conjugated linoleic acid (g/100 g of FAME);
[3] RCT= rennet coagulation time, mm:ss = minutes:seconds; k_{20}=curd firming time, mm:ss = minutes:seconds; a_{30}=curd firmness, mm=millimeters;
[4] DIM=day in milk; PA=parity; ML=month of lambing; TL=type of lambing; AL= altitude;
[5] r^2_{TD}= contribution of the flock-test date variance to the total variance;

Table 4. Least squares means and standard error of some factors affecting daily milk yield (l/d)

Effect	Levels	Milk yield (l/d)	SE
Parity	2	0.8910 [bc]	0.030
	3	0.9850 [a]	0.031
	4	0.9928 [a]	0.029
	5	0.9798 [a]	0.030
	6	0.9434 [ab]	0.032
	7	0.8853 [bc]	0.032
	8	0.8119 [c]	0.033
Lambing month	October and November	0.8794 [b]	0.023
	December	0.9350 [a]	0.025
	January	0.9785 [a]	0.035
	February-April	0.9152 [ab]	0.040
Altitude of location of flocks	Plain	0.9221 [ab]	0.033
	Hill	1.0015 [a]	0.024
	Mountain	0.8575 [b]	0.056

[a-c] means with different superscripts within the same effect are different (P<0.05)

Table 5. Least squares means and standard error of fat percentage for parity

Parity	Fat (%)	SE
2	5.85 [b]	0.121
3	5.84 [b]	0.123
4	5.95 [b]	0.116
5	5.99 [ab]	0.118
6	5.96 [ab]	0.126
7	5.95 [ab]	0.126
8	6.27 [a]	0.131

[a-b] means with different superscripts within the same column are different (P<0.05)

Table 6. Least squares means and standard error of protein and casein percentage for lambing month

		Protein (%)		Casein (%)	
Effect	Levels	Mean	SE	Mean	SE
Lambing month	October and November	5.33 [a]	0.032	4.10 [a]	0.026
	December	5.32 [a]	0.038	4.09 [a]	0.030
	January	5.20 [ab]	0.058	3. 10 [ab]	0.047
	February-April	5.02 [b]	0.064	3.85 [b]	0.052

[a-b] means with different superscripts within the same column are different (P<0.05)

Table 7. Least squares means and standard error of lactose, NaCl and with different parities

Parity	Lactose(%)	SE	NaCl (mg/100 ml)	SE	SCC, log_{10}	SE
2	4.85 [a]	0.026	142.67 [b]	3.436	2.38 [d]	0.062
3	4.79 [ab]	0.027	156.06 [a]	3.666	2.38 [d]	0.068
4	4.82 [ab]	0.025	146.35 [ab]	3.337	2.41 [cd]	0.061
5	4.79 [ab]	0.026	151.72 [ab]	3.409	2.52 [bcd]	0.062
6	4.78 [ab]	0.028	155.44 [a]	3.832	2.66 [ab]	0.071
7	4.77 [ab]	0.028	156.11 [a]	3.831	2.64 [abc]	0.072
8	4.76 [b]	0.029	155.72 [a]	3.983	2.76 [a]	0.075

[a-d] means with different superscripts within the same column are different (P<0.05)

Table 8. Least squares means and standard error of lactose, NaCl and MUN for lambing month

Lambing month	Lactose (%)	SE	NaCl (mg/100 ml)	SE	MUN (mg/100 ml)	SE
October and November	4.86 [a]	0.018	141.66 [c]	2.382	44.36 [a]	1.076
December	4.79 [b]	0.021	149.59 [b]	2.780	42.87 [a]	1.176
January	4.80 [ab]	0.031	152.26 [ab]	4.191	41.78 [a]	1.576
February-April	4.73 [b]	0.035	164.53 [a]	4.592	36.94 [b]	1.830

[a-c] means with different superscripts within the same column are different (P<0.05)

Table 9. Least squares means and standard error of PUFA for parity Parity

	PUFA (g/100 g of fat)	SE
2	5.46 [a]	0.127
3	5.44 [a]	0.128
4	5.31 [a]	0.123
5	5.27 [ab]	0.124
6	5.39 [a]	0.130
7	5.26 [ab]	0.130
8	5.01 [b]	0.133

[a-b] means with different superscripts within the same column are different (P<0.05)

Table 10. Least squares means and standard error of individual fatty acids for lambing month

Lambing month	VA (g/100 g of FAME)	SE	LNA (g/100 g of FAME)	SE	CLA (g/100 g of FAME)	SE
October and November	2.18 [a]	0.078	1.04 [a]	0.039	0.94 [a]	0.038
December	1.93 [b]	0.083	1.00 [a]	0.043	0.85 [b]	0.040
January	1.80 [b]	0.102	0.95 [a]	0.059	0.77 [b]	0.049
February-April	1.55 [c]	0.120	0.77 [b]	0.068	0.65 [c]	0.057

[a-c] means with different superscripts within the same column are different (P<0.05)

Table 11. Least squares means and standard error of k_{20} for lambing month

Effect	Levels	k_{20} (mm:ss)	SE
Lambing month	October and November	02:16 [b]	00:04
	December	02:29 [a]	00:05
	January	02:26 [ab]	00:07
	February-April	02:22 [ab]	00:08

[a-b] means with different superscripts within the same column are different (P<0.05)

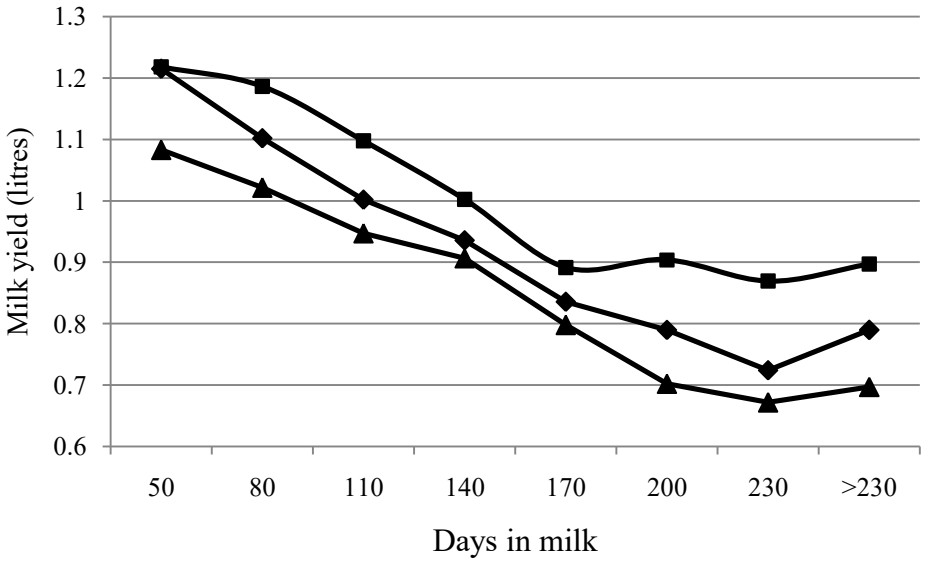

Figure 1. Lactation curves for ewes producing at different altitudes (♦ = plain; ■= hill; ▲= mountain).

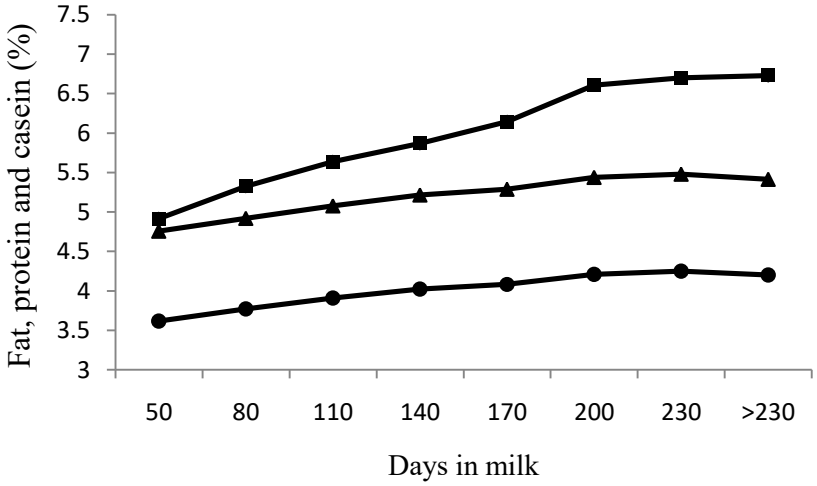

Figure 2. Lactation curves for fat (■), protein (▲) and casein (●) percentage during lactation.

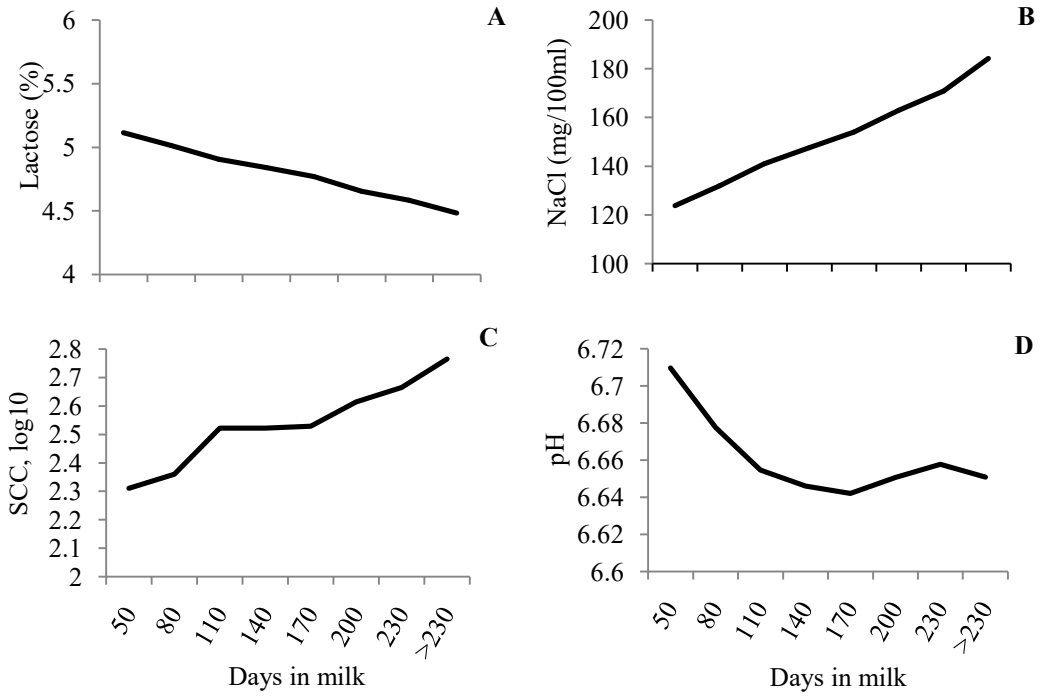

Figure 3. Changes in lactose, NaCl, log_{10}SCC and pH during lactation.

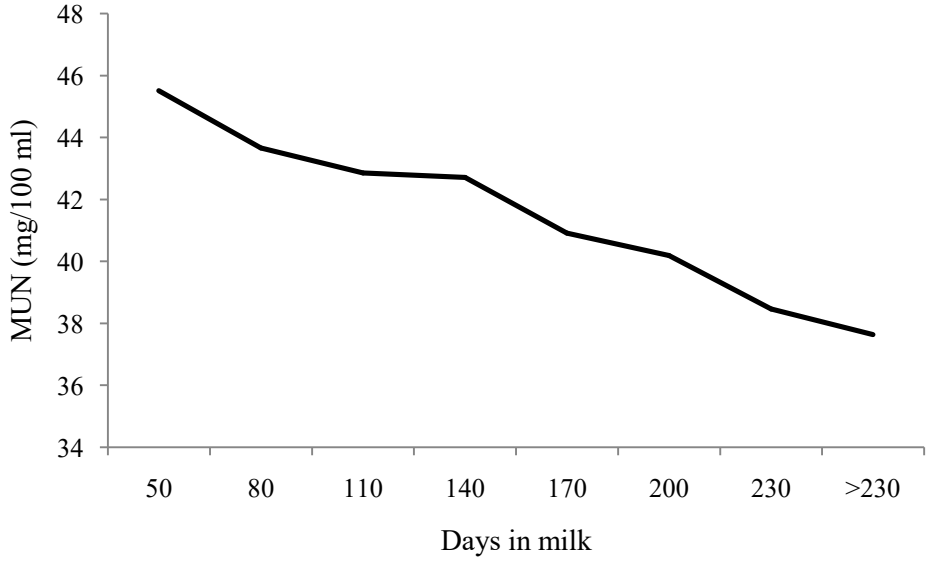

Figure 4. Changes in MUN during lactation.

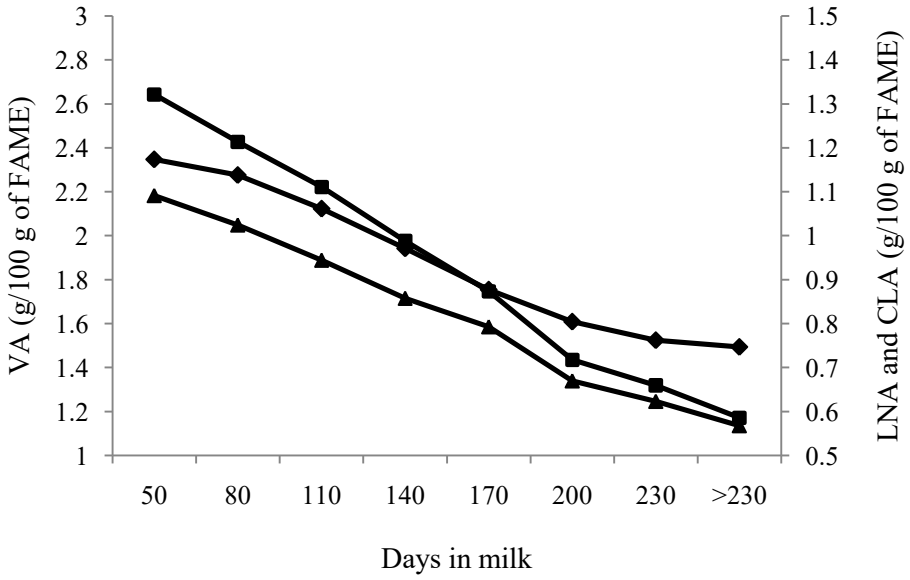

Figure 5. Evolution of some individual fatty acids:VA (*trans*-11 C18:1) (■), LNA (C18:3 *n*-3) (♦) and CLA (*cis*-9 *trans*-11 CLA) (▲), during lactation.

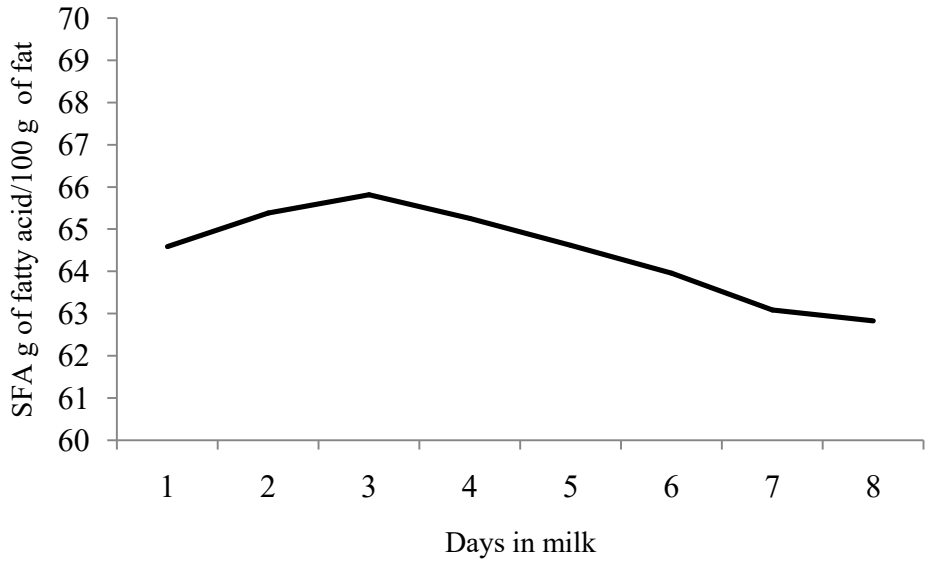

Figure 6 .Evolution of SFA during lactation.

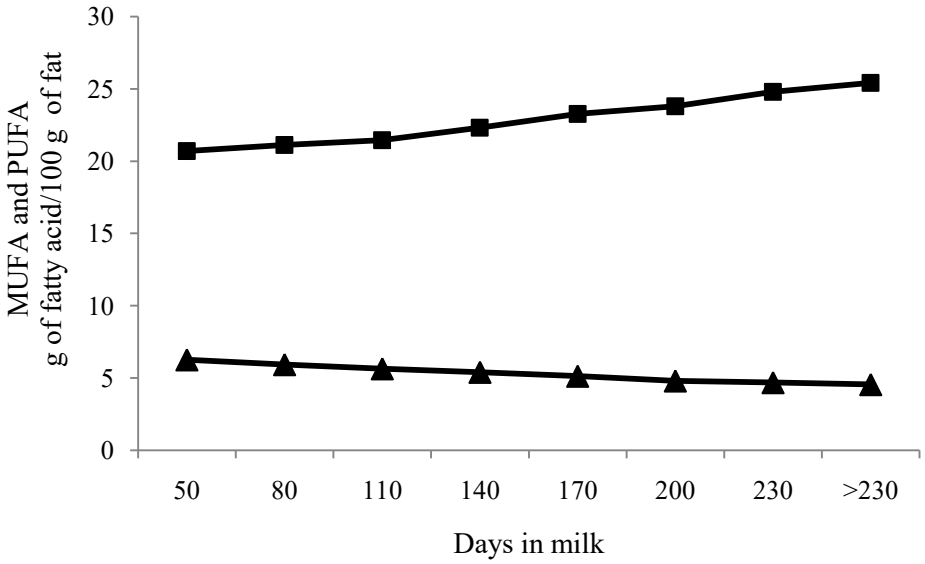

Figure 7. Evolution of MUFA (■) and PUFA (▲) during lactation.

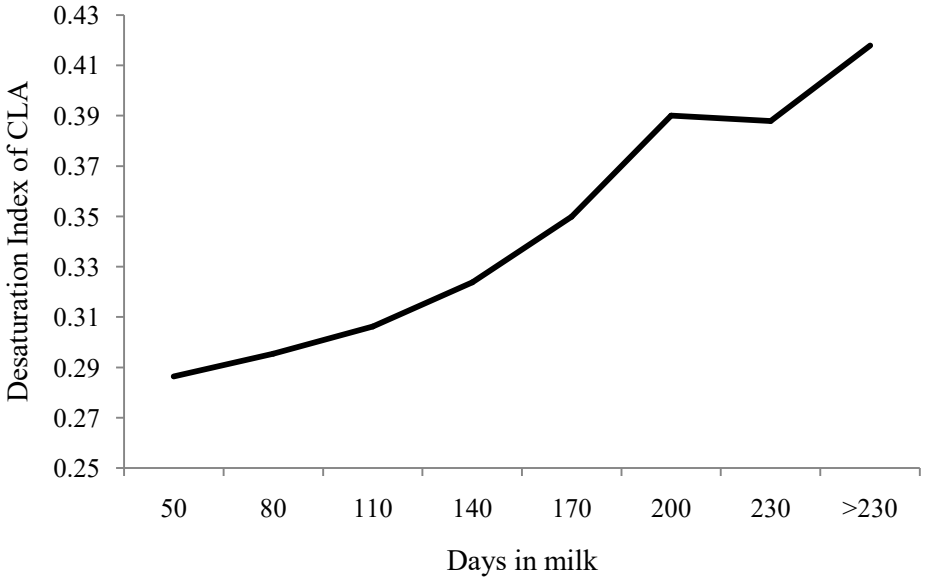

Figure 8. Evolution of desaturation index of CLA during lactation

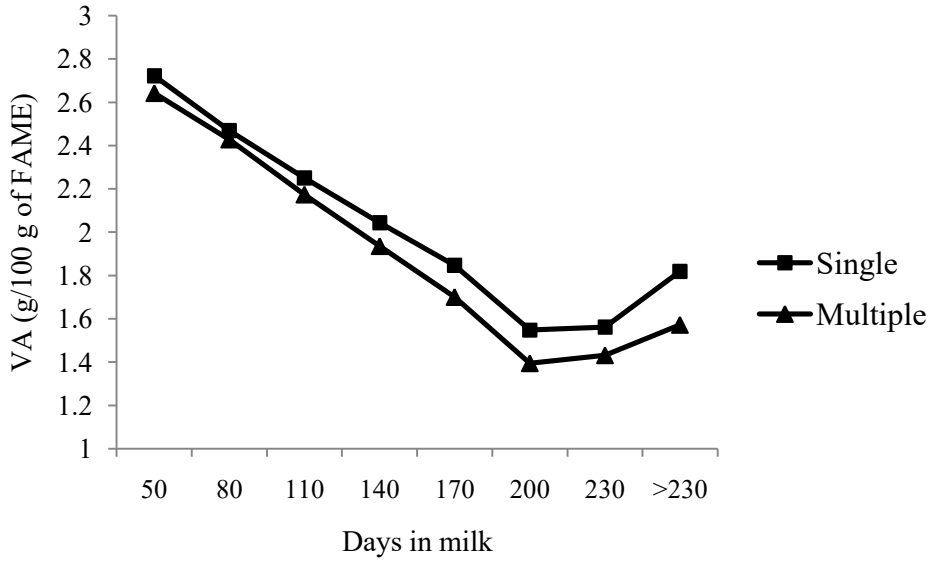

Figure 9. Lactation curves of VA (*trans*-11 C18:1) for type of lambing: single (■) and multiple (▲).

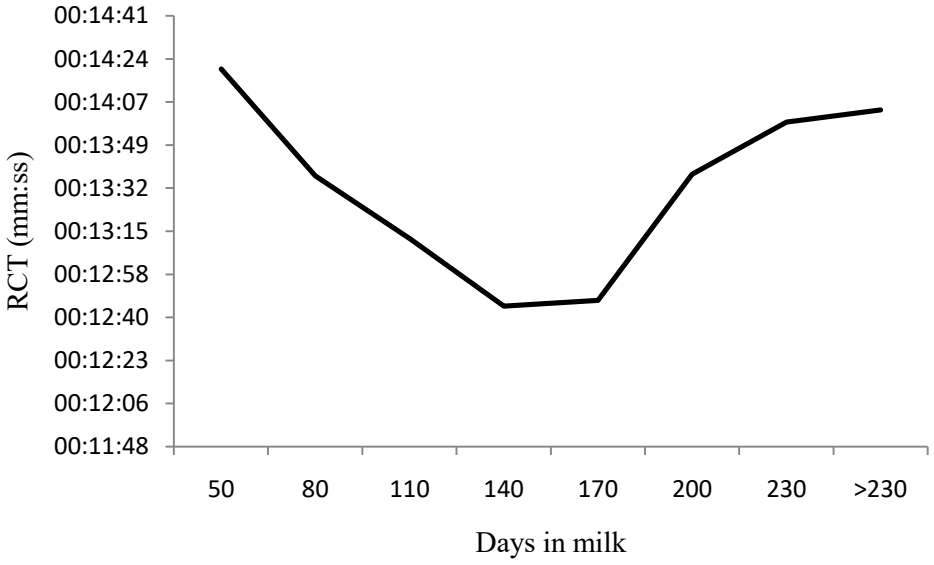

Figure 10. Evolution of RCT during lactation

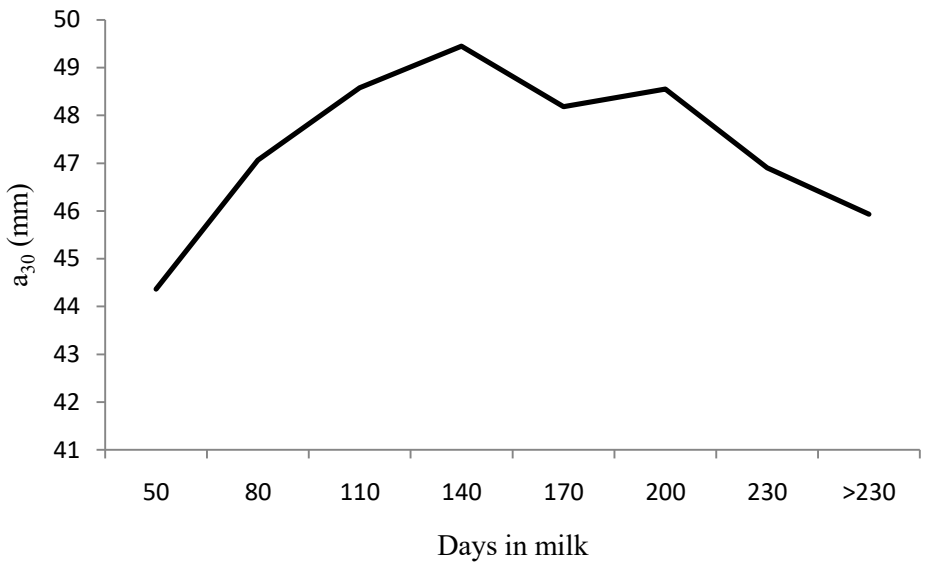

Figure 11. Evolution of a_{30} during lactation

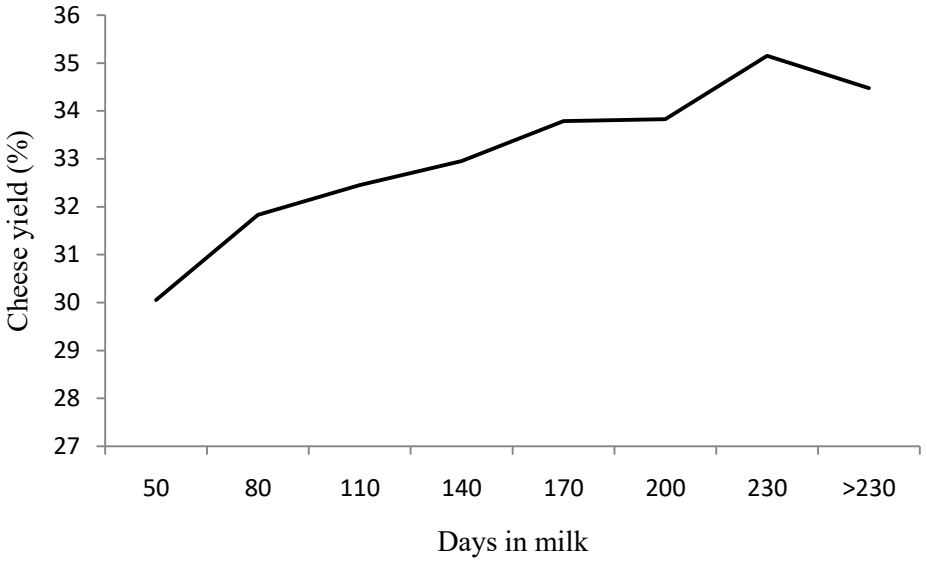

Figure 12. Evolution of cheese yield, during lactation.

CHAPTER 4

Genetic parameters for major fatty acids, groups and desaturation indices of fatty acids in sheep milk

4.1. Introduction

Milk quality is a topic of great relevance for the dairy industry due to the increasing consumer demand for safe, healthy and nutritionally valuable foods. In particular, milk fat content and composition plays an important role for human health. Sheep milk contains about 6.5 % of fat, most of which (98%) represented by triacylglycerols. The remaining part includes diacylglycerols, monoacylglycerols, free fatty acids and phospholipids, which consist of fatty acids (FA) and other minor lipid compounds. Ruminant dairy products, in particular milk and cheese, are the major dietary sources of some FA that have been found to have effects on human health.Among these *trans*-11 C18:1 (vaccenic acid, VA), *cis*-9,*trans*-11 CLA (rumenic acid, RA) and C18:3 n-3 (α-linolenic acid, LNA) can be mentioned. In particular, VA is metabolized into RA, which is the most abundant Conjugated Linoleic Acid (CLA). RA has beneficial effects as antiatherogenic agent (Banni et al., 2002). Omega 3- polyunsaturated FA (PUFA) play an important role in the prevention and treatment of the heart disease, hypertension, diabetes (Simopoulos, 2002). Therefore an improvement of milk FA profile would result in better healthy and nutritional qualities of milk.

FA profile of sheep milk is affected by many environmental factors such as diet composition (Nudda et al., 2011), season of production (Nudda et al., 2005; Mel'uchovà et al., 2008), and flock (De La Fuente et al., 2009). Diet is the most important source of variation of sheep milk FA composition (Nudda et al. 2014). Biondi et al. (2008) detected significant differences in the milk FA of ewes that change from stall to green pasture feeding. Some authors (Nudda et al., 2005; Mel'uchovà et al., 2008) observed a significant influence on CLA content in milk of spring compared with summer season. This observation could be related to different quantities of LNA in the pasture. Among animal factors that influence sheep milk FA can be mentioned the breed (Signorelli et al., 2008), the stage of lactation (De La Fuente et al., 2009), and parity (Mierlita et al., 2011). Several FA are de novo synthesized in the mammary gland by means of Stearoyl CoA Desaturase (SCD).This enzyme catalyzes the FA desaturation by adding of a *cis*-double bond between the carbons in 9^{th} and 10^{th} position (Pereira et al., 2003). Among substrates of SCD activity can be mentioned the VA, stearic acid (C18:0) and a group of saturated FA considered harmful for human health (lauric acid, C12:0; mirystic acid, C14:0; palmitic acid, C16:0), . Studies investigating the relationships between genetic polymorphism of SCD the variation of milk FA profile (Mele et al., 2007a; Moioli et

al., 2007), and the significant variation in milk fatty acids between breeds suggest that breeding strategies can be used to improve the nutritional value of milk.

In many sheep breeds, total milk yield per lactation represents the most important breeding goal. The study of genetic parameters of fatty acids profile and the evaluation of their genetic relationship with their production traits is an essential step for developing a breeding program for improving milk nutritional quality. Few studies have investigated the genetic parameters of fatty acids profile in small ruminant, especially in sheep (Sànchez et al., 2010; Boichard et al., 2014). The estimation of genetic parameters needs a reasonably larger sample size in order to obtain reliable estimates, but the number of analyzed samples is often lower due to the high cost of milk fatty acids analysis by gas chromatography. On the other hand, an option for implementing a large scale routine measurement of FA profile in sheep milk is represented by their prediction from data generated by mid-infrared spectroscopy (Ferrand-Calmels et al., 2014; Caredda et al., 2016).

The aim of this study was to estimate genetic parameters of major individual FA, principal groups of FA, and desturation indexes in sheep milk. Moreover, genetic correlations with milk production traits were estimated. Finally a comparison between FA measured with gas chromatograpohy and predicted on the basis of milk MIR spectra was performed.

4.2. Material and Methods

4.2.1. Animals and milk sample collection

The study was conducted on 989 Sarda ewes farmed in 48 flocks located in the four historical provinces of Sardinia. Some information about animal involved in this study are reported in Table 1. Individual milk samples were collected from April to July 2014 by the Breeder Provincial Association (APA) Ewes were in mid–late lactation (average = 156 ± 37.4 days). Samples were split into two subsamples. One subsample was analyzed at the milk lab of the Regional Association of Animal Breeders of Sardinia (Oristano, Italy) in order to determine milk composition and the other subsample was in part stored at -20°C for gas chromatographic analysis.

4.2.2. Laboratory analysis

Milk fat extraction and FA methyl ester (FAME) preparation were performed according to Nudda et al. (2005). FAME were determined by gas chromatography, using a 7890A GC System (Agilent Technologies, Santa Clara, CA, USA), equipped with an 7693 Autosampler (Agilent Technologies, Santa Clara, CA, USA) and a flame ionization detector (FID). A CP-Sil 88 capillary column (100 m × 0.250 μm i.d., 0.25 μm film thickness, Agilent Technologies, Santa Clara, CA, USA) was used to perform FAME separations. The oven temperature was programmed as follows: the initial temperature was set at 45°C for 4 min, increased at 13°C/min to 175°C, and held for 27 min; then it was increased at 4°C/min to 215°C, and held for 35 min. Helium (1 mL/min flow rate) was used as carrier gas with a pressure of 28 psi and 1μl of sample was injected. The split ratio 1:80. The injector and detector temperatures were set at 250°C. OpenLAB CDS GC ChemStation Upgrade software data system (Revision C.01.04, Agilent Technologies Inc., Santa Clara, CA, USA) was used to compute retention time and area of each individual FAME, which were identified by comparing their retention times with those of methyl ester standards and published isomeric profiles, as detailed in Nudda et al., 2005. Individual FAME were expressed as grams per 100 grams of total FAME.

Groups of FA were calculated as follows: short-chain fatty acids (**SCFA**), sum of the individual fatty acids from C4:0 to C10:0; medium-chain fatty acids (**MCFA**), sum of the individual fatty acids from C11:0 to C17:0; long-chain fatty acids (**LCFA**), sum of the individual fatty acids from C18:0 to C22:6 (DHA); **SFA**, sum of the individual saturated fatty acids; **MUFA**, sum of the individual monounsaturated fatty acids;

PUFA, sum of the individual polyunsaturated fatty acids; **TFA_noVA**, sum of individual trans fatty acids except VA; odd- and branched-chain fatty acids (**OBCFA**), sum of individual odd- and branched-chain fatty acids; de novo, sum of de novo synthesized FA (C6:0 + C8:0 + C10:0 + C10:1 + C11:0 + C12:0 + *iso* C13:0 + C14:0); moreover the n-6:n-3 ratio was calculated by the ratio between the sum of individual n-6 fatty acids (PUFA n-6) and the sum of individual n-3 fatty acids (PUFA n-3).

In order to study the extent of Δ^9-desaturase activity of stearoyl-CoA Desaturase, four indices (SCDI) were calculated according to Schennink et al. (2008) as follows:

C14 index **($SCDI_{14}$)** = [*cis*-9 C14:1 / (C14:0 + *cis*-9 C14:1)] × 100;

C16 index **($SCDI_{16}$)** = [*cis*-9 C16:1 / (C16:0 + *cis*-9 C16:1)] × 100;

C18 index **($SCDI_{18}$)** = [*cis*-9 C18:1 / (C18:0 + *cis*-9 C18:1)] × 100;

CLA index **($SCDI_{VA}$)**= [*cis*-9, *trans*-11 CLA /(VA + *cis*-9, *trans*-11 CLA)] × 100;

The mid-infrared analysis of milk, conducted in the lab of the Regional Association of Animal Breeds of Sardinia (Oristano, Italy) with a MilkoScanFT6000 (Foss Electric, Hillerød, Denmark), generated spectral data which were used, in the same lab, to estimate the milk content of 3 individual FA (*trans*-11 C18:1, C18:3 *n*-3 and *cis*-9, *trans*-11 CLA, named *trans*-11 C18:1_{FTIR}, C18:3 *n*-3_{FTIR} and *cis*-9, *trans*-11 CLA_{FTIR}, respectively) and four groups of FA (SFA_{FTIR}, UFA_{FTIR}, $MUFA_{FTIR}$ and $PUFA_{FTIR}$).

4.2.3. Statistical analysis

In order to assess the effects to be included in the mixed model for the genetic parameter estimation, a preliminary analysis was carried out by using the GLM procedure of SAS. Effects considered were days in milk class (DIM), month of lambing (LM), type of lambing, province, altitude of flock location, and parity. The effects of type of lambing and altitude of flock location resulted not statistically significant ($P > 0.05$); therefore these effects were not included in the final linear model.

(Co)variance components and genetic parameters were estimated by using restricted maximum likelihood methodology implemented in VCE v. 6.0 software (Groeneveld et al., 2010), using the following linear model:

$$y_{ijklmno} = \mu + PAR_j + DIM_k + LM_l + PROV_m + anim_n + ftd_o + e_{ijklmno}$$

where $y_{ijklmno}$ is the considered trait, with mean μ, explained by the fixed effects of the *j*th class of parity (*PAR*; *j* = 1, …,7 and >7), the *k*th class of days in milking (*DIM; k* = 1, …, 5; where *k* = 1: < 110 days; *k* = 2: 110 to 140 days; *k* = 3: 141 to 170 days; *k* = 4: 171 to 200 days; *k* = 5: >200 days), the *l*th class of lambing month (*LM*; where *l*1: January; *l*2: February to March; *l*3: October to November; *l*4: December), the *m*th province (*PROV*; *m* = 1, …, 4) and by the following random effects: *anim*, the *n*th additive genetic effect of animal (*n* = 1, …, 6,252), *ftd*, the *o*th cross-classified effect of the combination of flock-test date (*o* = 1, …, 66), and $e_{ijklmno}$, the residual term.

The additive animal genetic effect was assumd to be distributed as $\sim N(0, \mathbf{A}\sigma_a^2)$ where A is the additive genetic relationship matrix and σ_a^2 is the additive genetic variance; *ftd* was distributed as $\sim N(0, \mathbf{I}\sigma_{ftd}^2)$ where **I** is the identity matrix and σ_{ftd}^2 is the variance associated with the flock-test date; the residual term *e* was distributed as $\sim N(0, \mathbf{I}\sigma_e^2)$ where I is the identity matrix and σ_e^2 the residual variance.

The additive genetic relationship matrix was built using a pedigree file including 6252 animal records (supplied by the National Association of Small Ruminant Breeders, Rome, Italy).

The 3 random effects were fitted as factors with a normal distribution assumed as prior:

$[\text{anim}_1 \ \ldots \ \text{anim}_n]' \sim N(0, \mathbf{A} \otimes \mathbf{G})$;

$[\text{ftd}_1 \ \ldots \ \text{ftd}_n]' \sim N(0, \mathbf{I} \otimes \mathbf{F})$, and

$[\text{e}_1 \ \ldots \ \text{e}_n]' \sim N(0, \mathbf{I} \otimes \mathbf{R})$,

where *n* was the number of traits analyzed, **A** and **I** have previously been defined, and **G**, **F** and **R** were the *n* × *n* genetic additive, flock-test date and residual covariance matrices, respectively.

Heritability was estimated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{ftd}^2 + \sigma_e^2}$$

The proportion of variance due to the flock-test date was estimated as:

$$r_{ftd}^2 = \frac{\sigma_{ftd}^2}{\sigma_a^2 + \sigma_{ftd}^2 + \sigma_e^2}$$

Whereσ_a^2, σ_{ftd}^2 and σ_e^2 have previously been defined.

Moreover genetic and flock-test date correlations between traits were estimated as:

h^2 correlation = $\frac{\sigma_{a1,a2}}{\sqrt{\sigma^2_{a1} * \sigma^2_{a2}}}$

r^2_{ftd} correlation = $\frac{\sigma_{ftd\,1,ftd\,2}}{\sqrt{\sigma^2_{ftd\,1} * \sigma^2_{ftd\,2}}}$

Here $\sigma_{a1,a2}$ and $\sigma_{ftd\,1,ftd\,2}$ are the genetic and ftd covariances, respectively, between trait1 and trait2; σ^2_{a1} and σ^2_{a2} and $\sigma^2_{ftd\,1}$ and $\sigma^2_{ftd\,2}$ are the additive genetic and ftd variance, respectively, of trait1 and trait2.

4.3. Results

4.3.1. Descriptive statistics

Mean, SD, minimum, maximum, coefficient of variation of milk fatty acids, groups of fatty acids and desaturase indices are reported in Table 2. The results showed considerable variation in the concentration of milk FA among ewes. The coefficients of variation ranged from 5.80% (SFA) to 47.35% (n-6:n-3) among groups of FA, and from 11.43% (C16:0) to 55.76% (C18:3 n-3) among individual FA. The total concentration of the 15 FA reported was approximately 86%; the remaining 14% consisted of 86 FA which was not reported because of their low concentrations (mean of 0.15%, ranging from 0.01 to 0.86 %).

C16:0 and *cis*-9 C18:1 were the most abundant milk FA, representing the 25.95 and 17.23% of the total FA, and the 38.4 and 66.5% of SFA and MUFA, respectively. C18:0 and C14:0 showed similar concentrations (approximately 10%) and, together, contributed for the 31.2% to the SFA concentration. This group of FA was the most representative, accounting for 67.6% of the total FA. Among desaturation indices, those of long-chain FA ($SCDI_{18}$ and $SCDI_{VA}$) were, to a large extent, higher than that of medium-chain FA ($SCDI_{14}$ and $SCDI_{16}$). With respect to the individual and groups of FA obtained by mid infrared spectroscopy (MIRs) prediction, the results were similar to those of gas chromatography (GC) analysis, with the SFA_{FTIR} being higher than $MUFA_{FTIR}$ and $PUFA_{FTIR}$. The values of FA_{FTIR} groups were slightly lower than those from GC, whereas the three individual FA_{FTIR} where slightly higher than the corresponding FA obtained by GC.

4.3.2. Heritability of individual FA, groups of FA and desaturation indices

Contribute of flock-test-date to the phenotypic variance (r^2_{FTD}) and heritability of selected milk FA, groups of fatty acids and desaturase indices are reported in Table 3. With regard to the 15 individual FA investigated, low heritabilities ($h^2 < 0.20$) were estimated for most of them, including saturated (C6:0, C8:0, C10:0, C12:0 and C14:0) monounsaturated (*trans*-11 C18:1, *cis*-9 C18:1) and polyunsaturated (*cis*-9, *trans*-11 C18:2, *cis*-9, *cis*-12 C18:2 and *cis*-9, *cis*-12, *cis*-15 C18:3) FA. Moderate to high heritabilities were estimated for C4:0, C18:0, *cis*-9 C14:1, *cis*-9 C16:1 and C16:0; in particular the last FA showed the highest value of heritability estimated in this work ($h^2 = 0.48$). Considering the groups of FA and desaturation indices, high heritability values,

ranging from 0.27 to 0.31, were observed for MCFA, $SCDI_{14}$, $SCDI_{18}$ and $SCDI_{VA}$, whereas the other FA groups and $SCDI_{16}$ presented low heritabilities.

With respect to the four groups and the three individual FA obtained from FTIR prediction, only SFA_{FTIR} showed high value of heritability. This value was higher than that estimates for SFA obtained from GC analysis. In general, all the FTIR predicted variables showed higher values of heritability compared to the corresponding variables from GC analysis, despite all these values of heritabilities were low.

A generally high contribute of flock-test-date (FTD) to the phenotypic variance was observed for all investigated variables. The mean of the contribute of FTD was 49.7%, ranging from 21.94 to 85.76%. Most of the investigated variables yielded FTD contributions higher than the heritability estimates. Only C16:0 and *cis*-9 C14:1 showed FTD contribution lower than heritability estimates, $SCDI_{14}$ and SFA_{FTIR} presented differences closed to zero, and C4:0, *cis*-9 C16:1, MCFA, $SCDI_{VA}$, $SCDI_{18}$ and $SCDI_{16}$ showed low differences (< 20 or < 50%).

4.3.3. Genetic correlations of individual FA, groups of FA and desaturation indices

Genetic correlations of individual FA, groups of FA and individual FA vs. groups of FA are shown in Tables 4, 5 and 6, respectively. The C4:0 had a negative correlations with some short and medium chain SFA, and with some MUFA, but the highest negative correlation was with *cis*-9, *trans*-11 CLA (-1.00). A strong negative correlation was observed between C14:0 and C18:2 *n*-6 and LNA (-0.75 and -0.94), and a positive correlation with *cis*-9, *trans*-11 CLA. The C16:0 showed a negative correlation with all investigated individual FA, except for C4:0, C14:0, *cis*-9 C14:0 and *cis*-9 C16:1. The C18:0, *trans*-11 C18:1, C18:2 *n*-6 and C18:3 *n*-3 had moderate to high (0.47 to 0.94) negative genetic correlation with C14:0, C16:0 and their desaturation products, with a weak correlation of -0.02 between *trans*-11 C18:1 with C14:0. As expected, SFA were negatively correlated with MUFA and PUFA. Interestingly, SFA were also negatively correlated with FA with >18 carbon chains. SCFA showed a weak correlation (from -0.27 to 0.26) with all studied group of FA, except for *de novo* FA with which had a strong positive correlation (0.88). Content in milk of *de novo* FA group was also moderately correlated with PUFA (-0.44) and LCFA (-0.47). A strong positive genetic correlation (0.90) was observed between trans fatty acids excluded *trans*-11 C18:1 (TFA_noVA), and PUFA. TFA_noVA was also negative correlated with all desaturation indices (-0.56 to -0.78). Desaturation indices had the largest positive

correlations with each other and range from 0.75 to 0.98. The C4:0 to C14:0 FA were greatly correlated with SCFA and *de novo* FA, C4:0 with negative genetic correlation and the other FA positively. Unexpectedly, SFA showed a weak genetic correlation (-0.35 to 0.34) with individual FA which belong to the group, except for C14:0 (0.56) and C16:0 (0.63). Content of C18:0 in milk was strongly negatively correlated with all desaturation indices.I

Phenotypic and genetic correlations between FA measured by gas- chromatography and predicted by MIR (Table 7) were estimated. Moderate to high phenotypic correlations between measured and predicted by MIR FA were found, ranging from 0.41 to 0.77. The estimated genetic correlations between measures and predictions of SFA, MUFA and C18:3 *n*-3 were very large, and ranged from 0.76 to 1. The estimates of genetic correlations for *trans*-11 C18:1 and PUFA were moderate (0.59 and 0.61, respectively), whereas genetic correlations of *cis*-9, *trans*-11 CLA, was smaller (0.11).

4.4. Discussion

In general, average values of the milk FA observed in the present work were in accordance with previews works carried out on Sarda dairy sheep (Carta et al., 2008; Caredda et al., 2016). The high proportion of SFA, mainly composed by C16:0, C18:0 and C14:0, followed by MUFA, with *cis*-9 C18:1 being the most representative, and the relative low proportion of PUFA, were expected. Slight differences can be observed when compared with other studies, but it should be considered that most of the works reporting milk FA profiles have been conducted on lower number of animals than the present work, belonging often to the same flock. Furthermore, most of previous researches were aimed at studying variations in milk FA profile related to differences in dietary treatment (Mele et al., 2007b; Correddu et al., 2016), environmental conditions (Nudda et al., 2005), breed (Signorinelli et al., 2008) and physiological conditions of animals. The slightly lower mean values for the FA obtained by MIR prediction compared with those from GC can be explained by the different way of data expression. The first were expressed as g of FA on 100g of fat whereas the second were reported in term of g/100 g of total FA. Therefore, considering that milk fat is not completely constituted by FA (approximately 98%), a slightly underestimation was expected. In addition, considering the relative abundance of these FA groups (calculated by the % of each group on the sum of all groups), the values were almost identical to those obtained by GC (67.71, 32.38 and 6.41%; SFA_{FTIR}, $MUFA_{FTIR}$ and $PUFA_{FTIR}$, respectively). On the other hand, the individual FA_{FTIR} were expressed as g of FA on 100g of total FAME, therefore comparable with the corresponding obtained by GC. Therefore these results suggests that the used MIR method ensures a good prediction for the groups of FA, giving a slight overestimation for the individual FA.

With respect to the desaturase indices, the values were in agreement with those reported in previous works on Sarda sheep (Nudda et al., 2005; Carta et al., 2008), confirming that long-chain SFA are desaturated to a greater extent than medium-chain SFA (Bilal et al., 2012). Similar values of SCD indices have been extensively reported in studies on cattle, except for $SCDI_{14}$; compared with that observed in the present study (mean of 1.79%), higher value of $SCDI_{14}$ (approximately 8%) have been observed in cattle (Schennink et al., 2008; Mele et al., 2009).

Modifying the milk FA composition is of crucial importance from a quality-food point of view. Ruminant nutrition is one of the most important factor able to influence the

milk FA profile, and a number of studies have been conducted in this field. The milk concentration of some FA depends, also, by their enzymatic production in the mammary gland. Based on these considerations (evidence) the study of the genetic effect on milk FA profile has recently received the attention of researchers; however few studies are current available on this topic, most of them being conducted on dairy cattle. In addition, in most of them, data of FA were obtained by prediction of mid-infrared analysis; this make not easy the comparison of results among different studies. As evidenced by some authors and reported by Pegolo et al. (2016) heritability estimates among studies can vary due to several reasons: animal specific factors (e.g., breed, parity, stage of lactation), analytical methods (GC or MIR), method of FA expression, statistical analysis (e.g., the number of samples, the use of single or repeated records, sire or animal model) and estimate of heritability (e.g., intra-herd heritability or not).

The general higher contribute of flock-test-date (FTD) compared to that of genetic heritability was in accordance with previous studies on cows (Pegolo et al., 2016). However, in that work, more variables presented heritability higher than herd-date effect, compared with our results. As argued by Puledda et al. (2016), in sheep, the flock environment exerted a significant role, resulting in higher fraction of variance explained by flock in comparison with studies on cattle.

Regarding the individual saturated FA, the values of heritability estimates in the present work evidenced the different pathways of synthesis of C4:0 compared to the other short and medium chain FA. C4:0 (butyric acid), together with acetic and propionic acids (C2:0 and C3:0), is partly produced by the ruminal bacterial metabolism and it is mainly independent from the de novo synthesis occurring in mammary gland. Together with acetic acid, C4:0 represents the substrate for the synthesis of short and medium chain FA (C6:0 to C14:0 and almost half of C16:0), operated by acetyl CoA carboxilase and FA synthetase. Based on these considerations, a higher dependence from genetic control should be hypothesized for short and medium chain FA than for C4:0, as it was recently demonstrated in cattle by Mele et al. (2016). However, our results were in disagreement with this finding, being heritabilities estimates of C4:0 higher than short and medium chain FA. It is worth noting that C4:0 is not completely out of enzymatic control. The milk concentration of this FA depends on its incorporation into triacylglycerols, operated by the diacylglycerol acyl-transferase (DGAT) in the mammary gland, on the sn-3 position of a diacylglycerol (Parodi, 1982). In addition, the milk fat concentration

of C4:0 was found to be affected by DGAT1 gene polymorphism in sheep (Dervishi et al., 2015).

The results of our analysis showed that the FA groups, except MCFA, had low heritability. As pointed by Sánchez et al. (2010) this could be explained by the presence of individual FA of different origin in the same group, with possible genetic correlation of opposite sign, which can lead to a reduction of the overall genetic variance of the group. In addition, the FA groups presented in this work included, also FA identified in GC analysis that was not reported, due to their low concentrations, but that contributed to define the genetic variance of the corresponding FA groups. The heritability of MCFA is likely linked to that of C16:0 which, in this work, represents the 55% of the total group concentration, and that showed the highest value of heritability found in this work. From a genetic selection point of view this finding is of great importance. In fact, C16:0 represents the main FA of sheep milk, ranging in this work from 18.51 to 36.69% of total FA. It largely contributes to the milk content of SFA (mean of 38%), which are often associated with the risk increase of coronary heart diseases (CHD) events. Although a direct role of SFA and palmitic acid in CHD and other diseases are controversial and not totally convincing (Mozzafarian et al., 2010; Fattore et al., 2013), a general trend (interest) exists in reducing the dietary intake of these FA, also by decreasing their concentration in foods. Based on these considerations, the high heritability of C16:0 evidenced in this work indicated that its concentration and that of SFA, could be reduced by genetic selection.

High concentration of *cis*-9 C18:1 and *cis*-9, *trans*-11 C18:2 in foods are considered desirable, as several studied have evidenced their beneficial effects on human health. Oleic acid in milk fat derives mainly from the diet, and in part (about 50%) is endogenously produced in the mammary gland by the desaturation activity SCD on the C18:0 at the delta-9 position; rumenic acid is an intermediate product of the ruminal biohydrogenation of dietary PUFA, but its concentration in milk is mainly due (about 80%) to the SCD activity at the delta-9 position of the *trans*-11 C18:1 (Mosley et al., 2006). Other substrate for the SCD activity, reported in the present work, were C14:0 and C16:0, whose desaturated products are *cis*-9 C14:1, *cis*-9 C16:1, respectively. As expected, the milk concentration of SCD products and that of the desaturase indices, calculated to study the activity of SCD in the conversion of a substrate to the related Δ -9 desaturated product, showed high values of heritability. In accordance with our results, in the work of Garnsworthy et al. (2010), on cows, the heritability of $SCDI_{16}$

was lower than that of the other indices. The highest heritabilities among SCD indices observed for $SCDI_{14}$ was in accordance with previews studies (Mele et al., 2009; Garnsworthy et al., 2010; Pegolo et al., 2016). As pointed by Pegolo et al. (2016) the higher heritability $SCDI_{14}$ is related to the almost complete endogenous origin of C14:0 and C14:1c9, the first is of de novo synthesis and the second is produced by SCD activity. Surprisingly, together with $SCDI_{14}$, the heritability of $SCDI_{VA}$ was find to be the highest among the SCD indices, in contrast to other works, on cows, which report lower heritability of $SCDI_{VA}$ compared with the other indices (Schennink et al., 2008; Bilal et al., 2012; Pegolo et al., 2016). It could be supposed that in sheep the heritability of $SCDI_{VA}$ may be higher than in cows, suggesting a more suitable selection for this species, with the aim to produce milk with higher CLA content.

In accordance with previews works, very low heritabilities were estimated for linoleic and linolenic acids (Garnsworthy et al., 2010; Pegolo et al., 2016). These FA cannot be produced by endogenous biosynthesis, but they derive exclusively from the diet, and therefore can be for a large part modified by the biohydrogenation activity of rumen bacteria. The high contribute of FTD to the phenotypic variance LA and LNA was consistent with the high herd-date contributes found in by Pegolo et al. (2016) in cows; it likely reflects the dietary effects on the content of these FA in milk. Vaccenic and, in part, rumenic acids are intermediates of biohydrogenation of LA and LNA. Therefore, their low heritability estimates and high FTD effects were consistent with their origin, and in agreement with existing literature (Heck et al., 2012; Pegolo et al., 2016). In a work on Churra sheep, a significant heritability was found for vaccenic and linoleic acids (Sánchez et al., 2010); as the milk concentration of these FA depends on the biohydrogenation activity in rumen, the authors explained their result with a possible genetic variability in the determination of rumen microbial population structure and activity. This hypothesis was not confirmed by the our discussed results of VA and LA and by those of OBCFA, which showed low heritability and high FTD contributes. These group of FA are mainly derived from the activity of rumen microorganism (Fievez et al., 2012) therefore the genetic background was aspect to be of low importance.

With regard to individual and groups of FA predicted by MIR, only the SFA_{FTIR} showed high values of heritability. Overall, the heritabilities estimates of these FA were higher and FDT contributions were lower than those of the same variables obtained with GC analysis, supporting the considerations of Pegolo et al. (2016), pointing that heritability

estimates of FA predicted by MIR produces, often, higher values than those from GC analysis.

The study of genetic correlations of milk fatty acids is helpful to know the relationship that these traits have with each other. The selection programs have to take into account that the positive changes for some fatty acids may be negative for other. As said for the estimates of heritability, the limited number of previous studies about genetic parameters of milk fatty acids profile and the difference in analytical and statistical procedure used, make it difficult to compare the results. The estimates and the pattern of genetic correlations among individual fatty acids in this study, showed in Table 4, were similar with the results reported for cattle by Stoop et al. (2008). Individual fatty acids showed high genetic correlations with the corresponding length carbon chain group. The C6:0 to C14:0 fatty acids are generate *de novo* in the mammary gland, they are the only fatty acids whose synthesis is regulated strongly by the activities of acetyl-CoA carboxylase and the fatty acids synthetase complex from acetate and β-hydroxybutyrate, which come from the rumen (Barber et al., 1995). As found in recent report (Pegolo et al., 2016), C4:0 showed an opposite sign of correlation compared to other SCFA, confirming the independence of C4:0 concentration from *de novo* mammary fatty acids synthesis. These results may be explained by the dual origin of C4:0, via acetyl-CoA dependent and independent pathways (Palmquist et al., 1993). So, an increasing of C6:0 to C14:0 acids synthesis in the mammary gland coincides with a decrease of butyric acid. For the same reason, C4:0 was negatively correlated with *de novo* group. Crisà et al. (2010), reported that C4:0 concentration depend on growth hormone receptor (GHR) SNP polymorphism. Also, the high correlation among short and medium chain SFA could be explained with their common metabolic production pathways associated to fatty acids synthase activity. The C14:0 and C16:0 are among the most abundant fatty acids in milk, and are usually associated with a risk factor of cardiovascular disease (CVD) (Nicolosi et al., 1997). In contrast to what was claimed by Heck et al. (2012), a positive genetic correlation between C14:0 and C16:0 was found in this study, consequently it will be possible to decrease the concentration of both FA concurrently in order to improve the nutritional quality of milk. The negative correlation found between C16:0 and unsaturated C18:0 fatty acids and CLA, confirm the finding reported by Schennink et al. (2007), which associated the concentration of these fatty acids to the effect of DGAT1 K232A polymorphism on fat composition. Moreover, the concentration of short and medium chain saturated fatty acids (include C14:0 and

C16:0) might be negatively associated with the presence of long chain unsaturated fatty acids (Loor and Herbein, 1998). A theory of milk fluidity may be one of the reason (Gama et al., 2008). According this theory, the mammary gland maintain the milk fat fluidity balancing the concentration of unsaturated dietary fatty acids that have a lower melting point with de novo synthesis of saturated fatty acids and the desaturation of C18:0 by means of Δ9-desaturase enzyme (Gama et al., 2008; Chillard et al., 2000). The additive genetic correlations between groups of fatty acids, as reported in Table 5, were in agreement with the findings in Brown Swiss cows of Pegolo et al. (2016). The genetic correlations of unsaturation indices with each other are higher than those reported by Schennink et al. (2008). The high positive genetic correlation between *cis*-9 C14:1 and $SCDI_{14}$ may be attributed to the influence of the effect of AA SCD genotype on both traits (Mele et al., 2007). Moreover, recently was found that SCD polymorphisms had a significant effect on several desaturation indices such as $SCDI_{14}$, $SCDI_{16}$ and $SCDI_{18}$ (Rincon et al., 2011); these findings suggest that the desaturase activity could be include in breeding programs to improve the milk FA profile by decreasing of SFA and increasing unsaturated FA, such as CLA. Interestingly, all desaturation indices were negatively correlated with TFA_noVA; therefore genetic selection for this ratio in milk should have a beneficial effect of reducing the TFA, considered harmful for human health, in milk fat.

High phenotypic correlations between measures and MIR predictions of individual and groups of milk FA, could be explained with the use of FA values obtained by gas-chromatographic method to calibrate the MIR instrument (Caredda et al., 2016). The application of MIR predictions in breeding programs depends on the genetic correlation between the values of traits included as objectives of selection (e.g. fatty acids profile predicted by MIR) and the traits that really must be improved (milk fatty acids profile measured) (De marchi et al., 2014; Cecchinato et al., 2009). If a high genetic correlation exists between predictor trait and real phenotype of interest, an indirect selection could be implemented. In this study the genetic correlation between predicted and measured traits was higher than 50% for *trans*-11 C18:1, C18:3 *n*-3, SFA, MUFA and PUFA, for this traits an indirect selection would be advantageous. On the other hand, there is no advantage linked to the genetic selection on *cis*-9, *trans*-11 CLA, which showed a very low genetic correlation between predicted and measured values. Similar results are reported by Tabarelli (2014) in a preliminary study carried out on Italian Simmental cows.

4.5. Conclusions

Results of the present study gave interesting insights in the genetic determinism of FA profile in dairy sheep. The existence of genetic variability for some FA has been highlighted, in agreement with reports in dairy cattle, confirming the possibility to improve these traits by including them as breeding goals in selection programmes. The feasibility of large scale selection for some of these traits has been confirmed by the genetic correlations between gas chromatography and MIR predicted FA. On the other hand, the relevant effect of management and environment, summarized by the flock-test date factor, has been confirmed. The analysis of genetic correlations between FA detected the different metabolic pathways involved in the determinism of milk FA profile.

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4.7. Tables

Table 1. Distribution of animals involved in this study (=989) in different level of flocks, parities and provinces.

Item	class	Count
Flock	48	20.60[1]
Number of lactation	1	191
	2	123
	3	151
	4	164
	5	120
	6	95
	7	69
	>7	76
Province	Cagliari	321
	Nuoro	319
	Oristano	111
	Sassari	238

[1]means of ewes sampled per flock

Table 2. Mean, SD, minimum (min), maximum (max) and coefficient of variation (CV) of milk fatty acids, groups of fatty acids and desaturase indices in Sarda dairy sheep

Item	Mean	SD	Min	Max	CV
milk FA[1]					
C4:0	2.67	0.37	1.52	4.05	13.83
C6:0	1.75	0.37	0.46	2.65	21.02
C8:0	1.60	0.46	0.28	2.84	28.46
C10:0	5.52	1.76	0.87	10.18	31.86
C12:0	3.48	1.00	1.08	8.15	28.78
C14:0	10.81	1.54	5.28	18.42	14.23
cis-9 C14:1	0.20	0.08	0.04	0.68	42.43
C16:0	25.95	2.97	18.51	36.69	11.43
cis-9 C16:1	0.89	0.26	0.41	2.30	29.01
C18:0	10.29	2.51	1.37	21.00	24.38
trans-11 C18:1	2.06	1.03	0.46	5.77	50.21
cis-9 C18:1	17.23	3.64	5.37	34.75	21.11
C18:2 *n*-6	2.09	0.51	0.92	4.32	24.33
C18:3 *n*-3	0.89	0.50	0.20	3.35	55.76
cis-9, *trans*-11 CLA	1.03	0.47	0.28	3.16	45.52
SCFA	11.64	2.66	3.31	18.65	22.84
MCFA	47.19	4.09	33.54	70.35	8.67
LCFA	41.17	5.31	15.51	63.14	12.90
SFA	67.63	3.92	49.43	82.97	5.80
MUFA	25.90	3.64	11.95	45.26	14.04
PUFA	6.46	1.43	2.79	12.24	22.16
TFA_noVA	4.55	1.50	1.75	13.68	32.95
OBCFA	4.78	0.61	2.72	6.94	12.78
De novo	23.46	4.70	8.06	39.91	20.05
n6:n3	2.47	1.17	0.68	6.60	47.35
Desaturase index[2]					
$SCDI_{14}$	1.80	0.65	0.60	4.66	36.09
$SCDI_{16}$	3.31	0.76	1.59	6.43	22.86
$SCDI_{18}$	62.65	5.58	43.08	82.73	8.91
$SCDI_{VA}$	34.19	5.27	19.27	57.70	15.42
Milk FA predicted by MIR					
SFA_{FTIR}[3]	62.20	4.85	44.12	91.33	7.79
UFA_{FTIR}[3]	29.74	4.84	4.85	49.82	16.29
$MUFA_{FTIR}$[3]	23.74	4.59	7.79	40.70	19.34
$PUFA_{FTIR}$[3]	6.13	1.29	2.27	11.38	21.12
trans-11 $C18:1_{FTIR}$[4]	2.74	1.03	0.01	5.85	37.75
C18:3 *n*-3_{FTIR}[4]	1.18	0.38	0.01	2.96	32.66
cis-9,*trans*-11 CLA_{FTIR}[4]	1.26	0.57	0.01	3.04	44.87

[1]Expressed as g/100g of total FAME; SCFA = short-chain fatty acids, sum of the individual fatty acids from C4:0 to C10:0; MCFA = medium-chain fatty acids, included fatty acids from C11:0 to C17:0; LCFA = long-chain fatty acids included fatty acids from C18:0 to DHA; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; TFA = trans fatty acids; OBCFA = odd- and branched-chain fatty acids; De novo: sum of C6:0, C8:0, C10:0, C10:1, C11:0, C12:0, isoC13:0, C14:0; n6:n3 = ratio between PUFA n-6 and PUFA n-3.

[2]$SCDI_{14}$ = [*cis*-9 C14:1 /(C14:0 + *cis*-9 C14:1)] × 100; $SCDI_{16}$ = [*cis*-9 C16:1 /(C16:0 + *cis*-9 C16:1)] × 100; $SCDI_{18}$ = [*cis*-9 C18:1 /(C18:0 + *cis*-9 C18:1)] × 100; $SCDI_{VA}$ = [*cis*-9,*trans*-11 CLA /(*trans*-11 C18:1 + *cis*-9,*trans*-11 CLA)] × 100.

[3]expressed as g/100g of fat.

[4]expressed as g/100g of FAME.

Table 3. Estimates heritabilities and flock test date of milk fatty acids, groups of fatty acids and desaturase indices in Sarda dairy sheep (n=989)

	h^2		r^2_{ftd}	
trait[1]	mean	SE	mean	SE
milk FA[1]				
C4:0	0.21	0.08	0.24	0.05
C6:0	0.04	0.05	0.62	0.05
C8:0	0.09	0.05	0.64	0.05
C10:0	0.12	0.05	0.62	0.05
C12:0	0.12	0.06	0.54	0.05
C14:0	0.12	0.09	0.41	0.05
cis-9 C14:1	0.32	0.12	0.22	0.04
C16:0	0.48	0.10	0.37	0.05
cis-9 C16:1	0.29	0.09	0.32	0.05
C18:0	0.22	0.08	0.46	0.05
trans-11 C18:1	0.07	0.04	0.66	0.05
cis-9 C18:1	0.12	0.06	0.61	0.05
C18:2 n6	0.10	0.06	0.60	0.05
C18:3 n3	0.03	0.02	0.82	0.03
cis-9 *trans*-11 CLA	0.05	0.05	0.60	0.05
SCFA	0.08	0.05	0.64	0.05
MCFA	0.29	0.09	0.35	0.05
LCFA	0.14	0.08	0.47	0.05
SFA	0.12	0.08	0.45	0.05
MUFA	0.09	0.07	0.55	0.05
PUFA	0.09	0.04	0.64	0.05
TFA_noVA	0.13	0.08	0.58	0.05
OBCFA	0.02	0.05	0.61	0.05
De novo	0.09	0.06	0.59	0.05
n-6:n-3	0.10	0.03	0.86	0.02
Desaturase index[2]				
$SCDI_{14}$	0.31	0.10	0.31	0.05
$SCDI_{16}$	0.15	0.09	0.35	0.05
$SCDI_{18}$	0.27	0.08	0.39	0.05
$SCDI_{VA}$	0.31	0.08	0.42	0.05
Milk FA predicted by MIR				
SFA_{FTIR}[3]	0.32	0.09	0.32	0.05
UFA_{FTIR}[3]	0.18	0.09	0.39	0.05
$MUFA_{FTIR}$[3]	0.14	0.08	0.38	0.05
$PUFA_{FTIR}$[3]	0.15	0.08	0.48	0.06
trans-11 $C18:1_{FTIR}$[4]	0.13	0.10	0.50	0.06
C18:3 $n3_{FTIR}$[4]	0.13	0.08	0.38	0.06
cis-9, *trans*-11 CLA_{FTIR}[4]	0.09	0.06	0.52	0.05

[1]Expressed as g/100g of total FAME; SCFA = short-chain fatty acids, sum of the individual fatty acids from C4:0 to C10:0; MCFA = medium-chain fatty acids, included fatty acids from C11:0 to C17:0; LCFA = long-chain fatty acids included fatty acids from C18:0 to DHA; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; TFA = trans fatty acids; OBCFA = odd- and branched-chain fatty acids; De novo: sum of C6:0, C8:0, C10:0, C10:1, C11:0, C12:0, isoC13:0, C14:0; n6:n3 = ratio between PUFA n-6 and PUFA n-3.

[2]$SCDI_{14}$ = [*cis*-9 C14:1 /(C14:0 + *cis*-9 C14:1)] × 100; $SCDI_{16}$ = [*cis*-9 C16:1 /(C16:0 + *cis*-9 C16:1)] × 100; $SCDI_{18}$ = [*cis*-9 C18:1 /(C18:0 + *cis*-9 C18:1)] × 100; $SCDI_{VA}$ = [*cis*-9,*trans*-11 CLA /(*trans*-11 C18:1 + *cis*-9,*trans*-11 CLA)] × 100.

[3]expressed as g/100g of fat.

[4]expressed as g/100g of FAME.

Table 4. Additive genetic correlation between individual fatty acids in sheep milk

Traits[1]	C6:0	C8:0	C10:0	C12:0	C14:0	*cis*-9 C14:0	C16:0	*cis*-9 C16:1	C18:0	*trans*-11 C18:1	*cis*-9 C18:1	C18:2 *n*-6	C18:3 *n*-3	*cis*-9, *trans*-11 CLA
C4:0	-0.14	-0.56	-0.77	-0.93	-0.59	-0.42	0.15	-0.18	0.12	-0.32	0.17	0.40	0.62	-1.00
C6:0		0.82	0.71	0.53	0.03	-0.29	-0.31	-0.57	0.23	-0.12	-0.41	0.33	0.26	-0.66
C8:0			0.94	0.92	0.14	-0.06	-0.47	-0.35	0.13	0.06	-0.18	0.00	-0.06	0.05
C10:0				0.99	0.41	0.19	-0.27	-0.11	-0.09	0.08	-0.33	-0.17	-0.30	0.20
C12:0					0.42	0.23	-0.43	-0.14	-0.06	0.15	-0.08	-0.26	-0.35	0.50
C14:0						0.71	0.39	0.42	-0.49	-0.02	-0.28	-0.75	-0.94	0.65
cis-9 C14:1							0.48	0.89	-0.77	-0.49	0.16	-0.59	-0.50	0.05
C16:0								0.71	-0.56	-0.54	-0.37	-0.39	-0.56	-0.33
cis-9 C16:1									-0.76	-0.47	0.19	-0.53	-0.55	-0.02
C18:0										0.21	-0.07	0.36	0.51	-0.58
trans-11 C18:1											-0.40	0.43	0.30	0.50
cis-9 C18:1												0.09	0.43	-0.07
C18:2 *n*-6													0.54	0.30
C18:3 *n*-3														0.31

[1]Expressed as g/100 g of total FAME

Table 5. Additive genetic correlations between groups of fatty acids, desaturation indices and *n*6:*n*3 ratio in sheep milk

Traits[1]	MCFA	LCFA	SFA	MUFA	PUFA	*de novo*	n-6:n-3	TFA_noVA	$SCDI_{14}$	$SCDI_{16}$	$SCDI_{18}$	$SCDI_{VA}$
SCFA	-0.16	-0.18	0.26	-0.24	-0.16	0.88	0.15	0.23	-0.07	-0.10	-0.27	-0.23
MCFA		-0.94	0.70	-0.66	-0.62	0.22	0.50	-0.52	0.65	0.38	0.33	0.54
LCFA			-0.77	0.73	0.67	-0.47	-0.57	0.50	-0.62	-0.34	-0.27	-0.49
SFA				-0.98	-0.79	0.44	0.71	-0.33	0.16	-0.14	-0.31	-0.04
MUFA					0.65	-0.35	-0.73	0.15	-0.06	0.39	0.45	0.18
PUFA						-0.44	-0.26	0.90	-0.29	-0.27	-0.04	-0.22
De novo							0.37	-0.31	0.25	0.17	-0.07	0.12
n-6:n-3								0.14	0.17	0.02	-0.10	-0.20
TFA_noVA									-0.75	-0.78	-0.56	-0.65
$SCDI_{14}$										0.98	0.75	0.87
$SCDI_{16}$											0.75	0.82
$SCDI_{18}$												0.96

[1]Expressed as g/100g of total FAME; SCFA = short-chain fatty acids, sum of the individual fatty acids from C4:0 to C10:0; MCFA = medium-chain fatty acids, included fatty acids from C11:0 to C17:0; LCFA = long-chain fatty acids included fatty acids from C18:0 to DHA; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; *De novo*: sum of C6:0, C8:0, C10:0, C10:1, C11:0, C12:0, isoC13:0, C14:0; n-6:n-3 = ratio between PUFA n-6 and PUFA n-3TFA_noVA = trans fatty acids, except vaccenic acid (VA); $SCDI_{14}$ = [*cis*-9 C14:1 /(C14:0 + *cis*-9 C14:1)] × 100; $SCDI_{16}$ = [*cis*-9 C16:1 /(C16:0 + *cis*-9 C16:1)] × 100; $SCDI_{18}$ = [*cis*-9 C18:1 /(C18:0 + *cis*-9 C18:1)] × 100; $SCDI_{VA}$ = [*cis*-9,*trans*-11 CLA /(*trans*-11 C18:1 + *cis*-9,*trans*-11 CLA)] × 100.

Table 6. Additive genetic correlations between groups of fatty acids, desaturation indices, *n*6:*n*3 ratio and individual fatty acids in sheep milk

Traits[1]	SCFA	MCFA	LCFA	SFA	MUFA	PUFA	*De novo*	n-6:n-3	TFA_noVA	$SCDI_{14}$	$SCDI_{16}$	$SCDI_{18}$	$SCDI_{VA}$
C4:0	-0.56	-0.19	0.34	-0.35	0.33	0.27	-0.86	-0.07	0.52	-0.27	-0.31	-0.05	-0.29
C6:0	0.87	-0.28	0.00	0.21	-0.20	0.06	0.59	0.14	0.79	-0.27	-0.42	-0.52	-0.58
C8:0	0.98	-0.29	-0.04	0.17	-0.13	-0.16	0.83	0.06	0.11	-0.11	-0.08	-0.24	-0.17
C10:0	0.96	0.01	-0.31	0.34	-0.31	-0.25	0.96	0.15	-0.07	0.08	0.08	-0.14	-0.03
C12:0	0.88	-0.13	-0.12	0.14	-0.06	-0.25	0.93	0.14	-0.26	0.14	0.15	-0.06	0.09
C14:0	0.23	0.63	-0.75	0.56	-0.45	-0.68	0.67	0.67	-0.67	0.55	0.35	0.13	0.41
cis-9 C14:1	-0.01	0.70	-0.70	0.27	-0.15	-0.45	0.38	0.34	-0.88	0.98	0.93	0.64	0.83
C16:0	-0.36	0.96	-0.83	0.63	-0.62	-0.50	-0.10	0.36	-0.37	0.49	0.24	0.28	0.40
cis-9 C16:1	-0.31	0.76	-0.65	0.22	-0.08	-0.45	0.02	0.33	-0.70	0.93	0.86	0.66	0.78
C18:0	0.05	-0.67	0.67	-0.05	-0.01	0.08	-0.21	-0.28	0.32	-0.80	-0.68	-0.79	-0.85
trans-11 C18:1	0.02	-0.46	0.43	-0.39	0.25	0.71	0.05	0.26	0.97	-0.57	-0.27	-0.40	-0.64
cis-9 C18:1	-0.33	-0.39	0.48	-0.71	0.81	0.21	-0.31	-0.77	-0.51	0.27	0.62	0.64	0.50
C18:2 *n*-6	0.04	-0.58	0.62	-0.46	0.33	0.74	-0.40	0.27	0.56	-0.40	-0.55	-0.26	-0.44
C18:3 *n*-3	0.04	-0.79	0.86	-0.67	0.58	0.72	-0.54	-0.75	0.24	-0.30	-0.33	-0.24	-0.21
cis-9,*trans*-11 CLA	-0.13	-0.08	0.04	-0.53	0.40	0.78	0.38	0.09	0.77	-0.02	0.28	0.43	0.19

[1]Expressed as g/100 g of FAME

Table 7. Additive genetic and phenotypic (in brackets) correlations between some individual and groups of FA obtained with MIR and with GC.

	trans-11 C18:1 $_{FTIR}$	C18:3 *n*-3$_{FTIR}$	*cis*-9, *trans*-11 CLA $_{FTIR}$	SFA$_{FTIR}$	MUFA$_{FTIR}$	PUFA$_{FTIR}$
trans -11 C18:1	**0.59 (0.66)**	0.34 (0.22)	0.12 (0.60)	-0.33 (-0.24)	0.04 (0.12)	0.75 (0.54)
C18:3 *n*-3	1.00 (0.32)	**0.85 (0.41)**	0.78 (0.29)	-0.78 (0.01)	0.92 (-0.08)	0.09 (0.37)
cis-9,*trans* -11 CLA	0.29 (0.60)	-0.06 (0.21)	**0.11 (0.62)**	-0.31 (-0.28)	0.20 (0.20)	0.76 (0.53)
SFA	-0.84 (-0.40)	-0.48 (-0.09)	-0.82 (-0.37)	**0.76 (0.77)**	-1.00 (-0.75)	-0.74 (-0.22)
MUFA	0.60 (0.20)	0.46 (-0.06)	0.74 (0.17)	-0.71 (-0.75)	**1.00 (0.76)**	0.73 (0.04)
PUFA	1.00 (0.59)	0.37 (0.41)	0.66 (0.57)	-0.73 (-0.22)	0.79 (0.13)	**0.61 (0.54)**

[1]expressed as g/100g of fat.
[2]expressed as g/100g of FAME

GENERAL CONCLUSION

The main objective of this thesis was to investigate the genetic and phenotypic variability of milk composition, fatty acids profile and milk coagulation properties in dairy ewes.

The use of multivariate approach allowed obtaining new variables which were able to elucidate the relationship pattern between milk composition, MCP, and experimental cheese yield. This information could be used for management and breeding purposes.

The study highlights that stage of lactation, parity and month of lambing were the major environmental factors affecting milk composition and milk technological properties. Our results showed a worsening of cheese making aptitude of milk during lactation, as evidenced by a increase of rennet coagulation time and a corresponding decrease of curd firmness.

Genetic variability of major fatty acids suggests that some of these traits could be included in breeding programs in order to improve nutritional quality of sheep milk.