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Investigation on meteorological variables affecting sheep bulk milk quality in Sardinian farming systems

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Chapter 1: Introduction

Overview of world milk production

Humans started the consumption ruminant milk during in the Neolithic Age after the first agricultural revolution, when they evolved from a nomadic to a sedentary society, A consequence of such a transformation was the domestication of animals, used both for providing work and food. At a first stage, animals were used only to obtain meat, but afterwards all the other secondary products were known. Milk became a desired and appreciated product and an important source of nutrients, providing energy, high quality proteins, essential vitamins and minerals (Balthazar et al., 2017). Thus, dairy breeds were selected to obtain domesticated animals with higher milk yield than their wild ancestors (Barłowska et al., 2011). Sheep and goats were the first domesticated species about 10,000 years ago (Haenlein, 2007), because well-adapted to grazing or browsing (goat) of poor marginal lands.

Global production of milk worldwide in 2016 has been estimated in 798 million tonnes (FAOSTAT, 2016). Further of the total official milk production, unknown quantities exist that are destined for family or local consumption and processed directly by farmers or dairies. Most of world's (Figure 1) is produced by cows (82.6%), whereas the remaining amount derives from camel (0.3%), sheep (1.3%), goat (1.9%) and buffalo (13.9%).

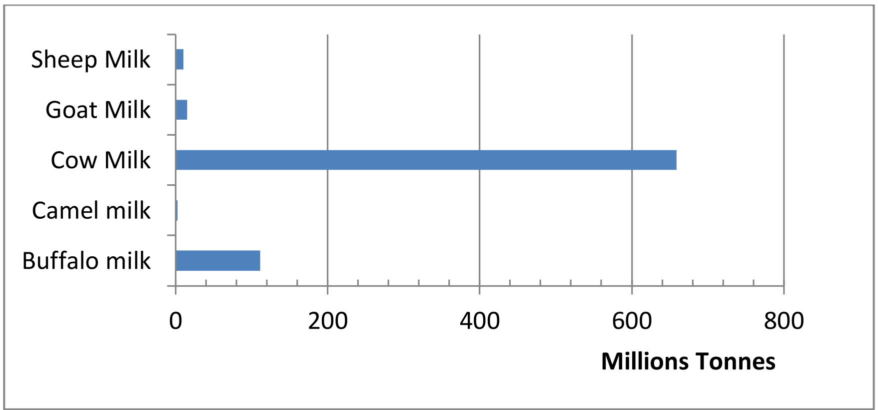

Figure 1 World milk production in 2016 (FAOSTAT)

Even if sheep milk has a lower importance compared to cows, ovine breeds are widely farmed in different regions worldwide. Sheep products contribute to the sustenance of a large number of people in rural areas and have high economic relevance. Most of the sheep milk produced in the world is processed into cheese, fresh sheep milk is seldom consumed or turned into yogurt. According to FAO statistics (2016) China (13.1%) is the major world's producer of sheep milk, followed by Turkey (9.0%) and Greece (6.9%) (Table 1).

Table 1 Top world sheep and goat milk producers in 2016 (FAOSTAT)

Sheep milk	tonnes	%	Goat milk	Tonnes	%
Asia	4,727,694	45.6	Asia	8,043,749	52.7
Europe	3,004,607	29.0	Africa	3,928,719	25.7
European Union	2,804,965	27.1	India	3,767,866	24.7
Africa	2,543,757	24.5	Europe	2,537,787	16.6
China	1,361,360	13.1	European Union	1,856,985	12.2
Turkey	929,432	9.0	Sudan	1,104,620	7.2
Greece	711,577	6.9	Bangladesh	1,051,493	6.9
Syrian Arab Republic	651,867	6.3	Pakistan	824,098	5.4
Romania	631,419	6.1	Americas	751,823	4.9
Spain	539,405	5.2	France	603,040	4.0
Mali	529,373	5.1	South Sudan	466,672	3.1
Italy	424,841	4.1	Spain	410,977	2.7
Sudan	403,008	3.9	Greece	384,903	2.5
Somalia	396,217	3.8	Somalia	377,733	2.5
Iran	326,555	3.1	Indonesia	375,453	2.5
France	292,945	2.8	Turkey	344,192	2.3
Algeria	282,759	2.7	Iran	317,910	2.1
India	204,104	2.0	Niger	289,702	1.9
World	**10,366,980**		**World**	**15,262,116**	

European Union produces itself a large amount (29.0%) of sheep milk, but many differences could be observed among the member states. The highest milk sheep production can be found in southern Europe, mainly in the Mediterranean area (Greece, Romania, Spain and Italy) (FAOSTAT, 2016). In 2016, Italy produced about 425 thousand tonnes of sheep milk, representing 4.1% of the global production (FAOSTAT, 2016). In particular, in Italy ovine milk production represents 1.1% of the Italian gross production value in agriculture, with 87,174 farms and 7,149,000 ovine head all over the country (Ismea, 2016).

Sheep breeding system and milk in Sardinia

According to Ismea (2016), about twelve thousand sheep farms are located in Sardinia, representing the 20% of the Italian total, and Sardinia itself produces the 65% of Italian sheep milk. Around 3,300,000 ovine heads, mainly belonging to the Sarda breed, are farmed (Istat, 2016). Sarda breed is an autochthonous and specialized breed of the Island of Sardinia. Its milk is almost all used for cheese production. In particular, three Protected Designation of Origin (PDO) cheeses are manufactured, Pecorino Romano, Pecorino Sardo, and Fiore Sardo, products that are very closely associated with Sardinia. Sarda breed ewes produce on average 216 L of milk per lactation, ranging from about 140 L in primiparous to 228 L multiparous (≥3 lactations) (ASSONAPA, 2017).

Figure 2 Examples of sheep breeding systems in Sardinia

Sarda sheep farming system is strongly influenced by the Mediterranean climate, with dry summer and mild winter. As rainfalls occur mainly in autumn and spring, grass is available only in these seasons (mostly in spring). The husbandry is mainly semi-extensive, with animals kept outdoors (Figure 2) and housed only during the night in the coldest 1-2 months. The flocks graze natural pastures and winter fodder crops. Supplementation with hay and concentrates is provided during autumn and winter, when herbage availability is low.

The productive cycle of Sarda sheep milk is strictly seasonal: with few exceptions, in the typical system ewes have one lambing per year, concentrated in autumn (October/December) for mature ewes and in late winter/spring for primiparous ewes, respectively. Lambing occurs when vegetative growth resumes and allows the optimal exploitation of the herbage growth cycle (Figure 3).

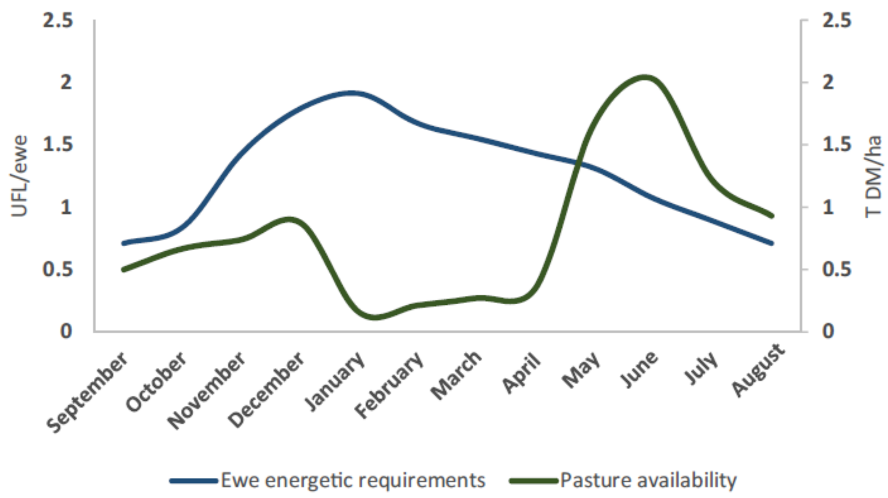

Figure 3 Energy requirements and pasture availability (from Sitzia and Ruiz, 2016)

Mating season starts in late spring for mature ewes and in early autumn for ewe lambs. After a suckling period of about 30 days, lambs are slaughtered or weaned. Ewes are usually milked twice a day from January to July. Despite of the period of lambing, drying-off happens in July and it is simultaneous for yearlings and mature ewes, when a pronounced fall of pasture quality happens for the summer drought (Carta et al., 1995; 2009; Cappio-Borlino et al., 1997a).

Sarda sheep breeding programme include selection for milk yield, and, starting from 2004, resistance to scrapie, a transmissible spongiform encephalopathy that affects sheep and goats (Carta et al., 2009).

Sheep milk composition

Many aspects of milk composition can influence milk technological properties. Milk consists of a liquid part, whose main component is water, and the dry matter. Dry matter is further composed by: a) fats and fat-soluble vitamins in emulsion; b) proteins and minerals linked to caseins in suspension; c) soluble proteins, fats and vitamins in solution; c) soluble carbohydrates, minerals, non-protein nitrogenous compounds (Pulina et al., 2001).

The average composition of milk varies among the species. While cow milk is expected to have minimal changes throughout the year, due to the year-round breeding, sheep and goat production is generally seasonal and changes occur across seasons (Balthazar et al., 2017; Barłowska et al., 2011). The chemical composition varies depending on animal feeding (dietary composition and availability), management, characteristics of the animal (breed, stage of lactation, body condition) and environmental factors such as cold and heat stress (Jaramillo et al., 2008; Nudda et al., 2014). Sheep milk contains higher total solids and fat and proteins than goat and cow milk. The higher content in fat and proteins makes it very suitable for cheese production. Although a large variability for both milk yield and composition can be observed across different breeds (Table 2). Carta et al. (2009) suggest caution in performing comparisons because a confounding effect can exist due to the genetic background of the breed, its management and their interaction.

Sarda breed milk, thanks to its high protein, fat and total solids concentrations, is very suitable for production of dairy products (Table 3), since cheese yield is strongly related to milk protein and fat.

Table 2 Milk in different sheep breeds

Breed	Fat, %	Protein, %	Casein, %	pH	Lactose, %	SCC, log cells/mL	References
Brogna	6.60	5.74	-	-	5.06	4.89	BITTANTE ET AL., 2014
Chios	4.97	5.18	-	-	4.66	2.40	KOUTSOULI ET AL., 2017
East Friesian	6.64	5.14	-	-	-	-	HERNANDEZ ET AL.,2014
Foza	5.45	5.71	-	-	5.28	4.35	BITTANTE ET AL., 2014
Guirra	8.72	6.64	5.17	6.60	4.59	5.51	JARAMILLO ET AL., 2008
Houtland	2.61	4.98	-	-	4.89	-	VITURRO ET AL., 2015
Karagouniko	5.51	5.35	-	-	4.47	2.72	KOUTSOULI ET AL., 2017
Lamon	6.69	5.51	-	-	5.08	5.96	BITTANTE ET AL., 2014
Manchega	8.68	6.39	4.97	6.72	4.21	5.23	JARAMILLO ET AL., 2008
Massese	6.44	5.71	4.73	-	4.49	3.05	MARTINI ET AL., 2008
Racka	4.48	5.83	-	-	4.57	-	VITURRO ET AL., 2015
Sarda	6.00	5.41	4.20	6.55	4.83	4.31	MANCA ET AL., 2016
Soay	4.49	5.71	-	-	4.71	-	VITURRO ET AL., 2015
Walachian	3.33	5.14	-	-	5.18	-	VITURRO ET AL., 2015

-. Not available

Table 3 Average composition of ovine milk produced in Sardinia in 2016 (ARA, 2017)

Milk Characteristic	Mean
Fat, %	6.55
Protein, %	5.59
Casein, %	4.34
pH	6.72
Lactose, %	4.79
Somatic cells count, cells/mL of milk	1010
Total bacterial count, CFU/mL of milk	230

Freezing point, °C	0.58
Milk Urea Nitrogen, mg/100mL	36.50

Milk proteins

Proteins are the main components of milk that influence milk suitability for cheese making (technological characteristics). Proteins can be divided into insoluble proteins (caseins) and soluble proteins (whey proteins) in lactoserum. Caseins are phosphoproteins synthesized in the mammary gland, whose function is to transport calcium phosphate, to provide a source of calcium, phosphorus and amino acids. Caseins are the most represented and the most important proteins in milk, constituting in sheep 76-83% of total proteins (Park et al., 2007). They are not homogenous substances and their behaviour changes in presence of calcium: while the calcium-sensitive caseins precipitate, the calcium-insensitive caseins are responsible for stabilizing the former against precipitation (Selvaggi et al., 2014a; 2014b). Precipitation occurs at pH lower than 4.6 or after rennet addition (Park et al., 2007). Encoded by genes mapped to chromosome 6, four types of casein exist: α_{s1}-, αs_2-, β- (calcium-sensitive caseins) and κ- (calcium-insensitive caseins). Their proportion and characteristics varies among species, mainly because of the existence of different genetic polymorphism, i.e. mutations in the nucleotide sequence of the gene that result in different amino acid sequences (Amigo at al., 2000; Selvaggi et al., 2014a). Casein and calcium phosphate are combined in the form of colloidal aggregates, termed micelles. Micelles are composite structures where casein molecules are held together by different interactions (hydrophobic and electrostatic) and hydrogen bonds (Hickey et al. 2015). The κ-casein is mainly sited on the surface of micelles, to stabilize them by means of its hydrophilic amino-acids. The micelle structure is similar in cow, goat and sheep milk, but the latter has caseins richer in calcium and with intermediate size between goat and cow (Park et al., 2007; Selvaggi et al., 2014a).

Whey protein represents 17-22% of total proteins and they separates from caseins during milk coagulation process. Whey proteins have high contents of sulfur amino acids, mainly methionine and cystine. Sheep milk has more whey protein than cow or goat milk. The principal whey proteins are β-lactoglobulin, α-lactalbumin and minor protein fractions as immunoglobulin, serumalbumin and lactoferrin (Nudda et al., 2003). β-lactoglobulin is the major whey protein in sheep, goat and mare milk, but its physiological function has not been well defined yet. α-lactalbumin is a calcium metalloprotein and it stimulates lactose synthesis in the mammary gland, playing a crucial role in milk secretion (Selvaggi et al., 2014a; 2014b). Lactoglobulins and immunoglobulins originate from blood and are highly hydrated. They show high heat resistance and act protective effect on caseins, impeding milk coagulation if their concentration is high.

Milk lipids

Lipids are the most important substances influencing the energetic value of milk and they account for many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and dairy products (Bauman et al., 2006). Lipids are emulsified in the aqueous phase as fat droplets, and can be found as singular globular shapes, coalesced fat globules or free fat. The number and average diameter of droplets vary depending on several elements, such as genetic, physiological and environmental factors (Martini et al., 2004). Comparing goat, cow and sheep milk, the latter has the smallest globules, with an average diameter of 3.30 µm (Park et al., 2007). Fat drops are rounded by a triple-layer membrane deriving from a portion of the mammary cell membrane, made mainly of phospholipids and glycoprotein. The membrane, thanks to its hydrophilic characteristics, has a major role in stabilizing droplets. The dispersion of fat influences the creaming rate and other important features of milk such as optical, rheological, and technological characteristics (colour, viscosity, conductance, separation rate, emulsion stability, and suitability for cheese and butter production) (Barłowska et al., 2011). Increased quantities of long-chain fatty acids accelerate the rising of fat globules and the formation of a cream layer (Sevi et al., 2002).

Triglycerides are the most represented lipids in milk (Table 4), representing more than 98% of sheep milk fat. Triacylglycerols are composed of glycerol and three fatty acids with different carbon chain lengths and degree of saturation, produced in the rough endoplasmic reticulum of alveolar cells. Furthermore, the lipid composition includes diacylglycerols, monoacylglycerols, cholesterol esters, phospholipids and liposoluble compounds (Park et al., 2007).

Table 2 Lipid classes content of cows, goats and ewes milk fat (adapted from Rodríguez-Alcalá and Fontecha, 2010).

Lipid classes (%)	Cow	Goat	Ewe
Triacylglycerides	97.75	97.32	98.11
Diacyglycerides+ Cholesterol+ Free Fatty Acids	1.81[a]	1.89[a]	1.45[b]
Cholesteryl ester	0.04	0.04	0.02
Monoacylglycerols	0.04	0.10	0.03
Phospholipids	0.36	0.65	0.39

Milk composition in terms of fatty acids is plastic and can be modified by modifying diet (Pulina et al., 2005; Vera et al., 2009). The final content of fatty acids in milk and meat is influenced by biohydrogenation of unsaturated fatty acids, mainly linoleic and linolenic acids, (Figure 4) and their transformation to saturated ones (stearic acid) as a result of microbial ruminal activity(Maia et al., 2007).

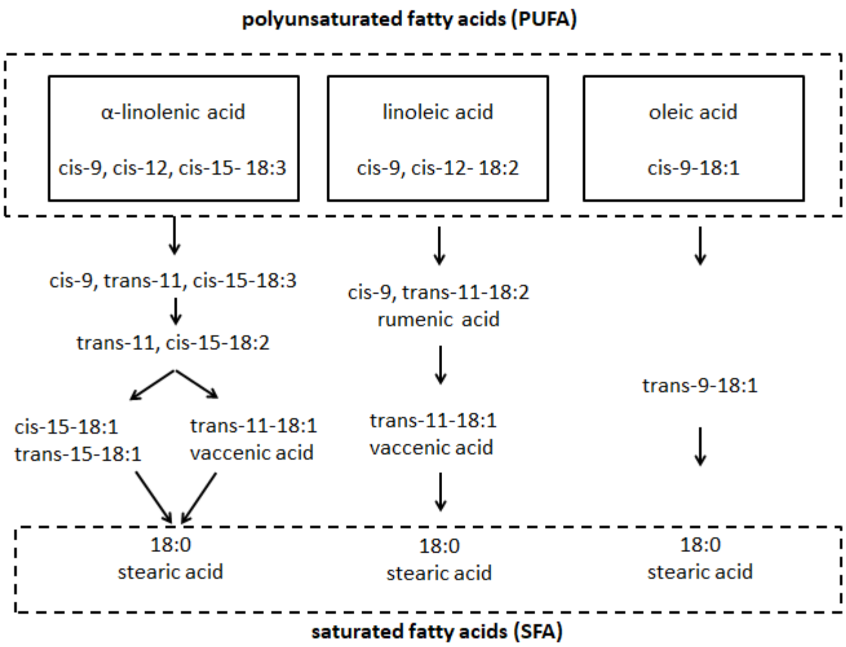

Figure 4 Biohydrogenation pathways of α-linolenic, linoleic, and oleic acids (modified from Jenkins et al., 2008).

Short chain fatty acids (C6-C10) are synthesised *ex novo* in the mammary gland, starting from acetate and β-hidroxybutyrate produced by rumen fermentation. Medium chain fatty acids, with a chain length varying from 12 to 16, can originate either by *ex novo* synthesis or uptake from bloodstream. Sheep milk contains large quantities of short and medium chain fatty acids (caproic, caprylic, capric, and lauric), that contribute to its aroma (Park et al., 2007). Long chain fatty acids (C>18) come from lipids circulating in blood, originated from mobilization of body adipose tissues or from the diet. Furthermore, both mammary gland and intestine can modify fatty acids with a chain length varying from 14 to 18 by means of desaturase enzymes (Nudda et al., 2014). For example, the vaccenic acid, the most abundant trans fatty acid in goat and sheep milk, is desaturated in the mammary gland into rumenic acid, the major conjugated linoleic acid (CLA) isomer in milk (Scintu and Piredda, 2007). The contribution of the different sources (mobilized body fat, *ex novo* synthesis by mammary gland, diet or rumen fermentation) to milk composition is influenced by the energy balance of the animal, lactation status and nutrient balance (Vera et al., 2009). More than 75% of fatty acids in sheep and goat milk are capric (C10:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), and total C18:1 (Park et al., 2007). Compared to cow, sheep milk and meat have a higher concentration of CLA positional and geometric isomers of linoleic acid, that have positive effects on human health (Park et al., 2007; Barłowska et al., 2011).

Lactose

Lactose is the main milk glucyde. It is entirely synthesized in the udder from precursors and in bovine it is responsible for 60% of the osmotic-coupled water flow that produces milk volume (Silanikove et al., 2000). The synthesis of lactose determines the rate of daily milk production, so its content shows little variation during lactation (Vera et al., 2009). The concentration of lactose follows the pattern of milk yield: it is low at the beginning of lactation in colostrum, rises and then decreases during lactation, with an opposite trend than that observed for protein, caseins and fat content (Bencini and Pulina, 1997; Sevi et al., 2000; Nudda et al., 2003). With the increase of number of lactations, Sevi et al. (2000) found a decrease in the lactose concentration in milk.

Somatic cell count

Different types of somatic cells can be found in milk: epithelial cells, blood cells and cytoplasmic particles. The proportions of the different categories vary during the lactation and they depend also on the health status of the animal. Indeed, most of the blood cells are lymphocytes, macrophages and leukocytes that reach the mammary gland as a response to a local infection (Bergonier and Berthelot, 2003; Albenzio et al., 2011). The Somatic Cell Count (SCC) can increase not only as a consequence of an inflammatory process (being the most important cause of increase), but also for non-pathological conditions due to physiological processes, as changes in milking frequencies (Nudda et al., 2002), oestrus or advanced stage of lactation, that is the main cause of SCC increase in healthy udder (Raynal-Ljutovac et al., 2007). SCC determination generally uses fluoro-optoelectronic method, automated by Fossomatic devices (Foss Electric, Hillerød, Denmark), that shows a good accuracy (Raynal-Ljutovac et al., 2007).

Milk mineral components

The main mineral compounds in milk are calcium and phosphorus (in the form of phosphate), but also sodium, potassium, chloride, iodine, magnesium, and small amounts of iron are contained. In sheep Ca, P, Mg, Zn, Fe, and Cu are present in higher contents than in cow, but their presence is more variable depending on changes in diet and period of the year.

Minerals in milk show high interest for their nutritional and healthy value. Calcium, sodium and phosphorous have a high bioavailability and they have a major effect on structure and texture of cheese. Minerals can be in soluble phase (as Na, K and Cl) or can be associated with micelles (Ca, Mn, P). This influences, during cheese-making process, their lost in the whey or retention in the curd. Milk mineral content, together with lactose, is responsible for the osmotic balance of a healthy udder and it is correlated to milk yield (Park et al., 2007).

Milk vitamins

Milk contains both water-soluble and fat-soluble vitamins. In sheep milk vitamin A, thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, biotin, vitamin B12 and vitamin C are higher than in cow and goat milk (Park et al., 2007). The greater content of vitamin A than cow milk corresponds to a lower quantity of β-carotene, resulting in a whiter colour (Barłowska et al., 2011). Sheep milk can be considered an important source of vitamin C, because of its concentration of about 4mg/100mL of milk.

Quality of sheep milk

Sheep milk quality is often considered as its capability to be transformed into cheese and dairy products. Cheese making requires milk coagulation, that occurs for the addition of rennet or acidification. The first step of rennet coagulation is enzymatic hydrolysis, where rennet cleaves k-caseins at Phe105-Met106 bond, originating para-k-Casein (residues 1-105) and casein-macropeptide (106-169). This transformation results in a reduction of repulsion, leading to the aggregation of the rennet-altered micelles (Lucey, 2002), that spontaneously expels whey. This process, termed syneresis, can occur by externally applying pressure onto the curd. The processing properties of milk, intended as the amount and quality of cheese that can be obtained from each litre of milk, can be evaluated through different methods. Since cheese yield is strongly related to milk protein and fat, it can be predicted through analytical determination of these parameters (Pirisi at al., 1994). Other methods refer to its clotting properties. Clotting properties are generally determined through the use of a mechanical lactodynamograph, as Formagraph instrument (Foss Electric A/S, Hillerød, Denmark), that provides a diagram illustrating formation and development of curd after the addition of rennet. Traditionally, milk-coagulation properties registered are: i) the renneting clotting time (RCT), which is the time when coagulation start appearing and casein micelles form visible flocks after the addition of rennet; ii) the curd firming time (k20), which is the rate of curd formation, and it measures how quickly the curd firms; iii) curd firmness (A30), which is the consistency of the curd measured 30 minutes after adding rennet (Bencini, 2002; Bittante et al., 2012).

Great variability exists in milk coagulation properties, depending on ruminant species (cattle, buffalo, sheep, and goats), breed within species, and individuals. The most of the studies refer to cows, while for small ruminants, and sheep particularly, milk coagulation properties are less known, even if they are heritable and their knowledge is very useful for the dairy industry. Processing properties of milk can be affected by various factors, that are endogenous (depending on milk characteristics) or exogenous (related to the process).

Factors influencing milk yield, composition and processing properties vary depending on several issues, some of which can be controlled by the farmer, as feeding or milking technique, while others are specific of the animal (i.e. breed, age and parity, stage of lactation).

Genetic factors affecting milk yield, quality and composition

Milk yield is currently the selection criterion in most of dairy sheep breeds. The selection scheme of Sarda ewes, realized since the early 1960s, provides a genetic gain of 2.0 L/year. In sheep, heritability for fat and protein percentages is about 0.50-0.60 while milk, fat, and protein yields is lower, around 0.30. The phenotypic and genetic correlations between milk and fat and protein yields are positive and range between 0.77 and 0.93, whereas genetic correlation between milk yield and content are negative and show high variability (Carta et al., 2009).

A genetic factor that influences greatly cheese-making properties is the polymorphism in ovine milk proteins, strongly associated with quantitative and qualitative milk parameters (Amigo et al., 2000; Carta et al., 2009; Selvaggi et al., 2014a). The genetic variant of β-casein (in dairy cows) or α_{s1}-casein (in goats) produces differences in texture and taste in cheese (Coulon et al., 2004). In sheep, α_{s1}-casein exist in eight different forms (A, B, C, D, E, F, H and I), seven variants have been found for α_{s2}-Casein (A, B, C, D, E, F, G) and five for β-CN (A, B, C, X, Y). κ- Casein is considered monomorphic in sheep (Selvaggi et al., 2014a).

The α_{s1}-casein variants differ by amino acid substitutions and degree of phosphorylation. The C variant shows higher total protein and casein content, smaller micelles diameters and higher clotting capacity. The D variant, detected also in Sarda breed, is associated with detrimental effects to ovine milk, with lower casein content and poorer technological properties (Amigo at al., 2000; Carta et al., 2009; Selvaggi et al., 2014a).

In Merino sheep, the GG genotype for β-Casein is associated with an increase in milk production but lower protein and fat percentages, whereas the AA has the highest milk fat and protein percentages (Corral et al., 2010).

Three variants of β-lactoglobulin exist (A, B and C), whose influence on composition and cheese-making properties is controversial and no conclusive evidence exist about their effect (Giaccone et al., 2000; Selvaggi et al., 2014a;). Nudda et al. (2003) noticed in Sarda breed a significant depressive effect of B variant on milk yield.

The main objective of molecular genetics studies is discovering and mapping genetic markers and genes that control production, quality, fertility and health traits for increasing both knowledge of genetic mechanism and to maximise the genetic gain. The use of microsatellites on the sheep linkage map allowed to identify Quantitative Trait Loci (QTL), highly specific for each population, that affect several traits (e.g. milk traits, SCC, nematode resistance, FA contents in milk fat, and udder traits). QTL for lactation persistency and longer period of milkability in sheep were studied, but results suggest that these traits are not controlled by the same genes that control milk composition (Jonas et al., 2011). Since fat and protein contents have negative genetic correlation with milk yield, the selection criterion would lead to a reduction of the genetic gain of each trait. In this view, the potential advantage of gene-assisted selection applied to sheep seems to be attractive (Carta et al., 2009). At the moment, genes that encode enzymes directly involved in FA metabolism are under study and two genes with polymorphism influencing the content of fat and protein in milk and CLA are identified (Carta et al., 2009). These QTL, obtained through traditional linkage mapping studies, are not used in selection programs because of low significance levels and large confidence intervals. More recently, the availability of high efficient single nucleotide polymorphism (SNP) typing technologies have made possible genome-wide association (GWA) analyses and genomic selection approach (Meuwissen et al., 2001) useful either to identify regions influencing traits of economic interest or to estimate genomic breeding values to score the selection candidates in breeding program, respectively. To date, only a few studies exist at genome wide scales in sheep aimed at quantifying the effects of single nucleotide polymorphism on milk characteristics and processing properties, whereas many more exist for cattle. The close phylogenetic relationship between sheep and cows promoted comparative studies on the Spanish Churra dairy sheep to assess the genetic variability of various genes influencing milk production traits in dairy cows (García-Fernández et al., 2011). Nevertheless, the associations between SNPs identified in these genes and milk production traits (test day milk yield, test day protein percentage and test day fat percentage) were not significant (García-Fernández et al., 2011), showing that outcomes of cattle cannot be completely referred to dairy small ruminants. Garcia-Gámez et al. (2012) conducted on Spanish Churra sheep a GWA analysis, identifying on chromosome 3 a QTL influencing milk protein and fat contents. The SNP with the highest significant association was placed on the α-lactalbumin gene, but further studies are required to establish if this SNP is the true causal polymorphism or it is in linkage disequilibrium with it. In Sarda breed, 29 SNPs are identified on the promoter region and 3'-UTR of the four casein genes, with a significant level of polymorphism. These SNPs may perform regulatory effects on genes associated with milk protein and casein contents and processing properties (rennet coagulation time, curd firming time and curd firmness) (Noce et al., 2016). For a greater quality in mapping QTL, the use of three approaches is suggested, one based on linkage analysis information, the other combing linkage disequilibrium and linkage analysis (LDLA) and the latter using a GWA analysis (Garcia-Gámez et al., 2013). In fact, while linkage analysis allowed to discover three significant QTL on sheep chromosome 2 influencing milk yield, protein yield, and fat yield, the LDLA method identified 34 genome-wise significant QTL regions (Garcia-Gámez et al., 2013).

Nutritional and non-nutritional factors affecting milk yield, quality and composition

Feeding

Feeding is the environmental factor that most strongly impacts sheep milk composition, particularly for highly productive animals. The most relevant factors influencing milk yield and composition are net energy intake, protein and fibre content of the diet.

Milk fat concentration is easier to modify by nutritional means than protein content. Ewe's net energy balance strongly influences fat content in milk, especially in the first part of lactation. Milk fat depends also on the dietary content of non-degradable fibre, that is positively correlated to milk fat content for a reduction in milk production. Fat supplements and their characteristics (like doses, physical form protecting against rumen fermentation, etc.) play an important role in increasing fat percentages and modifying the content in milk of fatty acids useful for human health, such as conjugated linoleic acid and omega-3 fatty acids (Nudda et al., 2001; Pulina et al., 2006; Voutzourakis et al., 2014).

Milk protein content is less alterable by diet. Generally, a higher energy content of the diet, and particularly a higher quantity of carbohydrates, corresponds to an increase of the percentage of milk protein, but diet protein and amino acid supplementation influence only to a limited extent milk protein amount and its characteristics (Nudda et al., 2001; Pulina et al., 2006; Voutzourakis et al., 2014).

Diet impacts also the content in vitamin A, carotenoids and tocopherols, minerals as Mn, Co, I, Se and other compounds as condensed tannins (Voutzourakis et al., 2014), especially in milk from pasture fed animals.

Physiological factors

Persistency of lactation, the rate of milk yield decrease after the lactation peak, depends on numerous physiological factors. Many hormones are involved in maintaining lactation in sheep, as growth hormone, prolactin and oxytocin. Growth hormone stimulates the production of insulin-like growth factor-I (IGF-I), recognized by IGF-I receptors in the mammary gland, with a positive effect on yield and characteristics of milk (Pulina et al., 2001; 2007). Prolactin influences milk synthesis by inhibiting the mammary apoptosis. Oxytocin influences myoepithelial cell contraction and milk let down and may also be involved in mammary cell maintenance and metabolism (Pulina et al., 2007).

Furthermore, milk constituents can modulate milk secretion. Some fractions of proteins contained in milk, if not removed by milking, carry out chemical inhibition and influence the rate of secretion when milk is stored in the secretory tissue (Pulina et al., 2007). This behaviour has been confirmed by experiments where unilateral frequent milking has no effect on milk secretion by the other udder half (Nudda et al., 2002). The degree of feed-back inhibition is determined by the concentration of inhibitor in the alveolar lumen (Wilde at al., 1988).

Plasmin-Plasminogen system is a physiological pathway affecting milk yield and characteristics able to carry on short-term regulation of milk secretion and induction of milk involution in case of milk stasis (Silanikove, 2016). Plasmin-plasminogen system is a complex protease-protease inhibitor system (Figure 5), formed by active and inactive forms, respectively Plasmin (PL) and plasminogen (PLG), modulated by the plasminogen-activator and the inhibitors and influenced by somatic cells (Silanikove et al., 2000, 2016; Kelly at al., 2006; Pulina et al., 2007; Ismail and Nielsen, 2010).

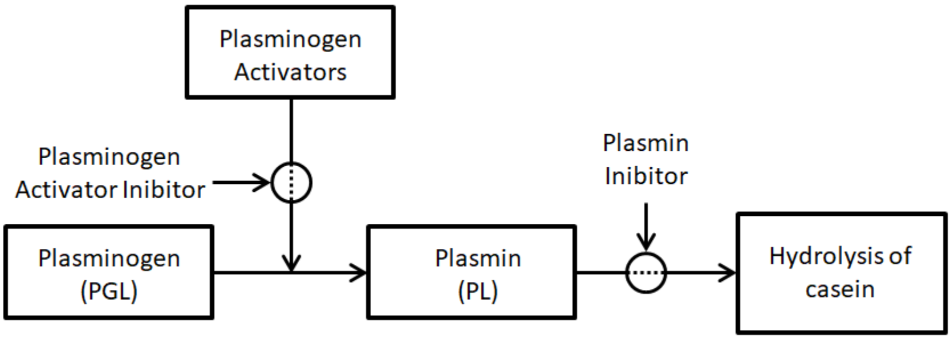

Figure 5 Plasminogen-plasmin system in milk (adapted from Ismail and Nielsen, 2010)

PL is a protease responsible in milk for the hydrolysis of α- and β- casein, leading to the formation of γ- casein and boiling-resistant peptides (proteose peptone), which then diffuse into the whey. This decomposition occurs both in the udder and during storage, modifying protein patterns of milk. PL originates from blood and enters milk in soluble form, then associating with casein micelles (Kelly at al., 2006), mainly in its inactive form, plasminogen (PLG).

Sevi et al. (2004) found in Comisana ewes significantly higher presence of both plasmin and plasminogen with the advance of lactation, regardless of the lambing season. Since plasmin plasminogen system seems to be involved in the gradual involution of the mammary gland, its effect increases as lactation proceeds. Levels of PL and PLG in milk are higher at the end of lactation, in older animals, and in mastitic milk, as it happens in dairy cows (Ismail and Nielsen, 2010).

Lambing season, parity and type of lambing

Lambing season can influence persistency of lactation because of the different availability and quality of pasture, particularly in extensive systems. If lambing occurs when the availability of forage is higher, a higher milk production is expected. The characteristics of milk produced by sheep that lambed in the spring can be related to the presence of high quantities of fresh forage in their diet (Martini et al., 2008). Considering that lactation occurs when days are lengthening, photoperiod can be another element of influence. In fact, in dairy ewes a higher number of hours of light seems to coincide to higher feed intake and milk production (Pulina et al., 2007). Carta et al. (1995) found that seasonal period of production had an important influence on milk yield in Sarda breed, ranging from - 332 mL in August to + 206 mL in April (-31% and +19% of overall mean). Seasonal effects were considerable on milk composition too, since fat yield varied from -19.2 g to +11.7 g (-27% and +17% of overall mean) and protein yield from - 17 g to + 11 g (-28% and +18% of overall mean). Furthermore, while fat percentages changed irregularly, protein percentages tended to remain constant.

Number of parity influences milk yield and the higher production is registered after the third or fourth lactation (Carta et al., 1995; Bencini and Pulina, 1997; Cappio-Borlino et al., 1997a, 1997b; Macciotta et al., 1999: Nudda et al., 2003; Jaramillo et al., 2008). The effect of the order of parity and age is difficult to be distinguished, but younger ewes produce less milk than older ones and milk quality is strongly affected by the increase of the number of lactations.

Higher milk yield is registered for multiple births. This can be explained by the influence of placental weight on the development of the mammary gland in ovine breed.

Stage of lactation

Stage of lactation is an important source of variation of milk yield and components. After parturition, milk production starts and daily yields increase rapidly for the first few weeks. Around the third to fifth week of lactation, peak yield is reached. The lactation peak is the highest daily yield reached during lactation. After that, lactation declines.(Figure 6) Generally, the greater persistency is, the greater the total milk produced all over lactation (Bencini and Pulina, 1997; Pulina et al., 2007).

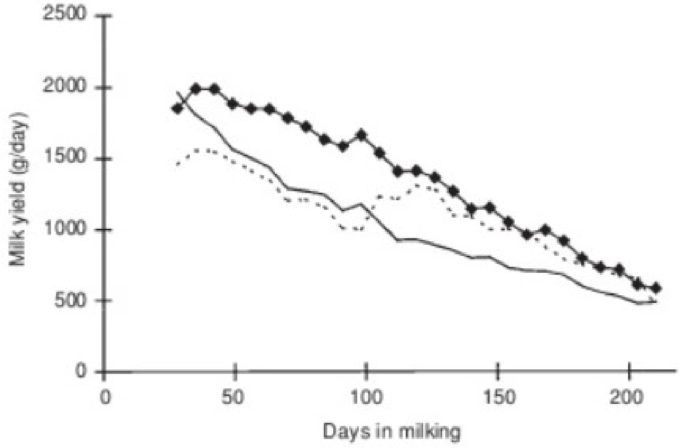

Figure 6 Lactation curve typologies in Sarda breed. Filled squares: classical lactation curve; solid line: atypical curve without peak; dashed line: double peak curve (from Pulina and Bencini, 2007).

With regards to composition, the concentration of fat (Figure 7a), proteins (Figure 7b), total solids and somatic cells is higher at the beginning and at the end of lactation than at peak lactation, while lactose follows lactation yield. Stage of lactation also affects mineral contents: chloride and magnesium tend to increase in milk, while potassium decreases during lactation (Bencini and Pulina, 1997).

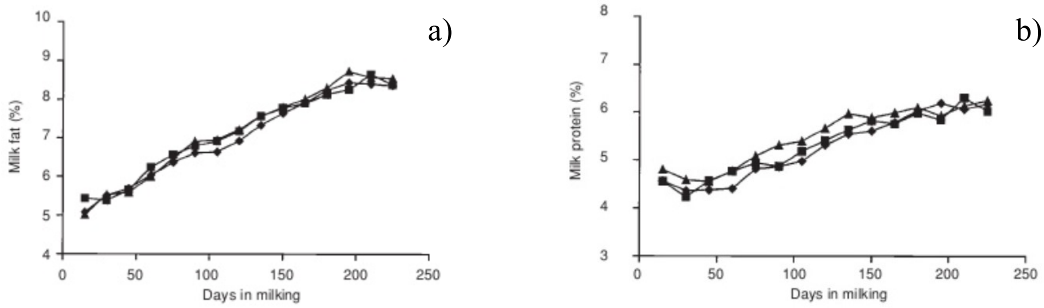

Figure 7 Milk a) fat and b) protein production along lactation (from Pulina and Bencini, 2007)

Weaning system, milking techniques and frequency

Weaning technique can affect milk yield after weaning. Studies carried out in the first 30 days of lactation showed similar length of average lactation period for different weaning systems. Nevertheless, higher milk yield was recorded when ewes were suckled for part of the day and machine milked once in the morning, after separation from lambs during the night. This was probably due to a more efficient udder evacuation by the suckling lambs combined with machine milking (Pulina et al., 2007).

The rate of milk secretion is also influenced by milking techniques and frequency. An high quality milk can be obtained with a regular and complete milking. A reduction of milking frequency can accelerate the involution process of the mammary gland and reduce lactation persistency, with losses in milk production, even if the response is not constant across breeds (Pulina et al., 2007; Castillo et al., 2008; Koutsouli et al., 2017). The reduction of milking frequency affects also milk composition, resulting in higher protein and lower lactose content and, for some breeds (e.g. Sarda), in higher SCC values (Nudda et al., 2002). The decline of milk yield leads on to losses in the total fat (Castillo et al., 2008; Koutsouli et al., 2017) and the total protein yield (Koutsouli et al., 2017).

Hand milking and machine milking do not produce differences on the concentration of protein and fat in the milk, but differences can occur because of worse hygiene conditions, resulting the former in higher microbial counts and SCC (Bencini and Pulina, 1997; Gonzalo et al., 2005).

Udder morphology and cistern dimension

The number of mammary secretory cells and their metabolic activity influence the yield (Wilde at al., 1987), but some milk constituents exert inhibiting action on the rate of secretion when milk is stored in the secretory tissue. Since these components are inactive in the cistern, their influence should be fainter in animals with wider cisterns, because less milk is in contact with the alveoli, while a large part keeps in the mammary cistern. Furthermore, mammary gland size and cistern dimension seem to have positive effect on milk production (Pulina et al., 2007). Because of the diffusion of milking machines, udder morphology has become an important trait influencing time and costs of milking. In fact low-implanted teats and low depth udders (i.e., close to the abdomen) are easier to be completely emptied. Selective breeding focuses on udder conformation traits and morphology to improve machine milkability, even if direct improvement of udder depth is difficult because of its high negative genetic correlation with milk yield (Carta et al., 2009).

Mastitis and somatic cell count

Mastitis is an inflammation of the udder that has a major role in the impairment of milk yield and quality. It has important economic and hygienic effects. Mastitis is generally caused by pathogenic bacteria belonging to various etiological groups, that exert physical damage to the epithelial cells of the mammary gland.

It is possible to distinguish between clinical and subclinical mastitis. Clinical mastitis have general and local signs (as, for example, fever, anorexia, udder inflammation and oedema) and the major pathogens causative are *Escherichia coli*, *Staphylococcus aureus*,and streptococci. Sub-clinical mastitis is more complicated to be detected, because of the absence of external symptoms, and it produces quantitative and qualitative functional modifications, as an increase in somatic cell count. It is generally caused by Coagulase-Negative Staphylococci (CNS) (Auldist and Hubble, 1998; Gonzalo et al., 2002; Bergonier and Berthelot, 2003; Rovai et al., 2014). Prevalence of clinical mastitis in dairy sheep is generally smaller than 5%, while subclinical mastitis infections range from 5 to 30% per lactation (Bergonier and Berthelot, 2003): these percentages make the latter a crucial phenomenon influencing milk yield and cheese-making. In fact, as often subclinical mastitis is not identified, milk coming from infected udders goes into the bulk milk tank, worsening its characteristics (Gonzalo et al., 2002). Numerous authors (Gonzalo et al., 2002; Bergonier and Berthelot, 2003; Martí De Olives et al., 2013) emphasized the need to prevent subclinical mastitis in dairy sheep to improve the quality of milk and minimize losses.

The global effect of an intramammary infection depends on different elements, such as infection severity, type of bacteria involved and number of infected glands (1 or 2). Gonzalo et al. (2002) found in Churra breed losses in milk yield that ranged between 2.6% and 10.1% in accord to type of pathogens and the uni- or bilateral distribution of the infection. Martí De Olives et al. (2013), in a study on Manchega ewes on a half-udder basis, found that the drop in individual milk production depended on the stage of lactation. When the onset of infection happened in the first week postpartum, losses were of 17% of production compared to a healthy sheep, while in ewes infected later during lactation losses were of 15%. An earlier onset of subclinical mastitis during lactation in Comisana ewes was described by Sevi et al. (2000) as number of lactations decreased. This may be explained by a less efficient natural defence mechanisms in younger ewes, probably because the endocrine-metabolic status and immune system development can play a role on susceptivity and sensitivity to mastitis (Albenzio at al., 2002).

SCC values can be useful to differentiate ewe milk for its overall quality and to establish whether a sheep is affected by mastitis or not, but huge disparity exist in the literature with regards to the SCC thresholds (Albenzio et al., 2002, 2011; Pulina et al., 2007; Raynal-Ljutovac et al., 2007). High values of SCC influence many characteristics of milk, and Albenzio et al. (2011) found in dairy sheep an impairment of udder efficiency starting from 300,000 cells/mL. The same authors suggested to consider the range from 300,000 to 1,000,000 cells/mL as a transition from normal to mastitic milk.

Even if the wider bibliography is referred to cow's milk, also in sheep and goats the negative relationship between a high SCC and milk yield and quality has been confirmed.

Nudda et al. (2003) found in Sarda breed a significant reduction of milk yield and lactose for SCC greater than 1,000,000 cells/mL, as well as in Churra and Comisana breed (Gonzalo et al., 2002; Albenzio et al., 2011).

Various aspects of milk composition are related to high SCC (Table 5).

Table 3 Changes in sheep milk composition associated with an increase of SCC

	Effect[1]	References
pH	↑	Bencini and Pulina, 1997; Pellegrini et al., 1997; Albenzio et al., 2004; Bianchi et al., 2004; Pirisi et al., 2007; Carloni et al. 2016;
	–	Albenzio et al., 2011; Caboni et al., 2017
Lactose	↓	Nudda et al., 2003; Bianchi et al., 2004; Albenzio et al., 2011; Caboni et al., 2017
Fat	↑	Caboni et al., 2017
	–	Nudda et al., 2003; Bianchi et al., 2004;Albenzio et al., 2011
	↓	Bencini and Pulina, 1997; Jaeggi et al., 2003
Total protein	↑	Nudda et al., 2003; Bianchi et al., 2004 ; Albenzio et al., 2011; Caboni et al., 2017
	↓	Jaeggi et al., 2003
Casein	↑	Bianchi et al., 2004; Caboni et al., 2017
	–	Nudda et al., 2003
	↓	Bencini and Pulina, 1997; Jaeggi et al., 2003; Albenzio et al., 2011
Casein/total protein	↓	Jaeggi et al., 2003; Nudda et al., 2003; Bianchi et al., 2004; Albenzio et al., 2011
α- casein	↓	Bianchi et al., 2004;
β- casein	↓	Bianchi et al., 2004;
γ- casein	–	Bianchi et al., 2004;
Whey protein	↑	Bencini and Pulina, 1997; Nudda et al., 2003; Bianchi et al., 2004;
β- lactoglobulin	↓	Nudda et al., 2003

Serum albumin	↑	Nudda et al., 2003
Immunoglobulin	↑	Nudda et al., 2003
↑ increase; ↓ decrease; − not significant effect		

High SCC in milk can perform an enhanced proteolytic and lipolytic activity through the release of enzymes, leading to modifications in the protein characteristics and resulting in reduction of cheese yield and quality (Auldist and Hubble, 1998; Hurley et al., 2000; Albenzio at al., 2004; Silanikove et al., 2006; Raynal-Ljutovac et al., 2007; Albenzio et al., 2011). For values of SCC greater than 1,000,000 cells/mL, higher contents and activity of plasmin and an increase of pH were found (Albenzio et al., 2004;2011; Bianchi et al., 2004). The effect of high SCC on the fat percentage in sheep milk has not been clarified yet but fat content seems generally to decrease with the increase of SCC (Bencini and Pulina, 1997; Jaeggi et al., 2003). Such a trend can be explained with a reduced synthetic and secretory capacity of the mammary gland and with an enhancement of the lipase activity in milk due to the release of lysosomal enzymes of somatic cells, as reported for dairy cows by Auldist and Hubble (1998). An increased lipolysis produces higher levels of free fatty acids, causing off-flavours. Furthermore, high SCC produces a reduction in casein concentrations, due to degradation of casein by proteinases originating from bacteria, leucocytes, leucocytes or the blood (Auldist and Hubble, 1998). SCC increases can modify the cheese-making aptitude of the milk also through changes in concentration of minerals.

Furthermore, bulk tank SCC had a statistically significant correlation with total bacterial count, suggesting the application of programs to improve hygiene and health quality in bulk tank milk involving both variables (Gonzalo et al., 2005).

Total bacterial count

Milk contains microorganisms that can be advantageous for transformation into cheeses, such as *Lactobacillus* spp., *Lactococcus* spp., *Streptococcus* spp., while others can be unfavourable. In fact, some microorganisms can cause human diseases (e.g. *Listeria*, *Salmonella*, *Brucella*) or can negatively compromise the maturation of the dairy products (e.g. Enterobacteriaceae, coliforms, psychrotrophs, *Clostridium* spp.) (Bencini and Pulina, 1997).

The total bacterial count (TBC) is the number of aerobic microorganisms which develop in a millilitre of milk at a temperature of 30°C, expressed as a number of colony-forming units (CFU/mL). The maximum thresholds for sheep and goat milk are ruled by the EU Directives. The routine analysis in dairy laboratories uses automated flow cytometry devices, such as Bactoscan FC, Foss Electric. This device does not allow differentiation of the milk flora (lactic, psychrotrophic, pathogenic bacteria) (Pirisi at al., 2007).

The microbiological safety of milk, the occurrence of undesirable bacteria and the quality control are delicate tasks involving different conditions. At the farm level, contamination can come from different sources such as flora and pathogens present in beds and getting in touch with the external surface of the udder and teats, milking facilities, wash water, milking system, or animal health (e.g. mastitis) (Elmoslemany et al., 2010; de Garnica et al., 2013). Contamination can be avoided by applying correct milking and milk handling procedures (Bencini and Pulina, 1997). In bovine, Jayarao et al. (2004) found that herd size and farm management practices had considerable influence both on somatic cell and bacterial counts in bulk tank milk. In fact, as the herd size increased, so did the use of automatic milking equipment that allows better hygiene standards. The lower values of TBC and SCC were found where automatic milking detachers, sand as bedding material, and strategies of mastitis prevention and control (such as dip cups for teat dipping instead of spraying, and pre- and post-dipping) were practiced. For instance, de Garnica et al. (2013) found in ovine milk

TBC higher than the ones reported in literature for cattle. An explanation can be found in the management practise in sheep, as the absence of teat washing before milking, that is not a usual practice in dairy ewes, and poorer facilities than those for cattle (Bergonier and Berthelot, 2003; de Garnica et al., 2013; Gonzalo et al., 2006). Furthermore, as hygiene can vary within the same flock depending on the season and management practices (as confinement versus grazing), in winter higher concentrations of psychrotrophic and coliform flora were noticed (de Garnica et al., 2013). Pirisi et al. (2007) pointed up that the lack of milking machines and refrigeration tanks at the farm and transport of milk in unsuitable vehicles have a notably influence on high TBC.

Stress

Sheep can face different sources of stress, that affect quantity and quality of milk produced, impairing the efficiency. In fact stress leads to the activation of the hypothalamus-pituitary-adrenocortical axis and liberates cortisol into blood plasma, resulting in a decrease of milk synthesis (Silanikove et al., 2000). Management practices are potential stressing factors, because sheep and goats suffer sudden from changes in rearing practices (e.g. time, place and stockmen involved in milking or when regrouping and relocation occur frequently) or excessive and rough handling (Sevi et al., 2009).

Heat stress

Weather can determine stressful conditions, producing a detrimental impact on production when environmental temperature and humidity fall outside the thermal comfort zone. The sensitivity to heat stress varies among species and within the same species, it depends on numerous factors such as the age or feeding characteristics. Effects of heat stress on production are widely studied on cattle, while only a few studies exist on sheep (Finocchiaro et al., 2005; Peana et al., 2007a; 2007b; 2017; Ramon et al., 2016; Sevi et al., 2001). In spite of that, it is widely accepted that exposure to adverse weather conditions has important economic effects, because it leads to losses in milk production, morbidity and mortality, impairing production and reproduction traits of the animal (Figure 6).

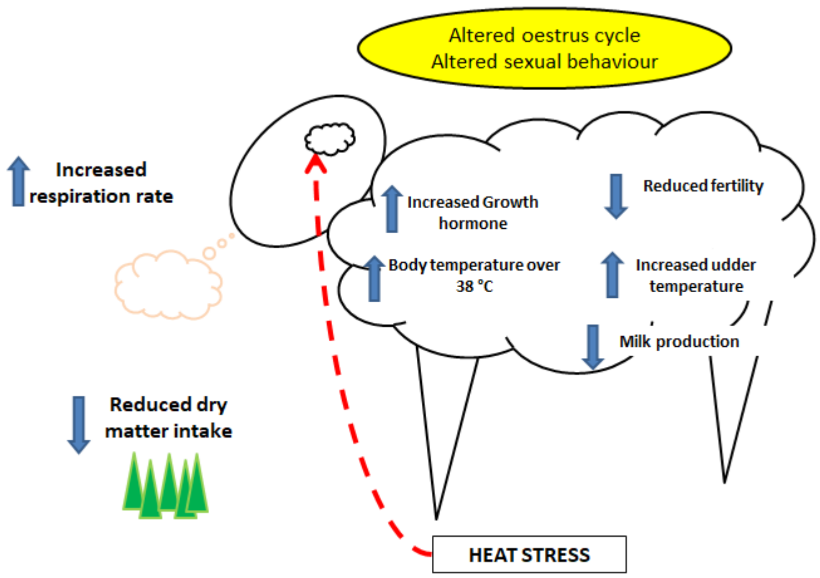

Figure 8 Heat stress influence on different activities in sheep (adapted from Sejian et al., 2017)

Although heat stress occurs more frequently in hot climates, even in Mediterranean environments animals experience this state. In fact, temperatures reached in Mediterranean area during the summer are above the comfort zone and often hot conditions happen quickly and they are prolonged, impeding the animal to acclimatize. Several studies (West et al., 2003; Finocchiaro et al., 2005; Sevi and Caroprese, 2012) suggested an improvement of animal welfare and productivity in hot climates by means of both management techniques for reducing heat stress and sheep breeding programmes including multi trait selection.

Thermoregulation and heat dissipation mechanisms

The control of cellular reactions and physiological responses of metabolism require homeotherms to keep their temperature variation in a fairly narrow range. The thermoneutral zone is the temperature interval at which the animal expends the less energy to maintain its temperature constant, and produces the less metabolic heat (Kadzere et al., 2002; Al-Dawood, 2017). Above the comfort zone, the animal has to apply behavioural and physiological strategies of thermic dissipation for increasing heat loss and reducing heat production with an increase in animal maintenance requirements, because part of the energy is used for thermoregulation. In spite of that, with the exposure to heat, dry matter intake decreases significantly. In dairy cattle, dry matter intake drops of 0.85 kg for each degree (°C) of increase in the mean air temperature beyond the thermal comfort zone (West et al., 2003). The decline in dry matter intake causes a reduction in metabolic heat generated by digestion. Other consequences are reduction of feed transit through the digestive tract, reduction of rumination and nutrient absorption, which result in altered performances and in a decrease of milk yield. Immediate responses as behavioural changes occur, such as increased water intake, sweating and panting, and heart rate reduction. If the exposure to heat is protracted, changes interest physiological processes: modification of rectal temperature, heart and respiratory rate; alteration of enzyme and hormones production; modification of metabolism of water, protein and mineral balances (Marai et al., 2007; Bernabucci et al., 2010; Al-Dawood, 2017). When the thermal stress on the animal is too high, body temperature rises up to hyperthermia and can lead to death.

Heat is transferred to environment through physiological processes. Four main ways for heat exchange exist: conduction, convection, radiation and evaporation. Conduction, convection and radiation are based on a thermal gradient, thus their efficiency is reduced when air temperature rises. On the other hand, evaporation is based on a vapour pressure gradient, and it is the most effective mean to lose heat at high temperature, sometimes compromised by high relative humidity. When heat stress occurs, external temperature rises and heat exchanges between the animal and the environment are inhibited. Sensitivity to heat stress can be increased by genetic selection for production traits: high productive animals produce higher metabolic heat and are more vulnerable and sensitive because of higher body weight, larger nutrient intake and more effective use of nutrients. The relationship between high production and susceptivity to heat could be explained also by the surplus of heat associated to the production of extra milk (Kadzere et al., 2002; Bernabucci et al., 2010).

For the majority of species, the thermoneutral zone can be placed between 13° C and 18°C (Peana, 2005). For lactating cows it is placed between 5 and 25°C (Kadzere et al., 2002; West, 2003). Sheep and goats, in spite of being considered animals adapted to harsh environment, are more sensible than how generally estimated, especially when high temperature persist for long periods (Peana et al., 2007a). Ramón et al. (2016) found in Manchega sheep a comfort region between 10 and 22°C for daily average temperature, and 18 and 30°C for daily maximum temperature.

The stressful effects of heat can vary depending on several environmental elements. Components such as radiation, air temperature and movement, humidity can influence the dissipation of heat. So, management precaution can produce differences in the effect of the same climatic conditions. Shade can improve welfare and production of animals, reducing solar accumulation, and protecting them from direct and indirect radiation (Armstrong, 1994; Sevi et al., 2001). At high temperature, air movement around the animal can modify the rate of heat loss, because high speed of wind can support heat dissipation by means of a more intense evaporation. Also precipitation has a positive effect in alleviating heat stress, because rain can wet animal coat. High air humidity can result in a discomfort, because it compromises evaporative cooling (West, 2003). The colour of the coat and its texture impact the heat gain. A black-coated animal will have a higher absorbance than a white one, influencing heat exchanges through radiation (Silanikove, 2000). Heat stress can be relieved by a correct diet and water availability (Marai et al., 2007), and changing of feeding time to the afternoon, to reduce heat production in the warmer hours of the day (Sevi et al., 2001). Water intake increases up to 50% during heat stress, while water losses in the faeces and urine decrease (Marai et al., 2007). In addition, minimum nocturnal temperatures are crucial in hot weather. In Holstein cows, if a cold period of less than 21°C occurs at night for 3-6 hours, the loss of heat gained is favoured and the effects of heat stress on milk production are reduced (Igono el al., 1992). In sheep, a reduction of THI below 72 for a number of hours relieves part of the stress associated with diurnal heat and humidity (Sevi et al., 2001).

Estimation of heat stress degree

Evaluation of heat stress can use different parameters. Direct measurements on animals, such as rectal temperature, respiration rate and heart rate, in spite of being very sensitive indicators of thermal stress, are really expensive to be defined and not feasible on a large scale. It is important to determine thresholds of heat stress in a cheap determination. Scientists developed biometeorological indices to predict when animals start experiencing heat stress. Many of these combine different environmental factors, but their use is reduced by the poor availability of data. An indicator of thermal climatic conditions often used is the Temperature Humidity Index (THI). The THI is a thermal index that represents the combined effect of humidity and temperature, quantifying heat stress in relation to these climatic parameters. Humidity and temperature are two variables that can be easily measured and obtained from meteorological services. In literature, a number of THI exist (Table 6), weighting differently the effect of temperature and humidity.

Table 4 Equations for calculating THI (modified from Berman et al., 2016)

THI Equation	Unit	Reference
$0.4\cdot(T_{db}+T_{wb})+15$	°F units	Thom, 1959
$1.8T_{db}-(1-RH:100)\cdot(T_{db}-14.3)+32$	°C units	Kliber, 1964
$(0.55\cdot T_{db}+0.2\cdot T_{dp})+15$	°F units	Cargill and Stewart, 1966
$(1.8T_{db}+32)-[(0.55-0.55\cdot RH):100]\cdot[(1.8T_{db}+32)-58]$	°C units	Kelly and Bond, 1971
$0.72\cdot(T_{db}+T_{wb})+40.6$	°C units	Maust et al., 1972
$T_{db}-(0.55-0.55\cdot RH)\cdot(T_{db}-58)$	°F units	Ingraham et al., 1974
$T_{db}+0.36\cdot T_{dp}+41.2$	°C units	Oliveira and Esmay, 1982
$0.8\cdot T_{db}+RH\cdot(T_{db}-14.4)+46.4$	°C units	Hahn et al., 2009

T_{db} dry bulb temperature; T_{dp} dew point temperature; T_{wb} wet bulb temperature; RH relative humidity

Humidity is an important factor influencing the evaporative loss through sweating and panting, because evaporative rate differs depending on the saturation of air. Air humidity can be expressed as wet bulb temperature (Thom, 1959; Maust et al., 1972), relative humidity (Kelly and Bond, 1971; Ingraham et al., 1974; Hahn et al., 2009) or dew point temperature (Cargill and Stewart, 1966; Oliveira and Esmay, 1982). Relative humidity (RH) gives information about the air saturation at a given temperature. It is expressed as a percentage and it is the ratio between the measured vapour pressure and the saturated vapour pressure at the same temperature: RH depends on the dry bulb temperature. Keeping constant the quantity of vapour (grams) in each kilogram of dry air, RH raises when T increases. In fact, at a lower air temperature, miscibility of air steam decreases. The dew point temperature (T_{dp}) is the temperature at which the measured vapour pressure is equal to the saturated vapour pressure and RH is 100%. Wet bulb temperature (T_{wb}) is the temperature that air would have if it were saturated through a process at constant enthalpy. It is measured by a thermometer whose bulb is covered by a water-wetted cloth. Air flow produces evaporation of water, removing heat from the bulb.

A recent study by Berman et al. (2016) showed that temperature and humidity have a different weight in the variance of THI values. Assessing by regression procedures the relative weights of the THI components, Berman et al. (2016) observed that the temperature resulted as the major component explaining from 82 to 95% of THI variance. In spite of accounting only for 5 to 12% of THI variance, relative humidity has a great importance in heat stress. At high temperature, a rise in RH reduces evaporation and increases the intensity of stress. This behaviour results in a higher rectal temperature, reduction of feed intake and reduced milk production, as demonstrated in studies that analyse the production at the same temperature but with different percentages of RH (Kadzere et al., 2003).

Temperature humidity index is often represented as a graphical scale (Figure 6), showing if the animal suffers a stress, depending on temperature and humidity, since different temperatures and humidities can produce the same THI.

	Relative Humidity, %																			
Temperature, °C	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
21	64	64	64	65	65	65	66	66	66	67	67	67	68	68	68	69	69	69	70	70
22	65	65	65	66	66	67	67	67	68	68	69	69	69	70	70	70	71	71	72	72
23	66	66	67	67	67	68	68	69	69	70	70	70	71	71	72	72	73	73	74	74
24	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75	76	76
26	68	68	69	69	70	70	71	71	72	73	73	74	74	75	75	76	76	77	77	78
27	69	69	70	70	71	72	72	73	73	74	75	75	76	76	77	78	78	79	79	80
28	69	70	71	71	72	73	73	74	75	75	76	77	77	78	79	79	80	81	81	82
29	70	71	72	73	73	74	75	75	76	77	78	78	79	80	80	81	82	83	83	84
30	71	72	73	74	74	75	76	77	78	78	79	80	81	81	82	83	84	84	85	86
31	72	73	74	75	76	76	77	78	79	80	81	81	82	83	84	85	86	86	87	88
32	73	74	75	76	77	78	79	79	80	81	82	83	84	85	86	86	87	88	89	90
33	74	75	76	77	78	79	80	81	82	83	84	85	85	86	87	88	89	90	91	92
34	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
36	76	77	78	79	80	81	82	83	85	86	87	88	89	90	91	92	93	94	95	96
37	77	78	79	80	82	83	84	85	86	87	88	89	90	91	93	94	95	96	97	98
38	78	79	80	82	83	84	85	86	87	88	90	91	92	93	94	95	97	98	99	100
39	79	80	81	83	84	85	86	87	89	90	91	92	94	95	96	97	98	100	101	102
40	80	81	82	84	85	86	88	89	90	91	93	94	95	96	98	99	100	101	103	104
41	81	82	84	85	86	88	89	90	91	93	94	95	97	98	99	101	102	103	105	106
42	82	83	85	86	87	89	90	92	93	94	96	97	98	100	101	103	104	105	107	108
43	83	84	86	87	89	90	91	93	94	96	97	99	100	101	103	104	106	107	109	110

Categories of Livestock Weather Safety Index associated with THI values:

Normal: ≤ 74 Alert: 75-78 Danger: 79-83 Emergency: ≥84

Figure 9 Temperature-Humidity Index (THI) chart (from Hahn et al., 2009)

Various THI can be calculated according to the use of different temperatures (average, minimum and maximum) and humidity (average, minimum or maximum) registered during the day (Ravagnolo et al., 2000; Finocchiaro et al., 2005; Peana et al., 2007b; 2017; Brügemann et al., 2012; Bertocchi et al., 2014). THI maximum includes daily highest temperature and minimum humidity and it is representative of the worst daily environmental conditions. THI minimum is calculated using daily minimum temperature and maximum humidity and it is generally considered as indicator of refers night conditions; while average THI utilizes daily average values of both temperature and humidity.

Furthermore, a delay in the full impact of climatic variables on production exists. This effect can be ascribed to the reduction of feed intake, deferring between intake and utilization of nutrients, or variations in the endocrine status of the animal (West, 2003; Bertocchi et al., 2014). For this reason, Finocchiaro et al. (2005) used meteorological data referred to a period of four days before the day of milk recording, even if little differences were found, probably because the stable weather in the sampling period. On the contrary, Ramon et al. (2016) ascribe the greater effect on milk yield to weather conditions on the test day and the two previous days, with a reduced effect of conditions in the preceding days.

Effects of heat stress on milk production

One of the main economic influences of heat stress in dairy animals is the decrease in quantity and quality of milk. THI threshold that indicates negative effects on milk yield can greatly vary across species, and within the same species across breeds. In cows, the critical values for minimum, average and maximum THI are estimated in 64, 72, and 76, respectively (West, 2003), even if reduction in milk yield for highly productive cows is registered at an average THI around 68 (Bernabucci et al., 2010). In dairy sheep, negative correlations are registered for milk yield and maximum daily temperature or THI and positive correlations with air humidity decreases. For Valle del Belice breed, losses in milk production are reported at a THI equal to 23 (calculated using the equation by Kelly and Bond, corresponding to a value around 73) and, beyond this value, each unit of increase in THI produced a decrease of 4.2% in daily milk yield. (Finocchiaro et al., 2005). In Manchega breed, the comfort region for milk yield is placed between 11.5-21.0°C for average temperature and 19.1-29.6°C for the maximum temperature, corresponding to values of 10.3-18.0 for average THI and of 13.9-22.0 maximum daily THI (calculated using the equation by Kelly and Bond). The decay in production was of 1-5 g/d for milk yield for each °C (or THI unit) above the threshold (Ramon et al., 2016). In Comisana breed not provided with shade in the summer, milk yield was approximately 20% lower when temperature exceeded 35°C, with values of THI greater than 80 (Sevi et al., 2001). In Sarda breed, milk yield decreased at average and maximum THI higher than 65 and 68 (Peana et al., 2017). A daily decrement of milk yield up to 15% (about 0.30 kg/d per head) was detected when maximum temperatures were higher than 21-24°C and mean temperatures overcome 15-21°C. When average THI passed from 60-65 to 72-75 the reduction of milk yield was of 20% (0.38 kg/d per head) (Peana et al., 2007b). These data show how sensitive sheep are to heat stress, in spite of the general conviction of their resistance.

In dairy cattle suffering heat stress, milk yield undergoes a rapid and acute (within 24 h) reduction and changes occur on its composition. Fat and protein percentages increase, even not compensating for the drops in yields caused by the fall in milk yield. The reduction in milk secretion originates from the increased activity of plasmin-plasminogen system: β-casein is cleaved and β-Casein-(1–28)-fragment is created, blocking the apical membrane of mammary epithelial cells (Silanikove et al., 2009). Also in ewe milk exposure to high temperature and direct solar radiation may produce changes in the lipid composition and a deterioration of the fatty acid profile. In Comisana breed an increase of short chain and saturated fatty acids, and a decrease in mono- and polyunsaturated fatty acids was registered, with a worsening of nutritional properties of the fatty acid profile: the contents of caproic, capric, lauric, myristic and stearic acids increased while contents of oleic, linoleic and linolenic acids decreased (Sevi et al., 2002). Furthermore, exposition to solar radiation increased milk concentrations of neutrophils, with augmented lipolytic and proteolytic enzymes into milk, resulting in lower yields of casein and fat and reduced clot firmness (Sevi et al., 2001). In Manchega breed, hot season causes an estimated annual decline of production for fat and protein yields ranging respectively from 0.09 to 0.2 and 0.08 to 0.1 kg/ewe. The decrease ranged from 0.1 to 0.3 g/d per °C (or THI unit) above the threshold of comfort (Ramón et al., 2016).

In summer, technological properties of ewe milk deteriorates due both to the advanced stage of lactation in late spring and summer and to the increase of environmental temperature. A negative effect on clotting properties is due to the increased pH of milk, because of the high amounts of CO_2 dissipated via the panting (Sevi and Caroprese, 2012). Moreover, plasma mineral imbalances produced by heat loads can bring to the reduction in calcium and phosphorous concentrations in milk (Sevi et al., 2004), that, together with a greater casein cleavage due to hydrolytic endogenous enzymes (Sevi and Caroprese, 2012), account for the deterioration of renneting parameters. Heat stress has also important consequences on and sheep udder health, since during summer the incidence of udder health problems increases, with negative effects on the hygienic quality of milk (Sevi and Caroprese, 2012).

Another factor that affects the response of the animal to heat stress is the stage of lactation. In cows, heat sensitivity is higher during in mid–lactation, maybe due to the strong support to milk production in this phase by feed intake. The efficiency in metabolic utilization of feed is lower than tissue store mobilization, thus resulting in more metabolic heat to be exchanged (Bernabucci et al., 2010).

Objectives of the Thesis

Several factors act on milk yield and quality, influencing its composition. Numerous studies exist, investigating the different factors acting on milk production and heat stress. Evaluation uses mainly direct measurements on animals and the results of a partial sampling are later generalized to the population. This method provides a clear overview of the phenomena, but it requires expensive indicators and it is not feasible on a large scale. The main objective of this thesis was to evaluate if it was possible to change the size of the samples collected: in spite of individual records, we want to use more general information and large scale investigation, considering as experimental unit the farm. Starting point was a dataset collected by the Sardinian Breeders Association (ARA), referred to the whole Sardinia (Italy), that reports both milk and farms' characteristics and data of the nearest meteorological station. The thesis consists of four main chapters.

This introductive Chapter 1 reviewed physiology and genetic of milk yield and composition. A discussion on the main factors influencing productive traits is developed, with a particular focus on the effect of heat stress.

In the Chapter 2, the data processing and descriptive statistical analysis at farm level of results of a survey carried out on a large sample of sheep farms in Sardinia will be provided with reference to milk production and quality. The main relationships among the available variables of interest are investigated and milk traits are analysed to detect how farm facilities and farm geographical characteristics affect milk quality.

The Chapter 3 is referred to meteorological variables and their connections with milk parameters. The objective of this chapter is to establish their main relationship at farm level and to demonstrate that heat stress can be harmful for bulk milk quality traits in Sarda sheep. Further, we wanted to quantify, if possible, losses in milk quantity and quality due to heat stress.

In the Chapter 4 we use mixed models to quantify at farm level the effect of THI and other management and environmental factors (and the relevant interactions) influencing milk characteristics and yield. The objective is to determine a model to forecast milk changes and the effect of heat stress, by means of farm characteristics and meteorological data, to assess stressful situations impairing milk production in a low-cost determination. Moreover with the use multivariate statistics on meteorological variables an alternative index attempting to describe the heat stress condition will be developed, providing also a comparison with the commonly used THI.

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Chapter 2: A survey on dairy sheep farming in Sardinia

Introduction

Sheep milk production in Italy in 2016 accounted approximately for 423,000 tonnes, 4.1% of global production (FAOSTAT, 2016). In Sardinia, sheep farming is a traditional agricultural activity, involving 45% of the Italian sheep population and approximately twelve thousands of farms, 20% of Italian total (Istat, 2016; Ismea, 2016). Flock management focuses mainly on milk production, afterwards transformed into hard cheeses, some of whom are Protected Designation of Origin (PDO), as Pecorino Romano, Pecorino Sardo and Fiore Sardo, and, to a lesser extent, into soft cheese and other typical dairy products. Sheep farming and its production chain play a seminal role in Sardinian economy, but it has also a strong social and environmental importance (Idda et al., 2010). Sheep are resistant animals and their farming has commonly exploited less-favoured areas: the high adaptability to difficult and marginal climatic and environmental conditions allows the exploitation of zones with low availability of plants and pasture, soil acclivity, reduced rainfall and water availability, widely spread in Sardinia (Carta et al., 2009). Production is influenced both by factors related to the ewe (for example age, year, season of yield and parity), and by effects related to the environment (such as variable forage availability). In fact, Sardinian dairy farming system has a strong relation with environment and climate, because it is generally semi-extensive, with animals kept outdoors and fed on grazing of fresh herbage (Cappio-Borlino, 1997). Mature ewes generally lamb in November, and they are milked up to July/August. Yearlings lamb generally later, in January, with a shorter lactation that finishes with the summer drought (Sitzia and Ruiz, 2016). In Sardinia a monitoring of productive system is conducted by the Sardinian Breeders Association (ARAS). In 2003/2004, nearly 4,700 farms were associated and controlled (ARAS, 2015).

This chapter analyses the dataset we were provided by ARAS. The dataset was edited and a large sample of farms were descripted by means of statistical analysis. This allows the illustration of their characteristics as a representative sample of Sardinian farming system. Furthermore, bulk milk parameters were analysed to check the general characteristics of sheep farms and their connection with milk production and quality and to assess their main relationships.

Materials and methods

Farm structural information and database editing

This survey was carried out on a large database provided by the regional breeder association (ARAS). The database includes information collected between 2003 and 2008. The ARAS database was composed by different type of information: geographic information, flock management variables, structural variables and bulk milk composition traits. Among the several variables, according to the record availability and to the relevance for this investigation a group of them were selected: altitude of the farm (m a.s.l.), province, Utilised Agricultural Area (Ha), flock size (no. head), organic or conventional farming, barn presence and farm equipment (milking machine and refrigerator tank), total annual milk production, sampling days of the year and milk composition traits. Data were initially available on 5,151 farms. Data editing procedures were performed to remove poor quality data, missing or outlier data. For instance, flock with a number of records lower than 10 in the entire period of study were removed. A total of 4,562 records were finally used (Table 1).

Table 1 Data editing

Datasets	Number of Records	Number of farm
Initial dataset	235,838	5,151
After Editing	218,170	4,562

Bulk milk quality in Sardinia

The routine determination of fat, protein, casein, and lactose contents, somatic cell count (SCC), total bacterial count (TBC), pH, freezing point and milk urea were determined in the laboratory of ARAS (Nuraxinieddu OR, Italy).

The bulk milk samples routinely collected by ARAS technicians were examined to provide information on milk composition by spectroscopic devices: in particular milk solids (g/100ml milk, approximate by %), pH, freezing point (°C) andmilk urea (mg/100ml) were determined by MilkoScanTM (Foss Electric, Denmark), SCC via automated flow cytometry devices using the FossomaticTM (Foss Electric, Denmark) and total bacterial load using BactoscanFC (Foss Electric, Denmark). (Table 2).

Milk sampling did not take account of individual lambing period and lactation stage, since analyses were referred to bulk tank milk. Depending on the character considered, a different availability of data occurs, varying from a minimum of 101,141 (pH) to a maximum of 216,748 records (logarithm of somatic cell count, SCC) during the whole period. The main average composition in the period of study was: fat 6.63±0.80, protein 5.81±0.33, lactose 4.86 ±0.31 (Table 2). These values are near to the values referred by ARAS for of ovine milk produced in Sardinia between 2009-2013 (data not shown).

Table 2 Milk mean characteristics in the dataset

Milk Trait	n[1]	Mean	Std	Min	Max
Bulk milk per head/year	7,690	158.18	59.33	18.87	437.50
Fat, %	216,716	6.63	0.80	0.61	12.55
Proteins, %	216,716	5.81	0.33	3.75	7.99
Caseins, %	190,234	4.52	0.27	3.01	6.43
pH	101,141	6.72	0.08	6.10	7.30
Lactose, %	212,962	4.86	0.31	2.51	5.72
Somatic cells count, log_{10} cells/mL of milk	216,748	3.17	0.25	1.26	4.45
Total bacterial count, log_{10} CFU/mL of milk	195,983	2.52	0.72	0.30	4.20
Freezing point, °C	213,165	0.58	0.01	0.35	0.78
Milk Urea Nitrogen, mg/100mL	194,453	40.55	11.22	0.40	78.90

[1]Total number of records

Statistical Analysis

Descriptive summary statistics were computed on structural variables. Frequencies were used for the categorical variables. Considering different sources of variability, the frequency distribution of analysed farm variables was provided. Main factor of variability considered were: temporal or geographic factors (years of sampling, province, altitude) and farm typology (farm size, organic farming, presence or barns of equipment). Correlation analysis were conducted on milk composition traits. Moreover the seasonal relationship between raw milk composition data and the days of the year were investigated. The effect of farm structural features on bulk milk quality was also assessed.

Results

Dataset characteristics

The dataset collected by ARAS, had 218,170 records belonging to 4,562 sheep farms located in Sardinia, Italy (Table 3). The period of study covers the years between 2003 and 2008. Records are equally distributed in the period, with 20% of data on average available per year. Since milk production starts in December and lasts up to August of the next year, five productive periods were identified from 2003 to 2008. Data referred to September, October and November were excluded because of their limited number. The period of study with the lowest number of records is 2004/2005, with 39,258 records concerning 3,276 flocks. Yearly, the average data available for each flock in the period of study varies from 11.9 and 12.9. The distribution of the average number of records per flock and years of sampling are reported in Figure 1. The number of records changes depending on the year and the farm. On average, for each farm contribute to the total dataset with ~48±23 records, with an average flock size of 248sheep together with a high variability(sd=222).

Table 3Dataset composition and within each characteristics considered.

Factors of variation	Records (%)	No. farms	Average data point[1](sd)	Average flock size (sd)
Years				
2003/2004	44,188 (20.3)	3,430	12.9 (4.2)	242.9 (184.5)
2004/2005	39,258 (18.0)	3,276	12.0 (4.3)	247.7 (225.7)
2005/2006	41,979 (19.2)	3,529	11.9 (4.6)	246.1 (175.3)
2006/2007	45,326 (20.8)	3,566	12.7 (3.2)	258.6 (287.6)
2007/2008	47,419 (21.7)	3,664	12.9 (3.1)	243.8 (216.3)
Province				
SS	94,138 (43.2)	1,943	48.4 (21.6)	255.1 (238.7)
NU	65,531 (30.0)	1,392	47.1 (24.3)	215.8 (170.7)
OR	22,457 (10.3)	480	46.8 (23.6)	247.3 (203.6)
CA	36,044 (16.6)	747	48.3 (19.7)	287.4 (260.0)
Altitude, m				
<300	50,027 (22.9)	998	50.1 (20.7)	307.6 (308.5)
>300	43,656 (20.0)	869	50.2 (22.4)	245.8 (180.3)
n.a.	124,487 (57.1)	2,954	42.1 (23.0)	224.5 (186.8)
UAA[1], ha				
<50	112,352 (51.5)	2,518	44.6 (22.3)	168.9 (142.1)
>50 <100	71,099 (32.6)	1,525	46.6 (24.0)	291.2 (205.3)
>100	28,182 (12.9)	592	47.6 (23.1)	454.3 (338.8)
n.a.	6,537 (3.0)	234	27.9 (19.6)	241.9 (189.6)
Organic				
Yes	6,403 (2.9)	163	39.3 (23.2)	287.2 (184.9)
T	9,551 (4.4)	217	44.0 (23.7)	318.5 (241.9)
No	202,216 (92.7)	4,283	47.2 (22.4)	243.3 (221.6)
Sheep Barn				
Yes	163,159 (74.8)	3,464	47.1 (22.9)	267.8 (225.3)
No	55,011 (25.2)	1,342	41.0 (22.6)	188.7 (201.5)
Milk Tank				
Yes	141,437 (64.8)	2,946	48.0 (22.5)	290.6 (222.7)
No	76,733 (35.2)	1,850	41.5 (22.6)	169.0 (198.3)
Milking Machine				
Yes	119,434 (54.7)	2,485	48.1 (22.2)	305.8 (230.1)
No	98,736 (45.3)	2,304	42.9 (23.7)	177.7 (189.9)
Total Count	**218,170 (100)**	**4,562**	**47.8 (22.4)**	**247.9 (222.2)**

[1]Utilised Agricultural Area

The 4,562 flocks are a reduced sample of the full dataset (summing 5,151 farms), because those with a number of records lower than 10 in the entire period of study were omitted. Farms were spread all over the four historic provinces of Sardinia, which are Sassari (SS), Cagliari (CA), Nuoro (NU) and Oristano (OR). Oristano includes a smaller area than the other provinces and it is characterised by lower number of data (22,457) and flocks (480), followed by Cagliari (36,044 records and 747 flocks).

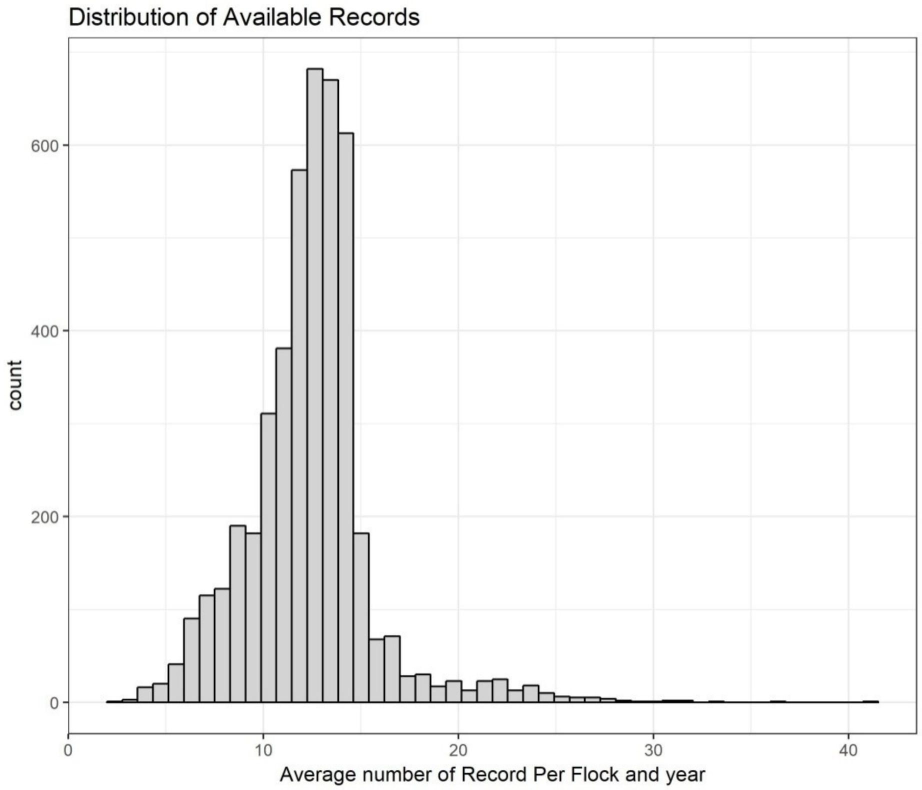

Figure 1Distribution of the average number of records per farms.

Part of the data was provided with altitude (meter above sea level). Two classes were identified: the first class included heights lower than 300 meters, the second higher heights.

Other general information available about farms refers to usable facilities and buildings, as milking machine, milk tank and sheep barn. In the dataset, the change of farms' characteristics during the period of 5 years can produce a variation in the number of farms:

when a farm improves its facilities or buildings, the total number of estates increases because of being counted twice. So, the total number of farms can be higher than 4,562, depending on the character considered. The majority of farms are provided with sheep barns (74.8%), milk tank (64.8%) and milking machine (54.7%),also as a consequence of the European Union's Common Agriculture Policy in the period of study, giving support to the development of modern farms.

The Utilised Agricultural Area (UAA) was used to define three classes, identifying small, medium and big farming systems. In this study 2,518 farms (51.7%), corresponding to 112,352 records, are small systems and have less than 50 hectares of UAA and average flock size of 168.9 heads. The less represented class refers to big farming systems, with UAA higher than 100 hectares, that are only 12.2%, whose average number of heads is 454.3. The dataset provided each flock with the total annual milk produced and the number of lactating heads. The relationship between flock size and total annual milk (Tons of milk/year) is linear, with total milk increasing as flock size rises (Figure 2).

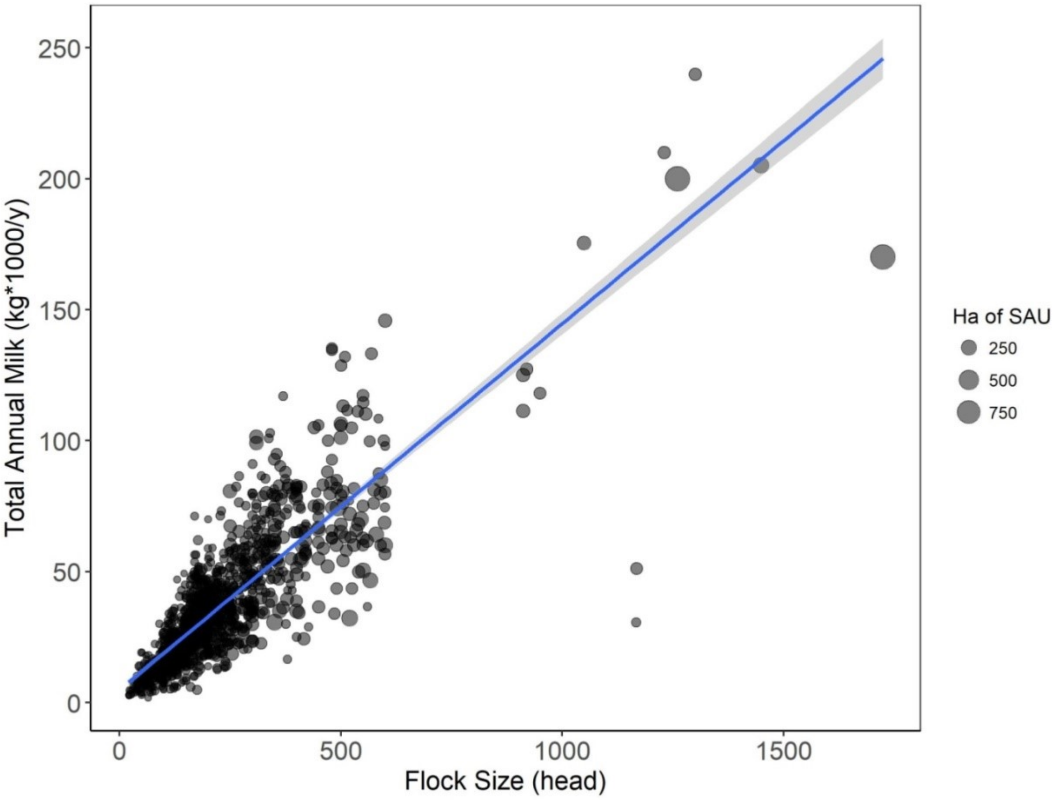

Figure 2 Relation between flock size, total annual milk and Utilised Agricultural Area (UAA).

Higher total production corresponds to larger flocks and, contemporary considering UAA, higher availability of land. This reflects the strong connection between production and pasture in Sardinian sheep farming systems, that are semi-extensive, with grazing animals integrated mainly in the milking period with grass hay and small amounts of concentrate feeds.

The frequency distribution of total annual production per flock and of the flock size for each year are shown in Figure 3. We can observe that most of farms produces very small quantities of milk. The majority of flocks are formed by less than 250 heads. Only a few big farming systems exist, producing an asymmetric distribution. Our dataset does not provide individual production, so we calculated an average value dividing the farm's total annual milk for the number of lactating ewes in the herd. The average annual milk produced during the sampling period was around 150 kg/year/head (Table 4), lower than the mean production of Sarda breed, the most represented in Sardinia, of 216 L/year/head (ASSONAPA, 2017). A small number of high productive flocks show extreme values, with average production per head higher than 400kg/year. The general pattern is similar in the four provinces, even if Nuoro and Sassari have thinner curves with higher peaks. Particularly, Nuoro has a higher number of flocks producing scarcely and big flocks are less represented than in Sassari, since Nuoro has wider mountain and hill areas.

Table 4 Average total annual milk per head.

Year	mean	sd
2003-2004	172.12	57.37
2004-2005	151.66	60.04
2005-2006	150.31	56.68
2006-2007	160.94	60.08
2007-2008	149.79	59.88
5-year	156.96	59.33

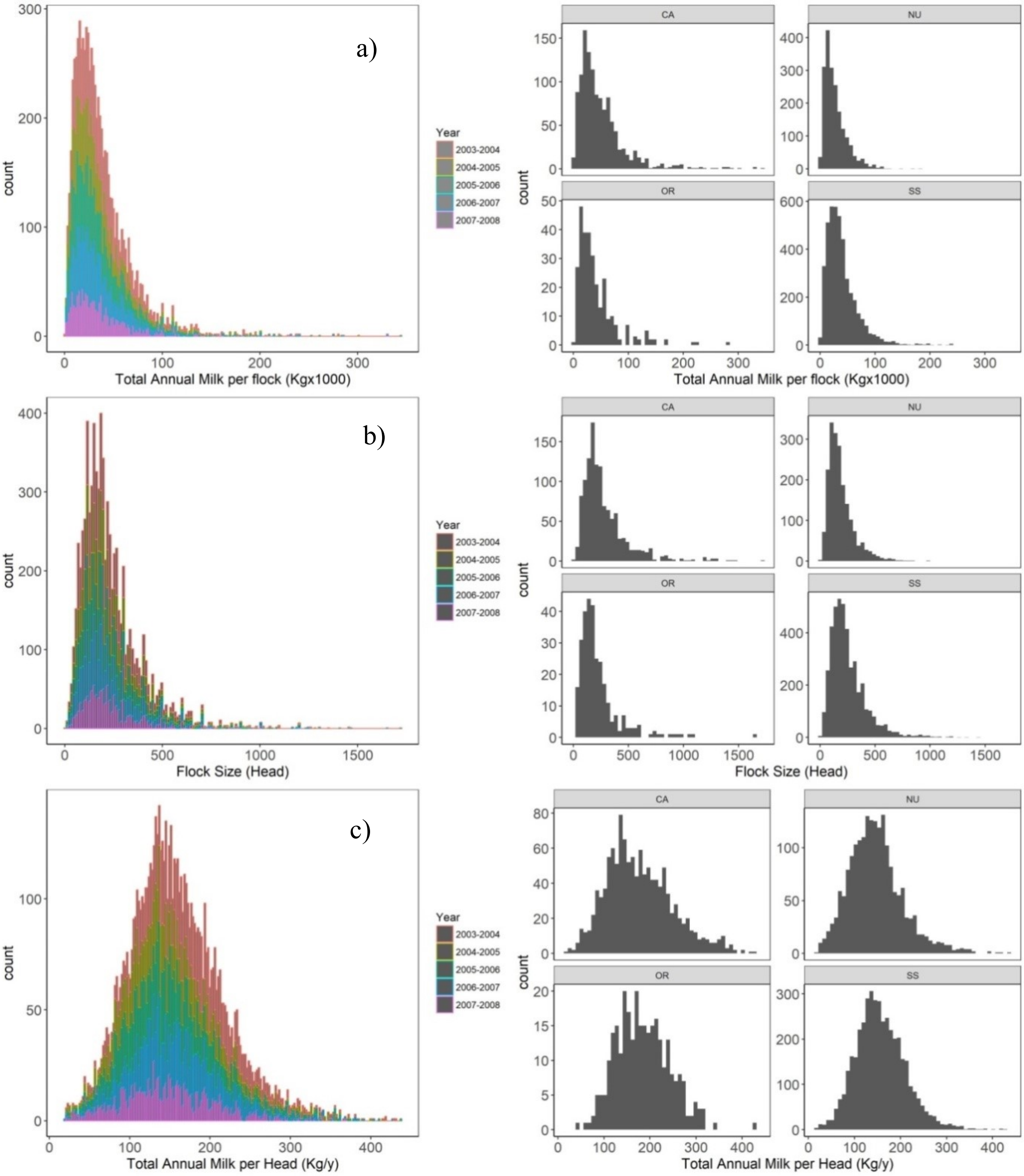

Figure 3 Pattern of milk production within year (left panel) or within province (right panel). a) Distribution of total annual milk per flock. b) Distribution of Flock sizes. C) Distribution of the total annual milk per head.

Bulk milk quality in Sardinia

Frequency distribution of records describes nearly a Gaussian curve for all the traits considered (Figure 4). However, according to the results of a Kolmogorov-Smirnov normality test, data were non normally distributed. Freezing point and pH show the smaller variability.

The correlations among the considered milk traits, calculated using SAS CORR procedure (SAS Institute, 2008), are reported in Table 5. All correlations were generally from moderate to low, a part from proteins and caseins (0.97), since the latter are insoluble proteins. Fat, protein and caseins percentages are negatively correlated with lactose due to the concentration effect occurring when the rate of milk production, pushed by lactose, decreases (Bencini and Pulina, 1997; Sevi et al., 2000; Nudda et al., 2003). As referred for Holstein cows, freezing point has a low negative correlation with fat but correlation with proteins and caseins have opposite sign than reported in literature (Otwinowska-Mindur et al., 2017),even though these values are very low.

Table 5 Correlation between milk characteristics

	Fat	Protein	Casein	Lactose	SCC	Freezing Point	TBC	pH	Urea N
Fat (%)	*	0.50	0.50	-0.58	0.21	-0.02	-0.03	0.14	-0.38
Protein (%)		*	0.97	-0.43	0.21	0.10	0.02	0.17	-0.24
Casein (%)			*	-0.36	0.17	0.10	0.01	0.15	-0.20
Lactose (%)				*	-0.29	0.28	0.00	-0.41	0.50
SCC (log_{10})					*	0.01	0.27	0.25	-0.19
Freezing P(°C)						*	0.03	-0.34	0.33
TBC							*	-0.02	0.00
pH								*	-0.27
Urea(mg/100m)									*

$P \leq 0.0001$

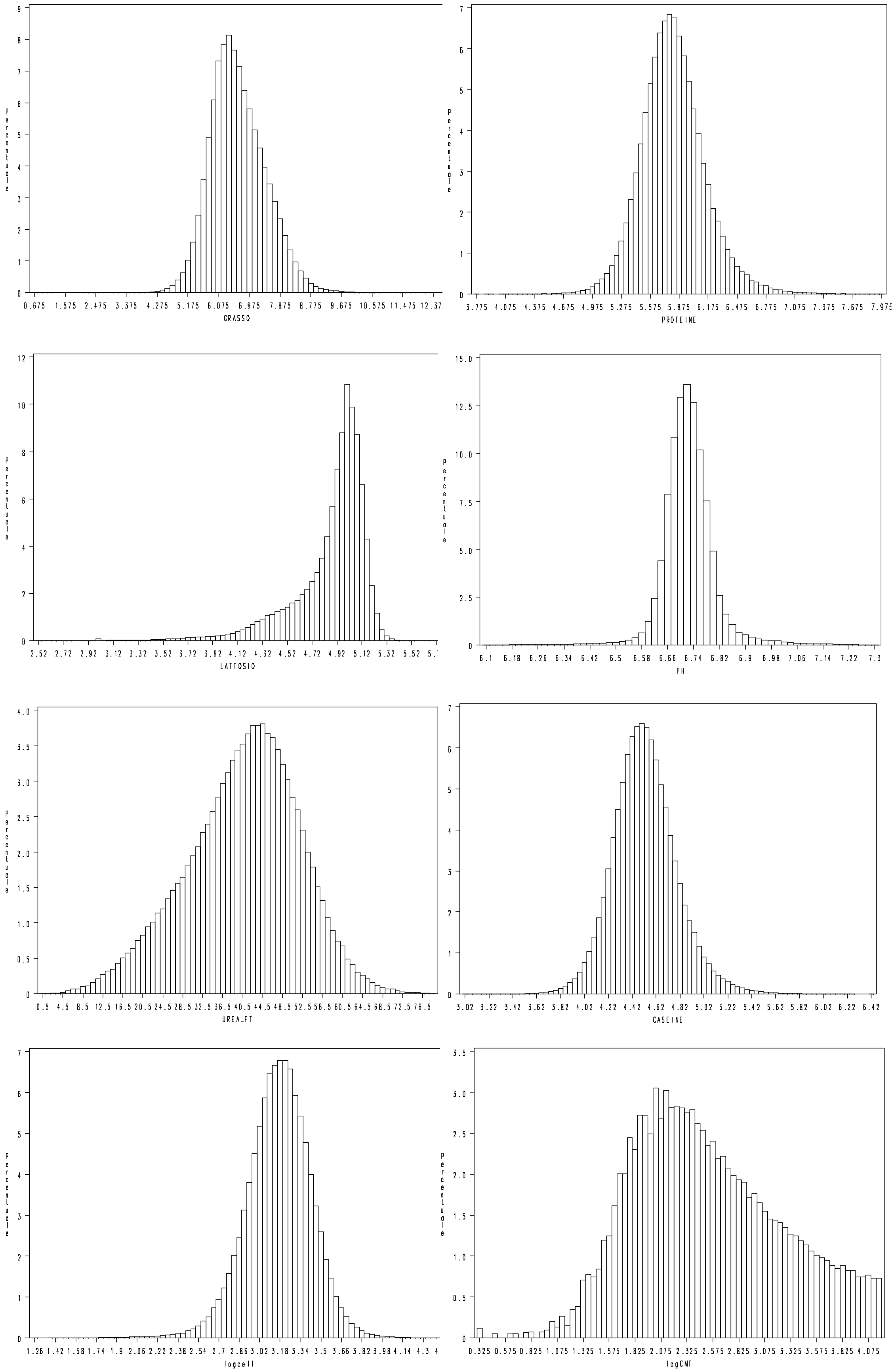

Figure 4 Milk characteristics distribution

The behaviour of milk parameters was analysed in relation to the productive period. Only slight differences were found between the years. In Figure 5 are reported the patterns of milk composition variables during the productive period. During all years, lactose percentage was constant upto March, then it exhibited a reduction till the end of lactation. This behavior is in agreement with the milk production pattern, since lactose determines the rate of daily milk production.

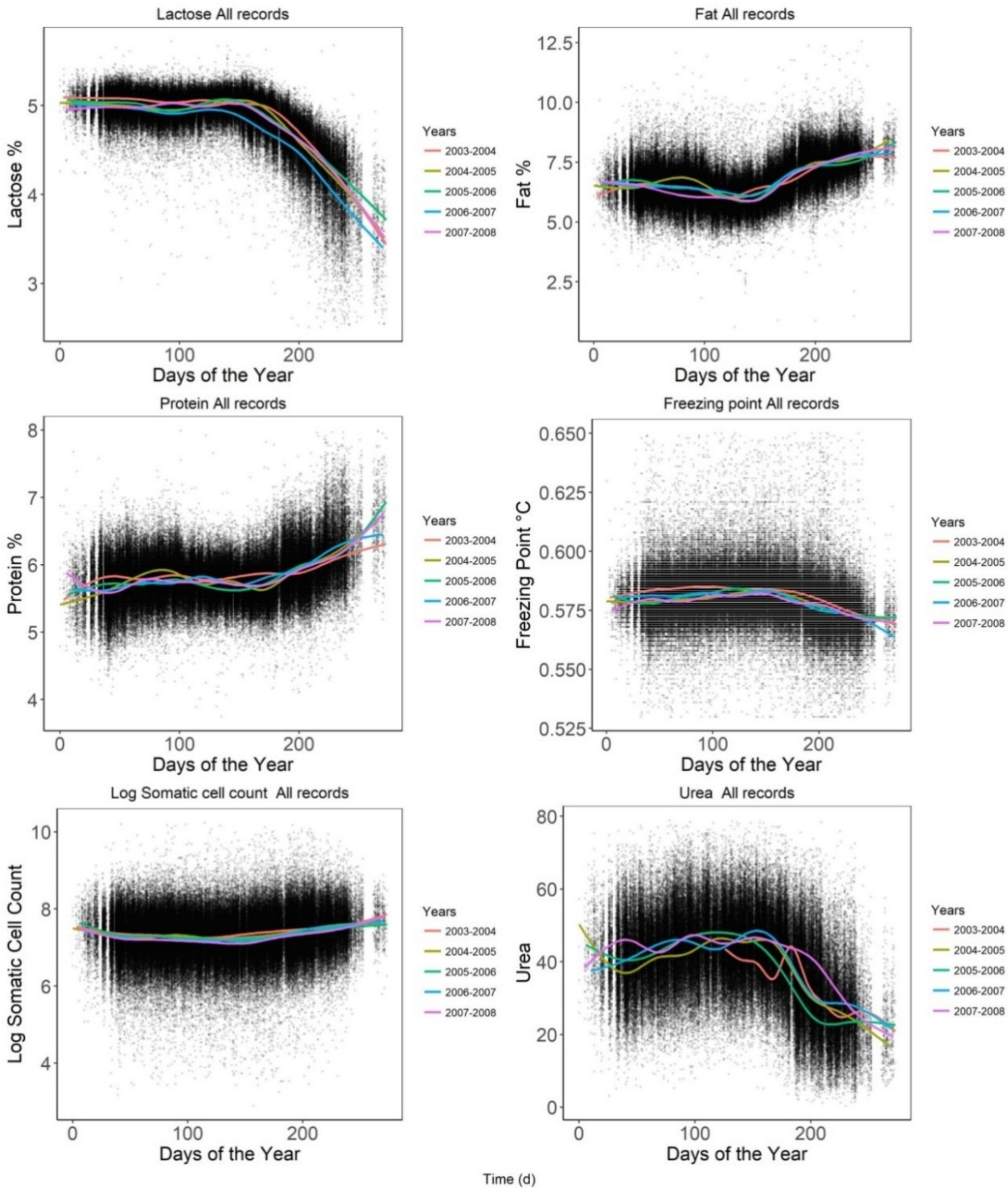

Figure 5 Seasonal changes in milk characteristics records along lactation

Freezing point is an important physical parameter that is checked to identify adulteration due to water addition in milk (Martini et al., 2008). It changes throughout lactation in a fairly narrow range, as a response to changes in milk composition. Since freezing point is mainly influenced by lactose, both of them have a similar pattern: at the end of lactation, as a result of reduced milk production, the increased dry matter content produces a decrease in freezing point (Janstova et al., 2013).

Fat, protein and caseins percentages showed an opposite trend. After a first period where they keep quite constant, they show an increase in the final part of lactation. Milk urea is a nitrogenous compound of milk whose concentration is a good indicator of protein metabolism and intake (Cannas et al., 1998). In our dataset, its content gradually increases during the production season, probably as a consequence of both a higher availability of grass in spring, and feed supplementation to allow higher milk production(Cannas, 2001). In fact, the availability of pasture in Mediterraean area progressively increases from September, after a period of stop in winter, and reaches its peak on April. Further it drops, corresponding the lowest availability of grass in the final part of lactation. So, at the end of the productive year, milk urea falls, likely for reduced availability of pasture and drop of milk production. Somatic cell count, represented as $\log_{10}$, keeps itself nearly costant, showing an increase in the final part of lactation. This agrees with literature, since the progression of stage of lactation is the main causeof somatic cell increase in healthy udder (Raynal-Ljutovac et al., 2007).

Factors influencing milk characteristics

As earlier told for Table 3, average values of milk composition traits are close to those observed in Sardinia. Milk composition traits were averaged by farm characteristics (altitude , barns etc), to assess their potential effect.

Geographical characteristics do not seem to influence milk parameters (Table 6). Considering altitude, freezing point, pH and caseins keep constant in the different classes, as told before. Only fat and proteins percentages show a slight increase as altitude grows. It is notable that the third class, where altitude was not available, show average values that are very similar to the ones in plain and mountain areas.

Table 6 Average milk characteristic and altitude

	Flock Altitude					
	< 300 m		≥300 m		Not available	
items	Mean	Std	Mean	Std	Mean	Std
Fat	6.61	0.77	6.67	0.78	6.63	0.82
Proteins	5.79	0.33	5.82	0.32	5.81	0.34
Lactose	4.86	0.31	4.88	0.30	4.85	0.31
Freezing point	0.58	0.01	0.58	0.01	0.58	0.01
pH	6.72	0.08	6.72	0.08	6.72	0.08
Caseins	4.51	0.26	4.53	0.26	4.52	0.27
Urea Nitrogen	40.36	11.07	40.63	11.06	40.61	11.33
log SCC	3.14	0.25	3.27	0.25	3.16	0.25
log CMT	2.45	0.72	2.60	0.72	2.53	0.72

[1]Total Number of total record used (Number of flocks ranged from x to y)

Even U.A.A. does not influence milk parameters (results not shown).

Milk composition data characteristics were merged with farm characteristics (Table 7), to analyse possible relationships. Slight differences were observed.. Sheep barn, milking machine and milk tank show very similar means and they do not seem to influence the studied variables. As told before, pH, freezing point, caseins and lactose show no difference across different levels of farm characteristics. Furthermore, also fat and proteins showed very little variation. Total bacterial count was the only trait that seemed to be influenced by farm features. It showed a reduction when milking machine and cooling system were available in the farm. This can be explained by improved hygiene conditions, generally corresponding to higher availability of equipment in the farm (Gonzalo et al., 2006). The effect of organic farming system and conventional system produces no significant differences on milk composition, in agree with previous studies (Pirisi at al., 2002).

Table 7 Average milk characteristic and equipment available in the farm

	Sheep barn			
	No		Yes	
Variable	mean	sd	mean	sd
Fat	6.70	0.82	6.61	0.80
Proteins	5.85	0.32	5.79	0.34
Lactose	4.87	0.30	4.84	0.33
Freezing point	0.58	0.01	0.58	0.01
pH	6.71	0.08	6.72	0.08
Caseins	4.55	0.26	4.51	0.27
Urea Nitrogen	40.69	11.33	40.51	11.18
log SCC[1]	3.20	0.26	3.16	0.25
log TBC[2]	2.60	0.74	2.50	0.72
	Milk Tank			
	No		Yes	
Fat	6.68	0.87	6.61	0.76
Proteins	5.84	0.34	5.79	0.33
Lactose	4.85	0.30	4.84	0.33
Freezing point	0.58	0.01	0.58	0.01
pH	6.71	0.09	6.72	0.08
Caseins	4.55	0.27	4.51	0.26
Urea Nitrogen	40.67	11.54	40.49	11.04
log SCC[1]	3.18	0.27	3.16	0.25
log TBC[2]	2.59	0.75	2.49	0.71
	Milking machine			
	No		Yes	
Fat	6.71	0.84	6.57	0.77
Proteins	5.85	0.34	5.78	0.33
Lactose	4.85	0.31	4.84	0.34
Freezing point	0.58	0.01	0.58	0.01
pH	6.71	0.09	6.72	0.08
Caseins	4.55	0.27	4.50	0.26
Urea Nitrogen	40.44	11.49	40.65	10.99
log SCC[1]	3.19	0.26	3.15	0.25
log TBC[2]	2.58	0.74	2.48	0.70

[1]Log of Somatic cells count, cells/mL of milk
[2]Log of Total bacterial count, CFU/mL of milk

A chi-squared test (χ^2 test) was performed between milk parameters and farm equipment, to determine if there was a significant relationship. χ^2test was performed on each milk parameter. Since this test of independence compares categorical variables, milk parameters were classified in classes through their quartiles. Low, medium low, high and very high levels of each parameter were created. As an example, fat results are reported (Table 8).

Chi-squared test allows to define if there is a significant difference between the expected and the observed frequencies of categorical variables. In our study, all tests performed were highly significant. Differences between expected and observed frequencies are very little, but the high number of data determines the statistical response. In fact, this test cannot provide any inferences about causation and a lot of contemporary effects act together: differences in buildings and equipment in the farm cannot explain by their owns these significant differences. A complete analysis of the outcome requires the use of mixed models, to be able to discriminate the different effects. We will go into the depth of this aspect in a further section.

Discussion

Sheep farming has played a crucial role in Sardinia's history and economy. With 12,669 farms registered, it is the most important livestock sector (Istat, 2010), spread all over the Island. The highest number of farms are in Sassari and Nuoro provinces, counting these two nearly 48% of the total (Istat, 2010). This distribution is noticeable also in our dataset, since the majority of flocks are located in these two provinces. Most of farms are not specialized in sheep farming, but they rear also goats, swine and cattle. Actually small sized flocks are the most common in Sardinia (Istat, 2010): 21% of flocks have less than 100 and only 28% flocks have more than 300 sheep respectively. Considering the whole sheep population, the medium sized flocks own approximately 56% of Sardinian ovine stock.

Table 8 χ^2 test performed between fat percentage quantiles and farm equipment and characteristics

Factors	N	Fat %,quartile								χ^2	P-values
		Q1 (<6.08)		Q2 (≥6.08 <6.55)		Q3 (≥6.55<7.15)		Q4 (≥7.15)			
Altitude, m											
<300	49,784	12,765	(12,046)	12,811	(12,823)	12,612	(12, 963)	11,596	(11,953)	135.7	<0.0001
≥300	43,286	9,754	(10,473)	11,161	(11,149)	11,622	(11,271)	10,749	(10,392)	DF 3	
Milk Tank											
No	76,102	19,485	(19,370)	16,230	(18,757)	18,891	(19,164)	21,496	(18,812)	1122.0	<0.0001
Yes	140,614	35,674	(35,789)	37,184	(34,657)	35,682	(35,409)	32,074	(34,758)	DF 3	
Milking Machine											
No	97,957	23,139	(24,932)	21,865	(24,143)	25,215	(24,667)	27,738	(24,214)	1585.8	<0.0001
Yes	118,759	32,020	(30,227)	31,549	(29,271)	29,358	(29,906)	25,832	(29,356)	DF 3	
Sheep Barn											
No	54,603	12,669	(13,898)	12,658	(13,458)	14,497	(13,750)	14,779	(13,497)	425.7	<0.0001
Yes	162,113	42,490	(41,261)	40,756	(39,956)	40,076	(40,823)	38,791	(40,073)	DF 3	
Organic											
No	200,883	51,356	(51,129)	49,384	(49,512)	50,499	(50,586)	49,644	(49,656)	28.9	<0.0001
T	9,483	2,336	(2,414)	2,370	(2,337)	2,470	(2,388)	2,307	(2,344)	DF 6	
Yes (V)	6,350	1,467	(1,616)	1,660	(1,565)	1,604	(1,599)	1,619	(1,570)		
UAA[1], ha											
< 50	111,590	28,734	(28,362)	26,524	(27,559)	27,358	(28,108)	28,974	(27,561)	307.6	<0.0001
≥50	70,614	17,801	(17,947)	18,079	(17,439)	18,139	(17,787)	16,595	(17,440)	DF 6	
< 100											
100	28,009	6,893	(7,119)	7,313	(6,917)	7,453	(7,055)	6,350	(6,918)		

[1]Utilised Agricultural Area

These figures are confirmed in the data considered in the present work: the average flock size was of 248 sheep with a huge standard deviation (222.2), that reflects the existence of a gtreat variability in flock size.

As far as milk production is concerned, Sarda breed ewe yields average from about 140 L/year in primiparous to 228 L/year in multiparous (ASSONAPA, 2017). Average milk production per ewe in the data set considered in the present work was 150 kg/year/head. This information can be explained considering that our farms rear mainly, but not only, Sarda sheep. Furthermore, flocks with less than 100 sheep herds smaller than 100 heads are often located in marginal areas, with very low yields reducing the general mean production. Another element that should be considered is that the traditional management system is based on semi extensive exploitation of pasture and grazing. It produces a strong relationship among farming and Mediterranean climate in Sardinia. Average annual rainfall is 756 mm (Usai et al., 2006), generally concentrated in winter and spring, and it largely varies depending on the year, area and altitude. This variability affects pasture availability and quality and also sheep welfare, with consequences on milk production, that suffers changes also due to the climate. Grasses, the principal constituent of diet, start growing after the first rains in autumn, but their growth reduces for low winter temperatures, and re-starts in spring, slowing down when water availability decreases. Because of these two peaks of growth, the nutritive value changes (e.g. in winter, during the first phase of growth, legumes availability is lower, being less tolerant to cold). Generally natural and semi-natural pastures, formed by annual self-regenerating forage species, are grazed by replacement lambs and non-lactating sheep, while forage crops are used in winter and spring for lactating ewes. The grazing method can range from a rationed to a continuous approach, to modulate the use of pasture and to limit its trample and wastage (Molle at al., 2008; Sitzia and Ruiz, 2016).

Farm location and altitude influence crop and husbandry systems by influencing pasture availability and climate. Sardinia counts 24,100 km^2, 19,648 of whom are classified as mountain or hill areas (Istat, 2017), in spite of reduced heights and the absence of mountain range. 78% of sheep are reared in hills and mountains (Figure 6).

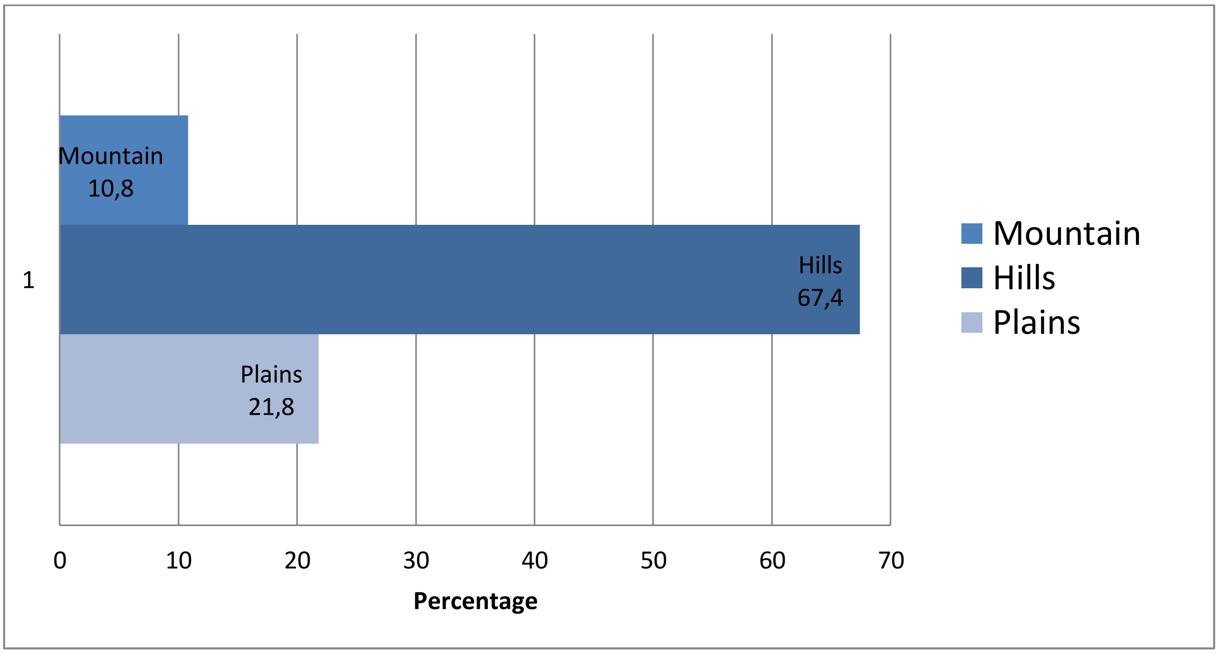

Figure 6 Sheep distribution in Sardinia depending on altitude (Istat, 2010)

Even if in our dataset nearly 60% of farms lack altitude, we decided to take it into account because of several studies referring the influence of altitude on production (Macciotta et al., 1999; Martini et al., 2008).

Our data are in agreement with official information about UAA. According to Istat (2010), only 14% (13% in our dataset) farms have more than 100 hectares, while 58% (52% in our dataset) have less than 50 hectares. The majority of farms are small, and 42% of them are placed in hill or mountain areas.

A large availability of information has permitted a fairly detailed description of sheep farm conditions in Sardinia, in terms of production and facilities. From this preliminary survey, it does not seem to exist a close relationships between farm structural variables and milk composition traits in Sardinian farms.

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Chapter 3: Influence of bio-meteorological variables on bulk milk composition of Sardinian farms

Introduction

Heat stress derives from the combination of environmental factors (mainly temperature and humidity) producing a rise beyond thermoneutral zone in the perceived temperature (Armstrong, 1994). The animal response is a complex phenomenon to neutralize the negative effects of heat stress, involving physical, biochemical and physiological processes. These include increase in respiration rate and rectal temperature, panting, drooling, reduced heart rates, sweating and decreased feed intake and milk production. The engage of homeostatic processes to keep stable temperature produces metabolic responses changing mineral, protein, energy and water metabolism and varying nutrient digestibility (Kadzere et al., 2002; Marai et al., 2007;Silanikove, 2000). Such changes produce a detrimental effect on production and reproduction performances. Even if sensitivity to heat stress varies among species and breeds, milk production is negatively associated to heat stress. Many studies exist on high producing cattle, that are highly sensitive, but only a few exist on small ruminants. In spite of this, sheep and goats often experiment high heat stress (Al-Dawood, 2017), due to direct exposition to solar radiation in Mediterranean semiextensive farming systems, since sheep are usually outside in spring and summer season. This happens contemporary to the late stage of lactation, thus bringing a decline in milk production and a worsening of its quality (Sevi and Caroprese, 2012). Differences in milk composition and reduced yield are revealed in goats and sheep (Finocchiaro et al., 2005; Hamzaoui et al., 2013; Peana et al., 2007a; 2007b;Ramon et al., 2016; Sevi et al., 2001).

The individual response to heat stress is partially caused by genetic variability. The genetic background determining the different interaction with the environment makes feasible the selection of animals with improved heat tolerance, even if it is a complex task. In fact milk yield and heat tolerance are antagonistic and negatively associated (Finocchiaro et al., 2005) and thermoregulation is a complex mechanisms determined by several factors

(Carabaño et al., 2017). Many studies have investigated the phenotypic expression of a genotype using reaction norm models, that describe a phenotype as a function of an environmental variable. They allow for estimating the magnitude of change in the phenotypic expression of a genotype because of the interaction with environment and its conditions, generally expressed as a combined effect of temperature and relative humidity. These models, applied at the population level for genetic purposes, provide estimations of heat tolerance thresholds and of the slopes of decay after the threshold. More accurate measures should use physiological traits related to the ability of coping with heat stress, as body temperature, rectal temperature and respiration rate, but their determination is highly expensive (Carabaño et al., 2017; Macciotta et al., 2017). On the contrary, individual milk production or milk composition can be easily obtained from dairy recording systems, even if different physiological stages or circumstances affecting production can produce a noise accompanying the information, resulting in biased estimates of the responses (Carabaño et al., 2017).

Other approaches use the principal component to analyse the total variance of the system across production traits at increasing levels of temperature and relative humidity (Macciotta et al., 2017). Single nucleotide polymorphism (SNP) typing technologies and genome-wide association (GWA) analysis have been used to investigate heat tolerance in dairy cattle (Dikmen et al., 2013; Macciotta et al., 2017) and in desert sheep and goats (Elbeltagy et al., 2016), to detect genomic regions associated with characters of interest. The use of GWA studies may help in the separation of genetics and environmental components of heat tolerance but it also looks at the issue of the best choice for heat stress quantification (Carabaño et al., 2017). In Holstein cows SNPs associated with genes of large effect on rectal temperature (Dikmen at al., 2013), slope for milk yield and composition (Macciotta et al., 2017) were identified.

The above mentioned approaches have been developed at individual animal level in order to investigate the genetics of heat stress phenomenon. Furthermore, the assessment of resistance of heat stress trough the dissection of the total phenotypic variation due environment and genetics and their interaction is crucial for selecting the best genotypes.

The current investigation looks at the heat stress and the use of THI from a different perspective. Indeed this study wants to analyse the effect of environmental and meteorological conditions in late spring and summer on sheep milk production at farm level in Sardinia (Italy). The variation in milk quality traits was firstly assessed looking at their sensitivity to heat stress by means of general characteristics of the farm and meteorological parameters. To accomplish the general objective, a very large sample of farm were analysed aimed at assessing the effects of THI on bulk milk yield, with special focus on milk composition traits.

Materials and methods

Data collection and database description

Database construction

Database used in this third chapter originates from merging the ARAS (OR, Italy) and ARPAS (Meteorological Department of Sardinia Environmental Agency, SS, Italy) datasets. The first dataset, with more than 4,000 Sardinian dairy ewe farms information (~220,000 records), included geographical, structural (no. hectares, flock sizes, facilities and equipment, organic or conventional farming system, etc.), productive (total annual milk yield per farm) and milk composition analysis information (e.g. lactose, fat, protein, somatic cell count, urea, etc.) collected ~ once a month over a period of 5 years (from 2003 to 2009). For further details see material and method of Chapter 1. The second dataset were kindly provided by ARPAS and it contained daily meteorological variables (e.g. temperature, relative humidity, wind speed, etc.) recorded by the network of 60 station spread around the regional territory. After being assigned to each farm based on a neighbourhood criteria, the final number of meteorological records ranged from 20,461 to 61,499, due to the presence of missing data in different variables.

Meteorological Variables and Temperature Humidity Index (THI)

Meteorological stations of the Sardinia Environmental Agency (ARPAS) provided the meteorological data. The network is formed by 60 stations spread all over the region (Figure 1): 14 were placed in Cagliari province, 20 in Nuoro, 6 in Oristano and 20 in Sassari.

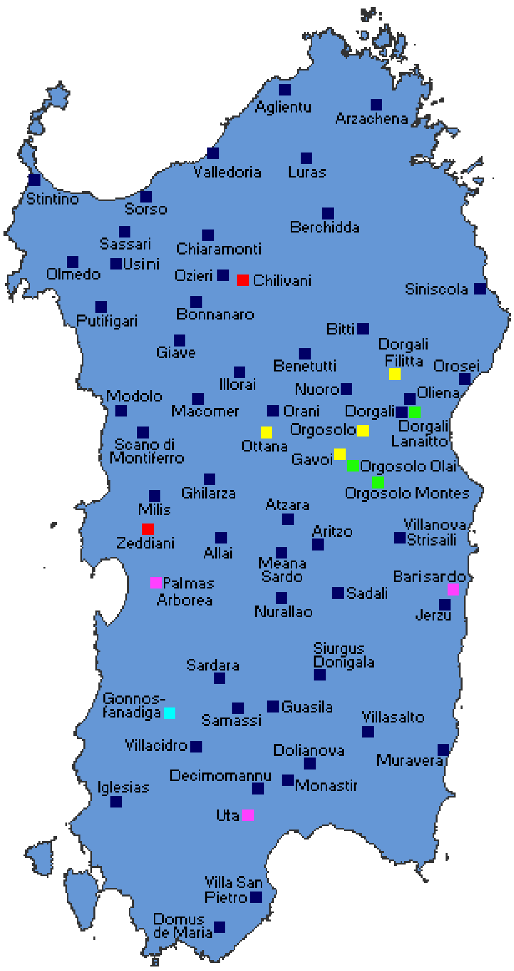

Figure 10 Network of meteorological stations in Sardinia (Picture from ARPA Sardegna – http://www.sar.sardegna.it/documentazione/strumenti/retestazioni.asp)

For each farm, milk composition data were merged with climatic variables coming from the nearest meteorological station. Depending on the considered year, a different number of flocks (and meteorological records) can be referred to the same station, ranging from 1 to 236 for a total of 4,093 farms (with 60,921 records, Table 1). Only 1,294 farm presented meteorological data referred to the whole period of study.

Table 5 Location and altitude of Sardinian meteorological network, number of farms within each Station by year of recording.

Meteo Station Information[1]				Number of Flock per Year[3]						
Station (n=60)	Prov (n=4)	Latitude (UTM n)	Alt[2]	2003 2004	2004 2005	2005 2006	2005 2006	2007 2008	Tot[4]	5-year[5]
GIAVE	SS	4479237	410	210	193	191	192	184	236	150
CHIARAMONTI	SS	4508715	368	160	121	133	143	152	212	81
OLMEDO	SS	4501382	31	144	139	144	151	141	194	96
SIURGUSDONIG.	CA	4386183	414	146	149	143	150	147	175	112
BENETUTTI	SS	4475850	284	136	118	129	122	134	169	88
BITTI	SS	4480550	782	116	100	103	114	108	149	64
CHILIVANIUCEA	SS	4495583	290	133	122	127	0	0	141	0
USINI MOBILE	SS	4500817	197	0	0	94	96	113	125	0
PUTIFIGARI	SS	4488593	422	101	105	99	97	102	120	83
SINISCOLA	NU	4492740	14	69	65	93	101	100	119	39
BERCHIDDA	SS	4514900	276	90	79	72	66	58	117	38
OTTANA	NU	4453749	160	85	78	82	94	94	116	47
MASAINAS	CA	4322389	57	56	98	0	0	0	102	0
GONNOSFANAD.	CA	4373392	146	74	79	85	82	82	100	57
OROSEI	NU	4468859	26	80	49	76	79	81	96	40
GHILARZA	NU	4441089	293	59	53	56	57	63	92	31
ILLORAI	SS	4469946	878	66	0	83	81	71	92	0
MODOLO	NU	4459270	241	71	66	79	72	0	90	0
BONNANARO	SS	4490098	345	74	74	70	71	69	90	51
GAVOI	NU	4446812	835	58	62	66	78	75	89	45
NURALLAO	NU	4406310	380	0	0	0	71	83	87	0
MACOMER	NU	4457378	665	56	52	77	66	65	85	40
S. MONTIFERRO	OR	4453110	491	71	71	0	0	0	80	0
SAN TEODORO	SS	4513069	15	45	35	55	70	70	80	26
DORGALI MOB	NU	4456432	156	76	61	0	0	0	78	0
ALLAI	OR	4422993	60	0	0	0	49	67	78	0
SARDARA	CA	4383164	189	53	45	44	44	45	62	33
VALLEDORIA	SS	4532148	2	35	36	52	52	52	62	26
IGLESIAS	CA	4352022	208	0	0	0	52	55	58	0
OLIENA	NU	4457432	132	28	32	41	48	49	53	25
GUASILA	CA	4375315	246	37	40	41	35	0	49	0
SADALI	NU	4407653	780	40	40	2	18	31	48	1
ZEDDIANI UCEA	OR	4426664	10	43	37	40	0	0	47	0
VILLACIDRO	CA	4366984	130	20	15	22	34	37	44	9
ATZARA	NU	4426815	620	30	31	30	31	33	44	18
SASSARI S.A.R.	SS	4509934	150	29	31	31	31	31	38	22
ORGOSOLO	NU	4450014	290	0	0	26	25	33	36	0

SILIQUA	CA	4350085	0	0	0	0	0	0	34	0
MEANA SARDO	NU	4422210	0	28	27	26	0	20	34	0
MURAVERA	CA	4363009	2	19	0	20	28	28	33	0
P. ARBOREA	OR	4412260	20	0	0	29	0	25	33	0
DORGALI FILIT.	NU	4469983	85	0	23	17	21	22	28	0
NUORO	NU	4465479	488	17	21	19	23	23	28	10
SORSO	SS	4519874	50	15	15	15	17	18	25	10
SAMASSI	CA	4375110	89	0	0	22	7	12	24	0
BARISARDO	NU	4411311	51	7	5	22	0	19	24	0
ARBOREA	OR	4412260	20	16	16	0	0	0	22	0
JERZU	NU	4404776	47	18	14	17	14	17	21	11
DOLIANOVA	CA	4359890	165	16	14	16	17	18	20	12
MILIS	OR	4434476	97	9	14	14	15	17	20	7
OZIERI	SS	4490655	238	17	18	17	15	17	19	13
UTA	CA	4343369	20	0	0	0	14	14	15	0
LURAS	SS	4531649	488	7	8	10	10	10	12	4
DECIMOMANNU	CA	4351919	29	0	8	6	8	0	9	0
STINTINO	SS	4532139	35	7	6	7	6	4	8	3
ARZACHEN MOB	SS	4545082	120	0	0	0	6	0	8	0
MONASTIR MOB	CA	4355655	96	3	4	2	2	3	5	2
VILLANOVA ST.	NU	4423612	813	0	2	2	2	0	4	0
ARITZO	NU	4422227	879	0	0	0	1	0	3	0
ARZACHENA	SS	4545082	83	0	0	0	0	1	1	0
Summary[6]	20:20:14:6	4443162±58504	248±254	2532	402	484	413	252	4083	1294

[1] In the first three columns the municipality and the province where the Meteorological Station are located. [2] Altitude, Meter above the sea level. [3] The number of farms for which both meteorological and milk production traits were available for each year; the same farm can be counted more than one time in different column if sampled in different years. [4] The total number of farm sampled at least one time. [5] The number of farms that have at least one record in each of the 5 year period. [6] The first three column represent respectively: count of the number of station by province in this order SS:NU:CA:OR; mean±sd of latitude coordinate and altitude where the meteorological stations are located.

Meteorological data consisted of daily maximum, minimum and average temperature (T, °C) and relative humidity (RH, %) recorded when bulk milk samples for chemical analysis were collected and in the previous three days, respectively. Descriptive statistics of the meteorological variables considered in this study (Table 2 and Supplemental Table 1) refer to the months from May to August for each year of study (from 2004 to 2008).

For some of the meteorological stations, maximum and average wind speed (m/s), solar radiation (MJ/m^2) and effective rainfall (mm/day) were also provided. In spite of their reduced availability, their knowledge is interesting because solar radiation and wind speed can strongly contribute to heat load (Bernabucci et al., 2010). Depending on the climatic factor considered, the number of data varies from a minimum of 26,143 (radiation two days before sampling) to a maximum of 61,491 (average temperature and humidity in the test day) during the whole period (Table 2).

Table 6Description of weather data on the test day

Meteorological Variable	n	Mean	Std	CV	Min	Max
T mean, °C	61,491	19.77	4.68	0.24	6.14	32.20
T max, °C	61,383	26.68	6.02	0.23	8.00	44.30
T min, °C	61,399	13.00	4.49	0.35	-2.00	26.60
RH med, %	61,017	37.58	18.23	0.49	7.00	100.00
RH max, %	61,491	63.12	16.44	0.26	11.00	100.00
RH min, %	61,273	88.04	13.46	0.15	13.00	100.00
Effective rainfall, mm/day	59,719	1.00	4.23	4.23	0.00	53.20
Solar radiation, MJ/m^2	26,717	21.16	5.61	0.27	1.00	31.00
Wind Speed mean, m/s	56,936	3.07	1.87	0.61	0.00	14.40
Wind Speed max, m/s	56,489	11.86	4.24	0.36	0.00	48.15

Temperature humidity index was calculated according to the approach of Kliber (1964), which has been used for Sarda breed in Mediterranean conditions in previous studies (Peana et al., 2007a;2007b; 2017):

$$THI = 1.8T_{db} - \left(1 - \frac{RH}{100}\right) \cdot (T_{db} - 14.3) + 32 \quad (1)$$

where T_{db}= Air temperature (°C) and RH = relative humidity. The formula (1) was applied to compute maximum, minimum and average THI by combining values of daily temperatures and humidity.

The onset of heat stress was fixed at 68 THI maximum (corresponding to 20°C at 100% humidity). According to the results Peana et al. (2017) that refer, for Sarda breed in Sardinia, milk yield decreases when maximum THI is higher than 68.The same threshold was determined for high-yielding dairy cows (Bernabucci et al., 2010). Considering the abovementioned threshold of 68, two classes of THI were created, the lower <68 identifying no stressing conditions and the higher ≥68 associated to heat stress.

Statistical analysis

Descriptive statistics of THI

The basic descriptive statistics were supplied for THI variables recorded the days of milk sampling and the three previous days. To explore the general pattern of THI variation, for each category (Max, Min and Mean), THI values – recorded in the meteorological station nearby to the farms under study – were plotted against the days of year when the milk samples were collected. A data smoothing procedure was applied to raw THI values, the LOESS regression (spanning parameter = 0.7) using R software (R core team, 2003). Furthermore, the frequency distribution of THI values was also plotted for each province.

To evaluate possible effects of geographic or temporal factors on THI, a χ^2 statistical test was used to asses different hypothesis on contingency-table built using THI class (2 levels, low < 68; high>68) and 3 other categorical variable: altitude (2 levels, low<300; high>300), geography (4 levels, one for each province) and year of recording (5 levels, from 2003-2008). To evaluate effect of altitude, THI max values were averaged by meteorological station and days of sampling and then plotted against the day of the year (from May to August). Furthermore for each year of data recording, box-whisker plot confronting THI values recorded at different altitude were also presented.

Exploratory data analysis of bulk milk composition and relationship with THI

The relationship between bulk milk composition traits (lactose, fat, protein, casein percentage and log Somatic Cell Count) and bio-meteorological indexes was assessed computing the product moment Pearson correlations in the whole dataset. To try to highlight common pattern in bio-climatic and milk composition variables, data were summarized using different averaging procedure: i) daily mean values of each meteorological station collecting data from all the neighbour farms; ii) daily mean values across years; iii) within year (and meteorological station) data averaging.

In order to assess eventual differences in milk composition under heat-stress condition, box-whisker plots of milk traits were drawn starting from the average value of each meteorological station across 5 year period, both in potential stress (THI>68) and no-stress condition (THI<68). Then raw bulk milk and bio-climatic (THI_max) data were averaged by days of sampling and plotted against days of the year; then a smoothed regression was fitted to data in order to highlight common temporal pattern between the two classes.

The analysis of bio-climatic and composition variables were also conducted within years of study (from 2004-2008). Plots of daily average of THI_max and milk traits were provided, as well as their detailed daily averages for each of the 60 meteorological stations for which data were available. Finally, the simultaneous relationship among milk composition, seasonal variation within year and bio-climatic indexes were also provided.

Regression analysis

To better quantify potential effects of THI on milk yield and composition, two series of linear regression analysis were conducted.

The first approach was aimed at quantifying the effect of THI on milk yield (and composition). For each farm (or meteorological station) in the whole period, the effect of THI quantified on milk yield was expressed as the deviation of the annual milk yield/ewes from the 5-years average. Thus, for each farm (or meteorological station) milk deviations were regressed onto the average values of THI recorded for that particular farm (or meteorological station) in the same year. Two regression lines were fitted, one for no-stressing (low THI<68) and the other for possible heat-stress condition (high THI>68).

In the second approach, daily milk composition values were regressed onto the day of the year, both within and across years. As well as for the first approach, two regression lines were fitted for no-stressing (THI<68) or possible heat stress conditions (THI>68).

Results and discussion

Descriptive statistics of THI

Meteorological data refer to the whole island in the late spring and summer and low values of temperature and high humidity are recorded. In fact, minimum temperature ranges from -2°C to 26.6 °C. Lower temperatures are generally reached in mountain areas, where frost may occasionally occur also in May and June. Highest summer temperatures are reached in the central part of the island, in a wide plain with continental climate. Considering temperatures and humidity in the days before sampling, the reduced daily weather variation makes clear the stable nature of the weather in Sardinia (see Supplemental Table 1).

The influence of different weather conditions was assessed by means of the bio-meteorological index (THI). Average, maximum and minimum THI were reported in Table 3. Average THI uses average temperature and humidity during the day; maximum THI is based on maximum temperature and minimum humidity; minimum THI is based on minimum temperature and maximum humidity on the day. Our THI did not accounted for the length of the heat period and the application of strategies to relieve the negative effects of heat stress.

Table 7 THI on the test day and three days before milk recording

Variable	n	Mean	Std	CV	Min	Max
THI med	61,491	65.06	6.19	0.10	43.89	86.52
t-1	60,331	65.02	6.19	0.10	43.89	86.52
t-2	59,735	64.78	6.20	0.10	43.93	85.31
t-3	59,874	64.76	6.18	0.10	44.32	85.31
THI max	60,921	71.48	6.05	0.08	48.46	103.64
t-1	59,896	71.54	6.03	0.08	48.46	103.64
t-2	59,422	71.42	5.99	0.08	48.46	103.64

t-3	59,532	71.42	5.93	0.08	48.92	103.64
THI min	61,193	55.27	7.51	0.14	28.40	74.35
t-1	60,034	55.11	7.58	0.14	28.40	73.75
t-2	59,523	54.75	7.55	0.14	30.92	74.22
t-3	59,627	54.68	7.54	0.14	30.92	74.22

t-1, t-2,t-3 mean value of THI measured one, two or three days before milk recording

Potential discomfort produced by cold is highlighted by several values of minimum THI lower than 65, corresponding to a temperature round 18°C at 100% humidity. The range of maximum THI shows the existence of stressing conditions, with high values imposing dangerous situations for animal and its survival.

We analysed the changes in the maximum THI with days going on in the different years (Figure 2). Generally, THI values follow a relatively predictable pattern passing from late spring to summer, as a consequence of the increase of temperature, that decline only in August, when climatic summer finishes. In the first part of 2004 the thermal regime was moderately cooler than normal, producing lower values of THI,which were later in line with the climatological average values (ARPAS, 2004). Detailed description of temperature and humidity data for each meteorological station is provided in Supplemental tables 2-4.

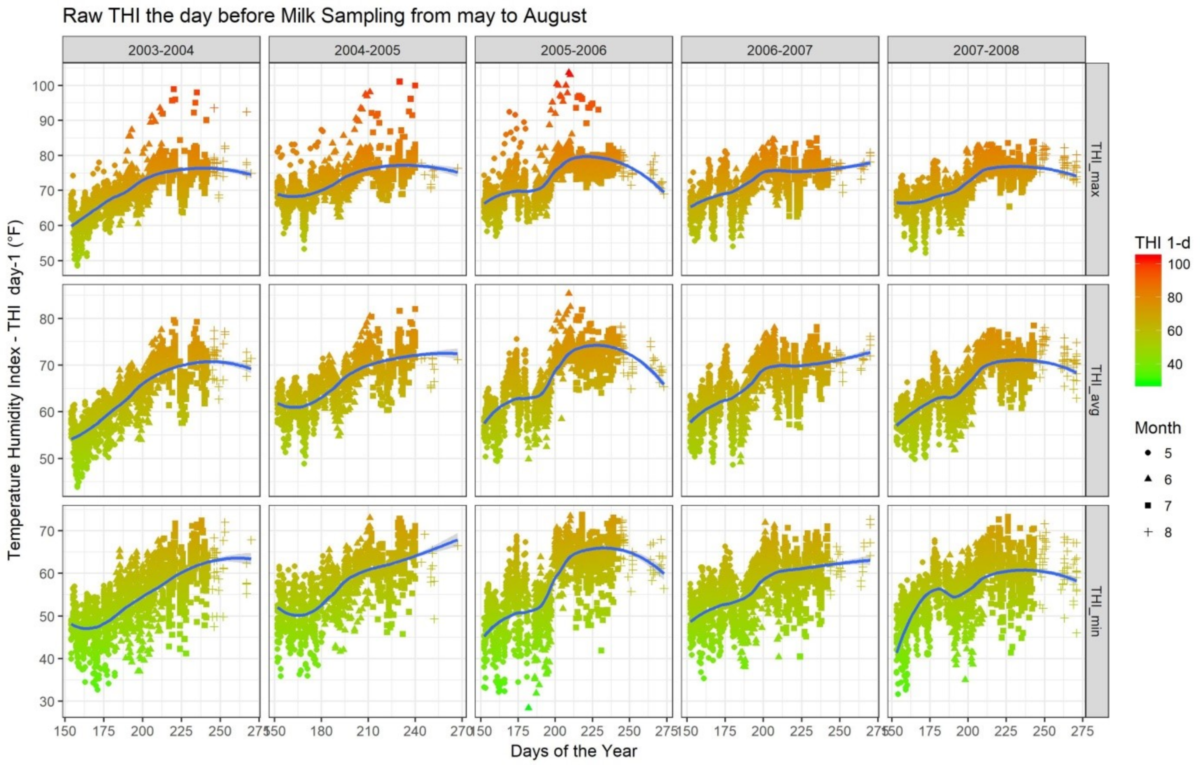

Figure 11 Changes in THI (max, min, and mean) recorded in Sardinia during the period of study.

In 2005, after a first period in May with mild temperatures, peaks higher than 40°C were reached during July and August, resulting in very high values of THI (ARPAS, 2005). In 2005/2006, temperatures increased suddenly passing from May to June, with higher values than the averages (ARPAS, 2006). This situation lasted also in July. Only in August temperature decreased to lower values than the normality. This behaviour is reflected by the maximum THI. The pattern in 2006/2007 reflects the increase in maximum temperatures in July and August 2007 in the whole region (ARPAS, 2007), producing a nearly constant pattern of THI in throughout these months. Then, in August, temperatures higher than 40°C in several localities produced a growing curve. Differently from the previous, 2008 was not a hot year (ARPAS, 2008).

Chi-squared tests(χ^2 test) on contingency-table between geographical characteristics and the classes of bio-meteorological index were highly significant (Table 4). Differences between expected and observed frequencies, in spite of being small, are significant. The statistical response, due also to the high number of data, describes a strong relationship between geographical features of the site and THI and it reflects the existence of strong differences in Sardinian environments. Further, the lower class of THI is less represented because of the growing trend of temperatures passing from May to August.

Table 8χ^2 test on THI class and geographical characteristics

Factors	n	THI threshold				χ^2	P-values
		No stress(<68)		Stress(≥68)			
Altitude							
<300	12856	2724	(3302)	10132	(9554)	269.4942	<0.0001
>300	13114	3946	(3368)	9168	(9746)	DF 1	
Province							
CA	8392	1602	(2304)	6790	(6088)	563.2194	<0.0001
NU	19067	6059	(5234)	13008	(13833)	DF 3	
OR	5151	1120	(1414)	4031	(3737)		
SS	28311	7942	(7771)	20369	(20540)		
Years							
2003/2004	13199	5274	(3623)	7925	(9576)	1730.3756	<0.0001
2004/2005	10559	2036	(2899)	8523	(7660)	DF 4	
2005/2006	11861	2630	(3256)	9231	(8605)		
2006/2007	12032	2765	(3303)	9267	(8729)		
2007/2008	13270	4018	(3643)	9252	(9627)		

The cumulative distribution of maximum THI by province (Figure 3) reflects the different constitution of the area from a orographic point of view. Oristano and Cagliari, provinces that include mainly costal and plain areas, have a wider range of maximum THI. Particularly Oristano, in spite of its reduced area, include very high values due to its geographical characteristics. On the opposite, Nuoro has a thinner and higher curve, because of many intermediate values, even if also here numerous extreme values are registered.

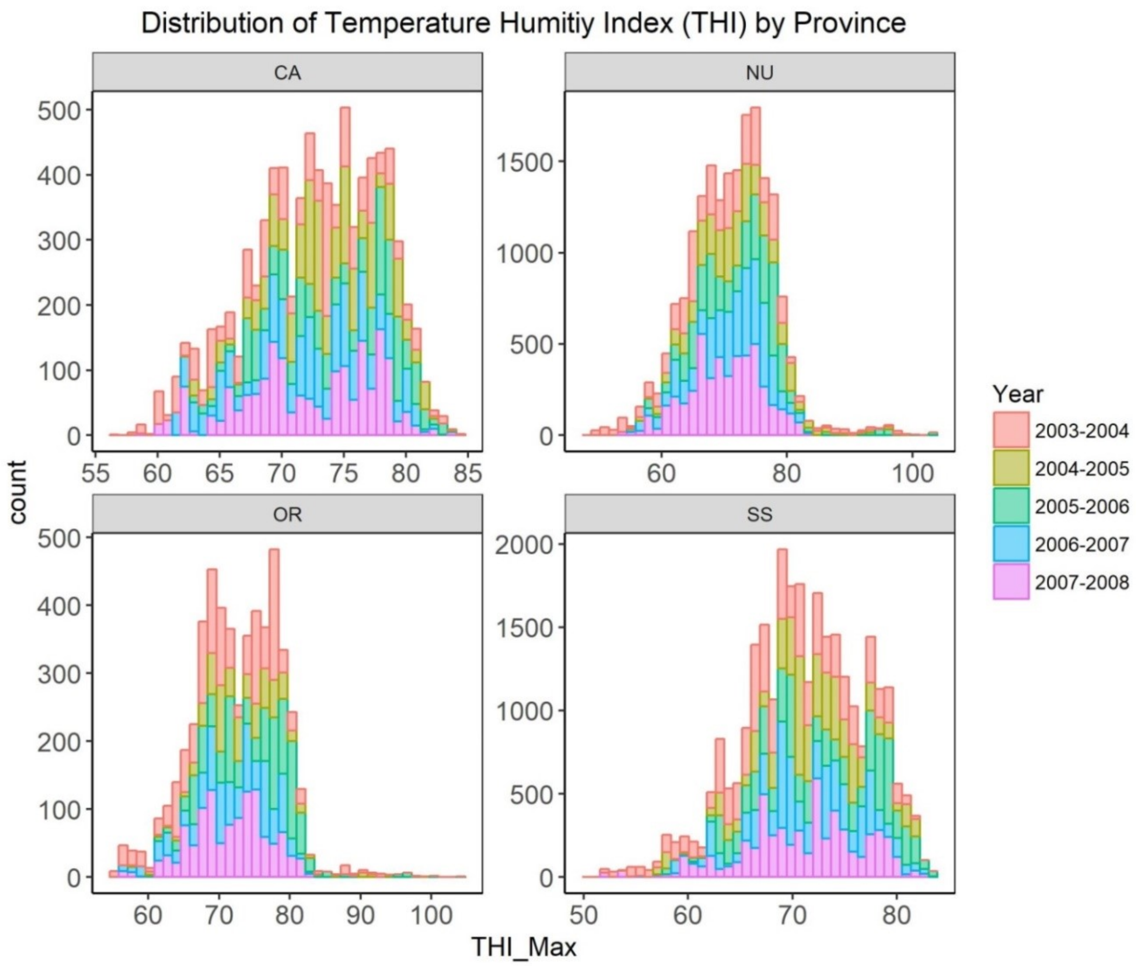

Figure 12 Distribution of maximum THI by province and year of recording

Evaluating changes in meteorological variables on the basis of the altitude of the meteorological station (Supplementary material, Figure 2, 3, 4), lower temperatures and higher humidity and, consequently, lower THI values are observed as altitude increases. As told in the previous chapter, two classes of altitude were created, considering heights lower and higher than 300m. Analysing how the maximum THI is influenced by altitude without any distinction of year, we can notice that flocks in the plains and hilly areas experiment higher values and thus more stressing conditions than flocks reared in the mountains (Figure 4), but it must be stressed out that average values are close in both classes. These results were expected and generally confirmed, year by year, by box-whisker plot distribution.

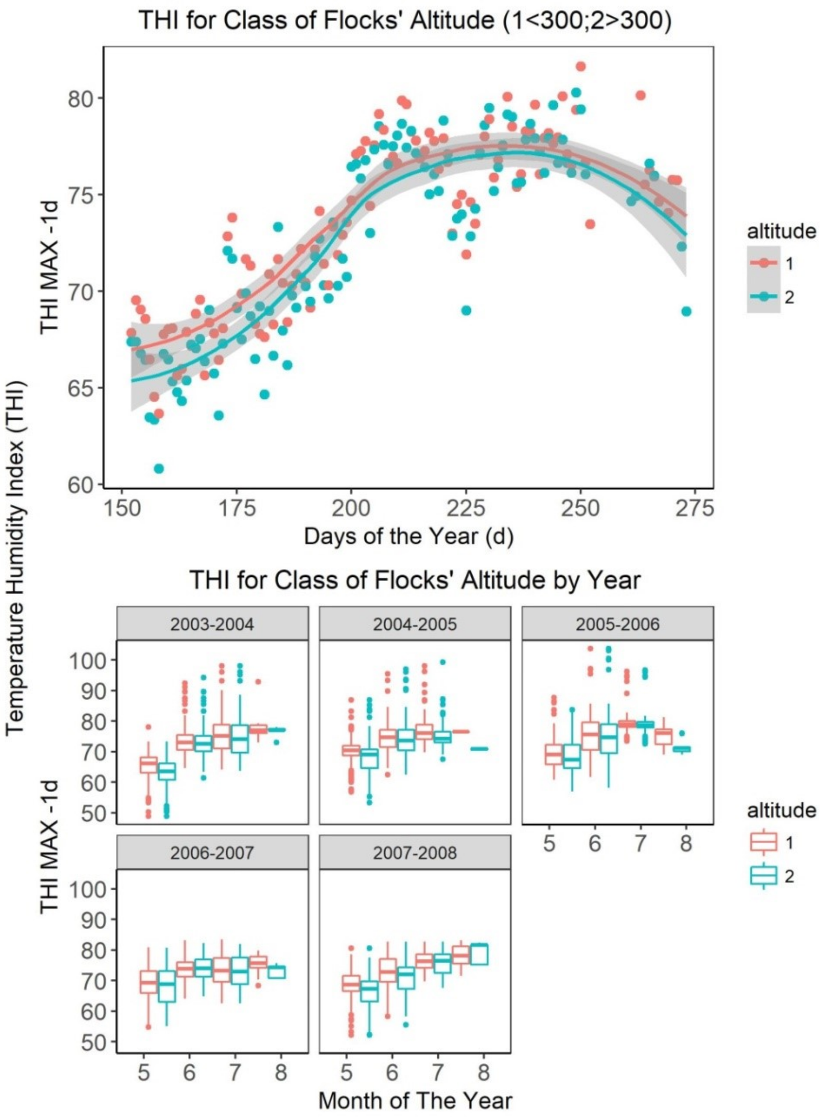

Figure 13 Pattern of THI maximum as function of Altitude class.

Bulk milk composition and relationship with THI

The raw correlations among bulk milk composition traits and bio-meteorological variables are reported in Table 5. In general, the coefficients were low to moderate, spanned from 0.03 (logSCC) to 0.55 (Lactose %), with a decreasing absolute magnitude for lactose, fat, protein and logSCC, respectively. The lagged effect of THI (days of lag) does not seem to have a relevant influence on correlation coefficients, conversely to what generally observed in literature (Finocchiaro et al., 2005; Peana et al., 2007a, 2007b; Ramon et al., 2016) where strongest effects of heat stress on milk production were recorded two days before sampling. In the present study no direct measure of milk yield was available, since only the total annual milk yield was recorded. However, the lactose could be used as proxy of milk production since it follows the same temporal pattern. In general, this study will focus primarily on change in milk composition due to possible effect of bio-climatic variables.

Table 9 product-moment Pearson correlations between productive and bio-meteorological index

THI[1]	Lactose%	Fat%	Protein %	Casein %	LogSCC[2]
Max	-0,47	0,35	0,20	0,16	0,08
t-1	-0,49	0,33	0,19	0,15	0,08
t-2	-0,49	0,35	0,21	0,18	0,08
t-3	-0,49	0,35	0,22	0,18	0,08
Min	-0,49	0,32	0,23	0,20	0,04
t-1	-0,48	0,32	0,23	0,20	0,05
t-2	-0,48	0,35	0,25	0,22	0,03
t-3	-0,48	0,35	0,24	0,22	0,03
Mean	-0,54	0,39	0,24	0,20	0,07
t-1	-0,55	0,38	0,23	0,19	0,08
t-2	-0,55	0,39	0,25	0,22	0,07
t-3	-0,55	0,40	0,26	0,22	0,08

t-1, t-2,t-3 mean value of THI measured one, two or three days before milkrecording[2] Log of somatic cell count

In Figure 5 the evolution of milk characteristics as a function of THI is presented, considering data averaged for each meteorological station. When no stressful condition occurs (low THI < 68, THI class=1), lactose content is higher whereas fat, proteins and caseins percentages are lower. This suggests a possible effect exerted by bio-meteorological factors on milk composition traits.

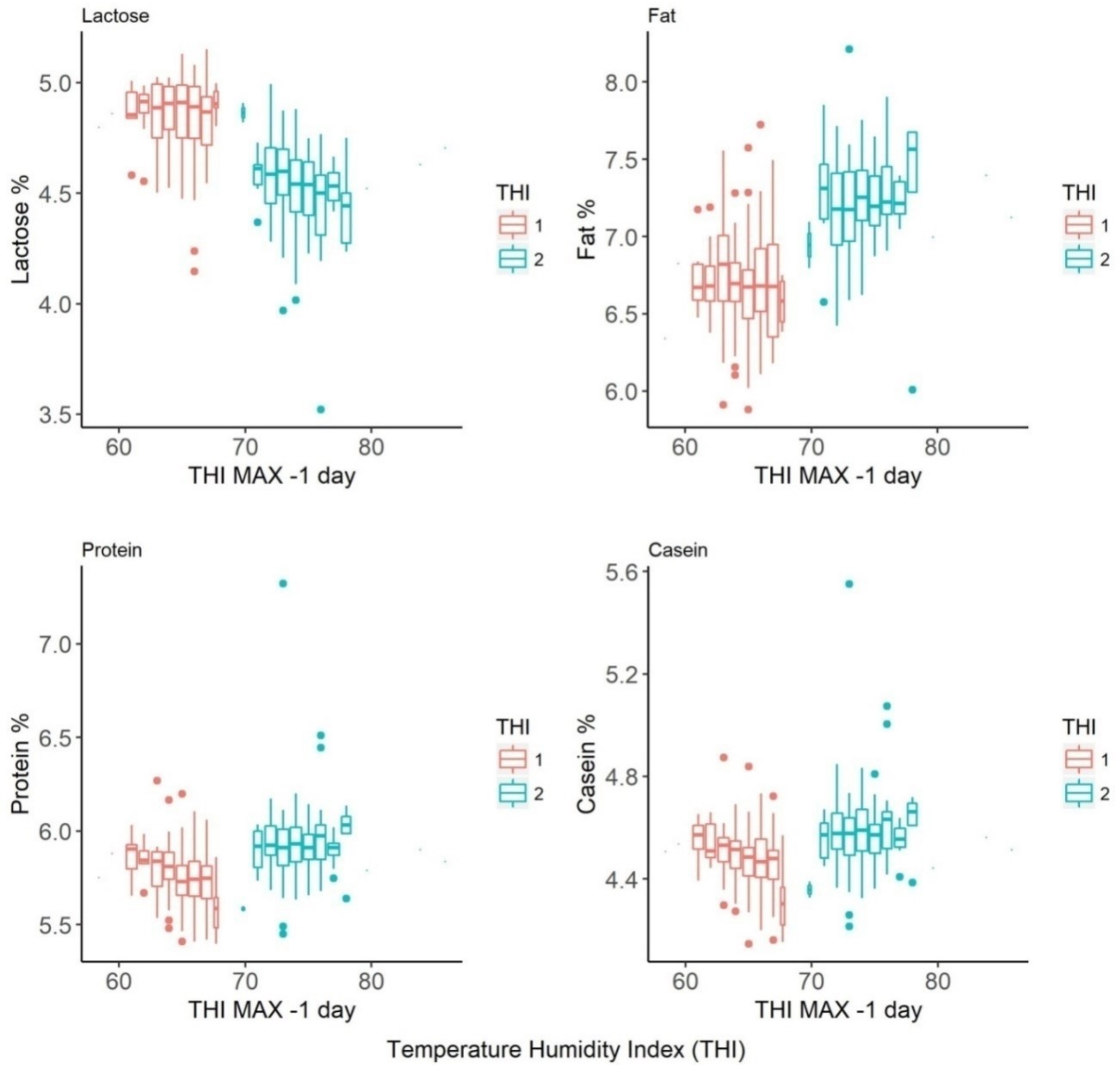

Figure 14 Lagged effect of THI classes on milk characteristics

Temporal evolution of milk bulk composition is reported in figure 6. How it is possible to argue from data, bio-meteorological information was available from 150° days onwards, whereas the milk composition was available the whole year long. Daily average and bulk milk variables were merged with maximum THI (Figure 6a). The decline of milk characteristics corresponds to the increase of THI. Fat, protein and casein contents increase, while lactose diminishes. If we analyse in detail the temporal changes in milk composition and THI, they shared a common pattern (Figure 6b)

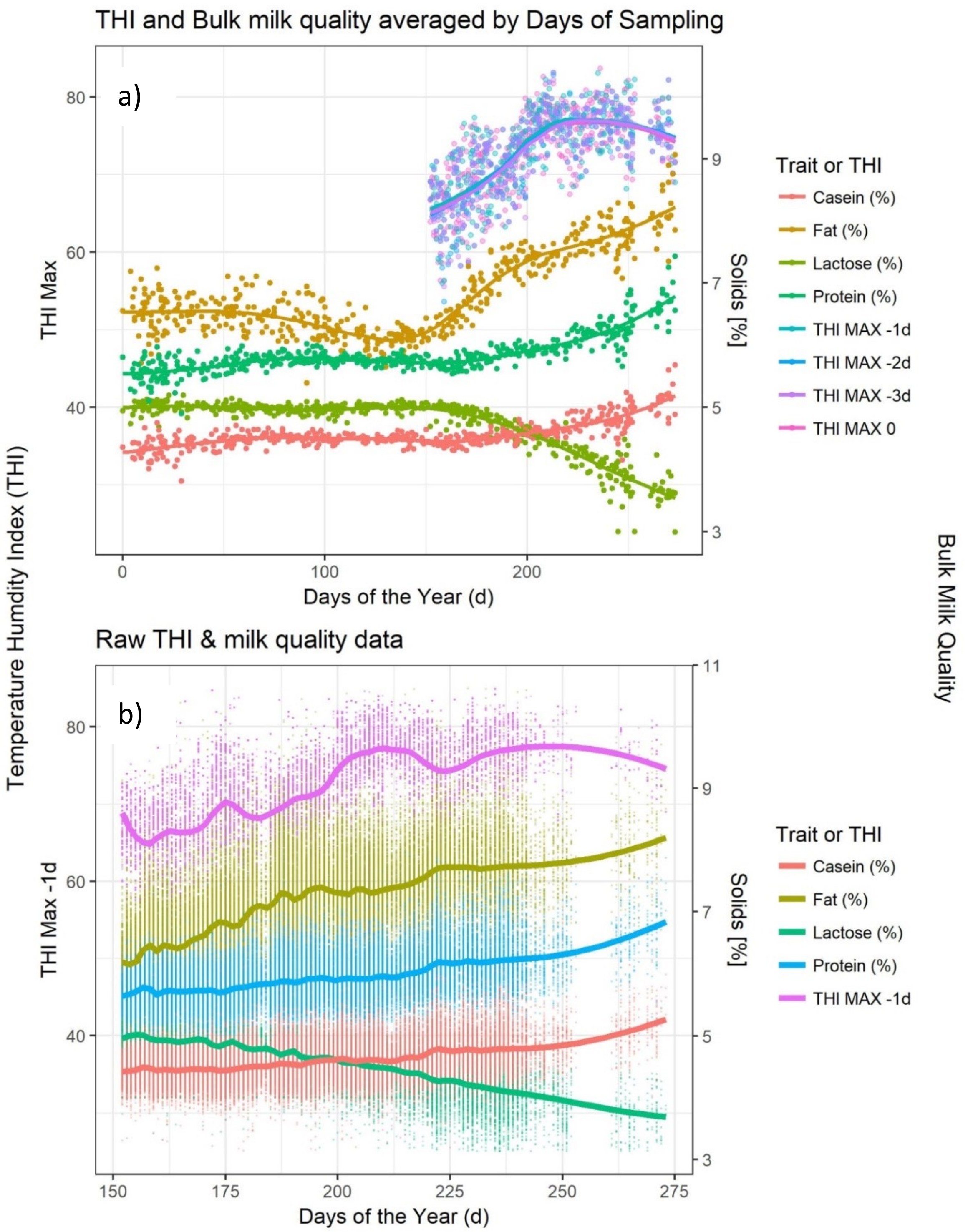

Figure 15 Milk characteristics and THI changes in the period of study

It should be pointed out that in the period from May to August there are lots of confounding factors influencing milk yield and composition. Changes in pasture availability, lower quality of forage, supplementation and changes in meteorological factors add their effect to the physiological phase of the end of lactation, negatively influencing milk characteristics (Sitzia and Ruiz 2016; Molle et al. 2008). All these factors act in the same direction, making difficult the identification of THI effect on milk characteristics. Similar behaviour is observed when comparing milk traits with days of the year (Supplemental Figure 5). Further confounding effect can derive from the low number of data referred to THI<68 (absence of heat stress). The analysis of daily average of bio-climatic and milk composition variables for each year separately is presented in Figure 7.

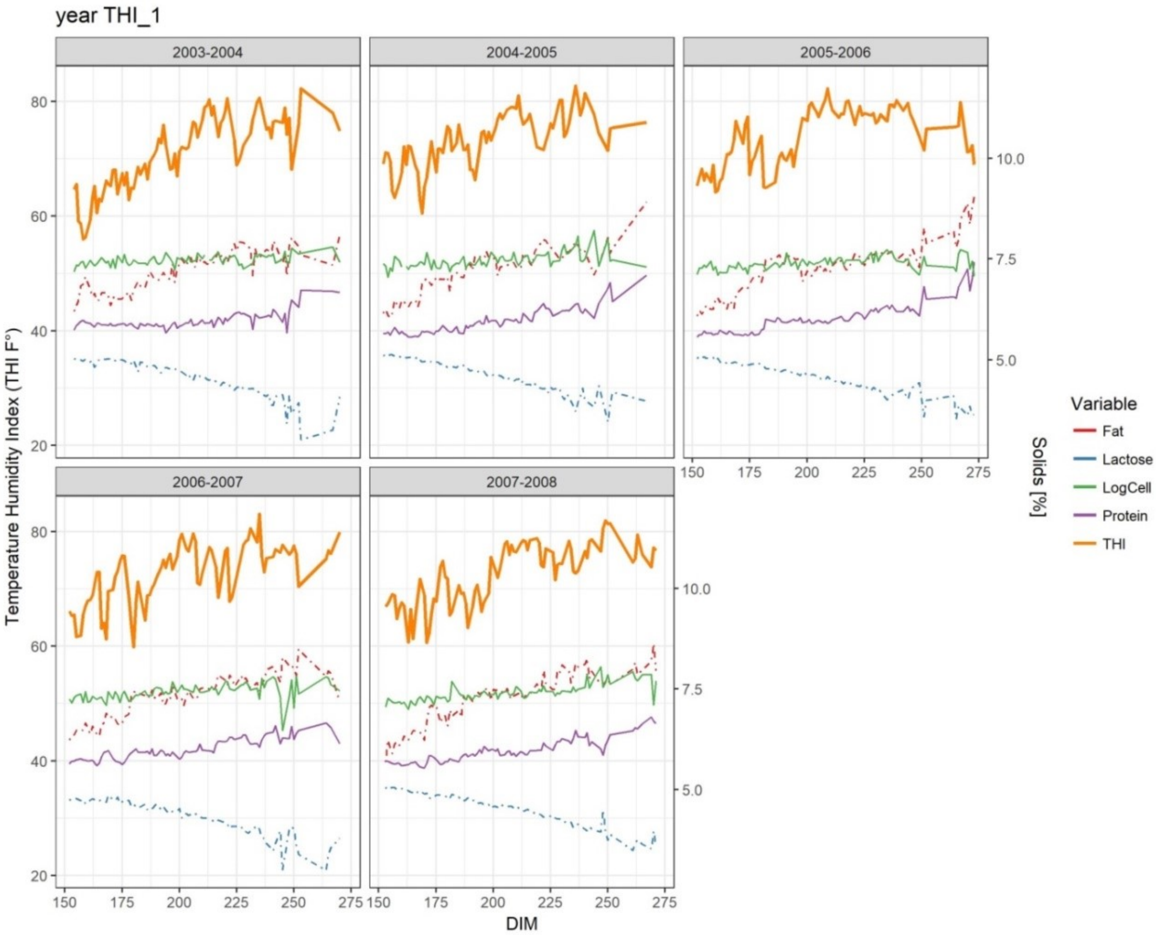

Figure 7 General behaviour of THI and milk characteristics along the summer in different years

This plot allows to highlight both the contemporary effect on milk traits of increased THI, the progress of lactation and the within and across year variability of THI values. The local variation in milk composition may be related to the change in the observed THI. Moreover, due to the reproductive seasonality of sheep, late lactation occurs in the warmest period of the year, producing itself a deterioration of milk properties. The simultaneous visual inspection of meteorological and productive data allows observing a confounding effect of physiological and environmental factors, different from temperature and humidity index. It makes difficult to separate these effects and determine which magnitude is caused by the increase of environmental temperatures and heat stress, and which part is ascribable to the final part of lactation.

Aimed at finding peculiar patterns observable from daily average, an analysis of data within each available meteorological station have been performed (Figure 8). If we consider the years 2005-2006 (chosen being the hottest in the five year period) for each meteorological station, we have generally the same growing trend of THI in each of them. Some graphs present higher degree of data availability or larger width of variation. Stands out the station placed in Ottana (NU), that, in spite of its 160 m (a.s.l.), has the highest values of temperature in Sardinia. What needs to be determined is whether the differences in the drop of lactose and rise of fat and proteins exist among stations and how much of these variations can be attributed to the THI. Although difficult to quantify, it is also common to observe local temporary variation both in THI and in milk composition.

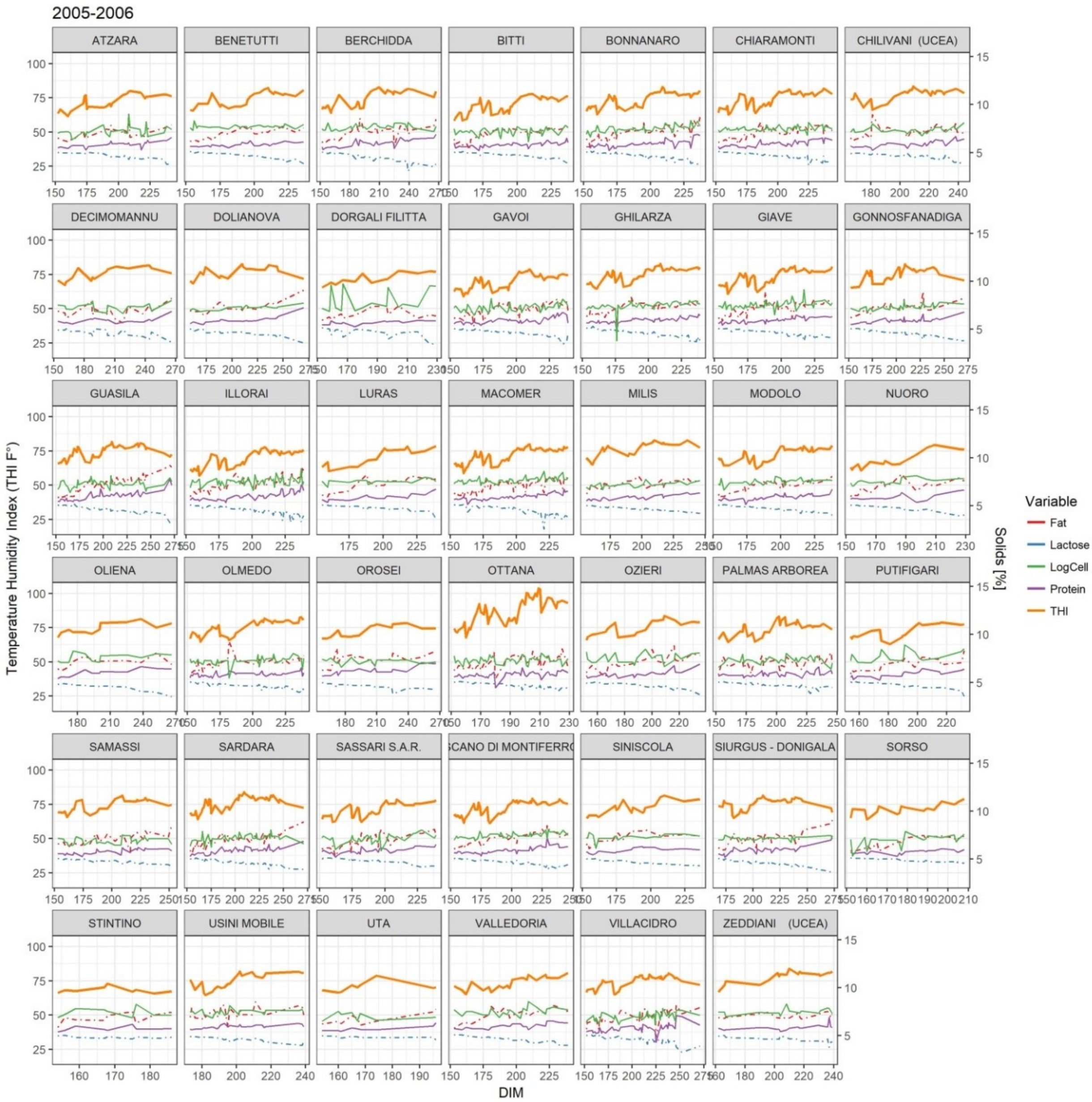

Figure 8 Daily averages of milk solids and THI in each meteorological station

Our dataset did not provide milk yield, it was available only the total annual milk produced by each flock and the number of ewes. Using these information, it was determined the total annual milk for each head, averaged for each meteorological station (Figure 9). In spite of being a reduced information, when it is represented as a function of THI (Figure 9a), this production shows a decreasing trend when THI goes beyond 65-68. This behaviour may be due to the higher number of flocks still milked in the mildest months, that produced a higher density of points in the central part of the graph. As days pass and temperatures grow, only a few flocks carry on with production.

Considering milk composition, lactose percentage drops as THI increases (Figure 9b). Furthermore, the lowest values correspond to the warmest months. This behaviour corresponds to the decrease in milk production. Fat and protein percentages rise as THI increases (Figure 9c;d), with a distribution consistent with the final part of lactation. In fact points showing the highest percentages of milk fat and proteins are referred to June, July and August, when, in late lactation, their contents rise before dry period. Furthermore, the central part of the curve consists of a minimum value, corresponding approximately to the period of April. This confirms the simultaneous effect of lactation stage and meteorological situation.

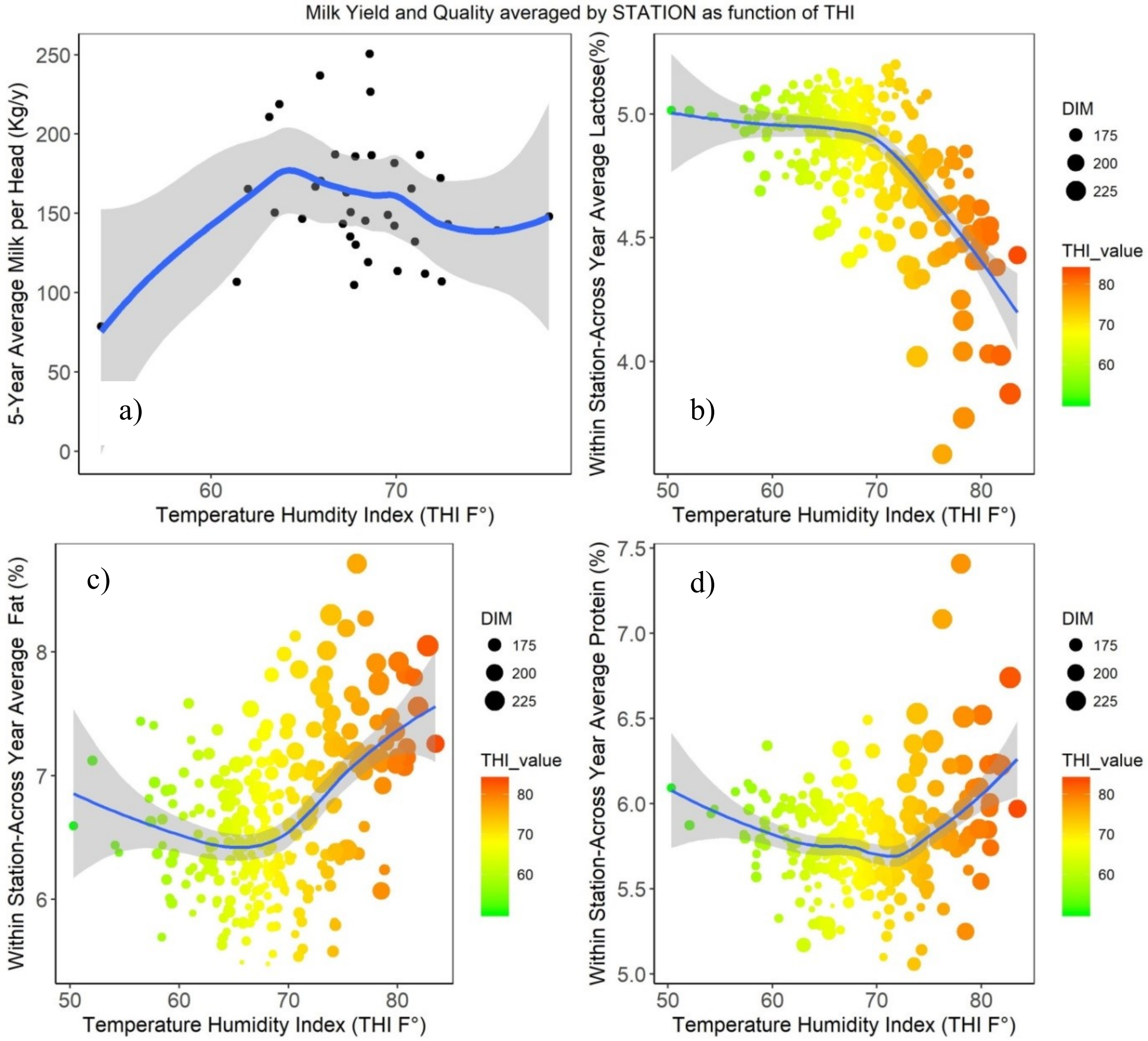

Figure 9 Bulk milk, lactose, fat and protein percentages as a function of THI_{max}

Regression analysis

The regression analysis of bulk milk deviation from 5-year means on THI was reported in Table 6 and Figure 10. Milk yield derives the total annual milk divided the total number of ewes of each farms, and some caution in discussing these results is due. No significant effect exerted by stressing condition (THI>68) on milk yield deviations was observed. When considering the whole THI variation (Table 6 and Supplemental Figure 6) milk deviations experienced a weak drop as THI values increase. Compared to the average milk production assumed as 100% a drop of 5% of milk every 10 THI point was estimated (β_{Overal}within flock). As far as the milk composition deviations concern, significant effects are played on fat, protein and lactose in stressing condition (β_{High}) both within flock and within meteorological station averaging scenarios. The lowest and highest effects were registered respectively on protein and fat percentage.

Table 6. Slopes of the regressions of average milk traits deviations % (mean value is equated at 100%) on average THI value for low (β_{Low}), high THI (β_{High}) or in the whole dataset ($\beta_{Overall}$).

			Milk traits[1]							
Scenario[2]	THI[3]	n[4]	Milk (l/y/head)		Lactose (%)		Fat (%)		Protein (%)	
1. Within Flock	β_{Low}	516	-0.99 (0.22)	***	-0.01 (0.04)	NS	-0.33 (0.08)	***	-0.32 (0.04)	***
	β_{High}	521	0.03 (0.17)	NS	-0.41 (0.04)	***	0.67 (0.08)	***	0.15 (0.03)	***
	$\beta_{Overall}$	1037	-0.49 (0.09)	***	-0.16 (0.02)	***	0.13 (0.04)	***	-0.09 (0.09)	***
2. Within Station	β_{Low}	40	-0.61(0.71)	NS	0.07(0.13)	NS	-0.39(0.23)	NS	-0.33(0.12)	**
	β_{High}	49	-0.02(0.55)	NS	-0.74 (0.22)	**	1.40(0.33)	***	0.29 (0.13)	*
	$\beta_{Overall}$	89	-0.51(0.31)	NS	-0.20 (0.08)	*	0.27(0.14)	NS	-0.02(0.06)	NS

$H_0\beta=0$ *** <0.001, ** <0.01, * <0.05, NS >0.05; se=standard error of β. [1]Annual deviations from 5-year average (%)within Flocks or within Station. [2]A total of 374 farms contributed to the 1st scenario. A total of 29 Meteorological Station contributed to the 2nd scenario.[3] Low (max THI <68), high (max THI >68);[4]Total number of records used.

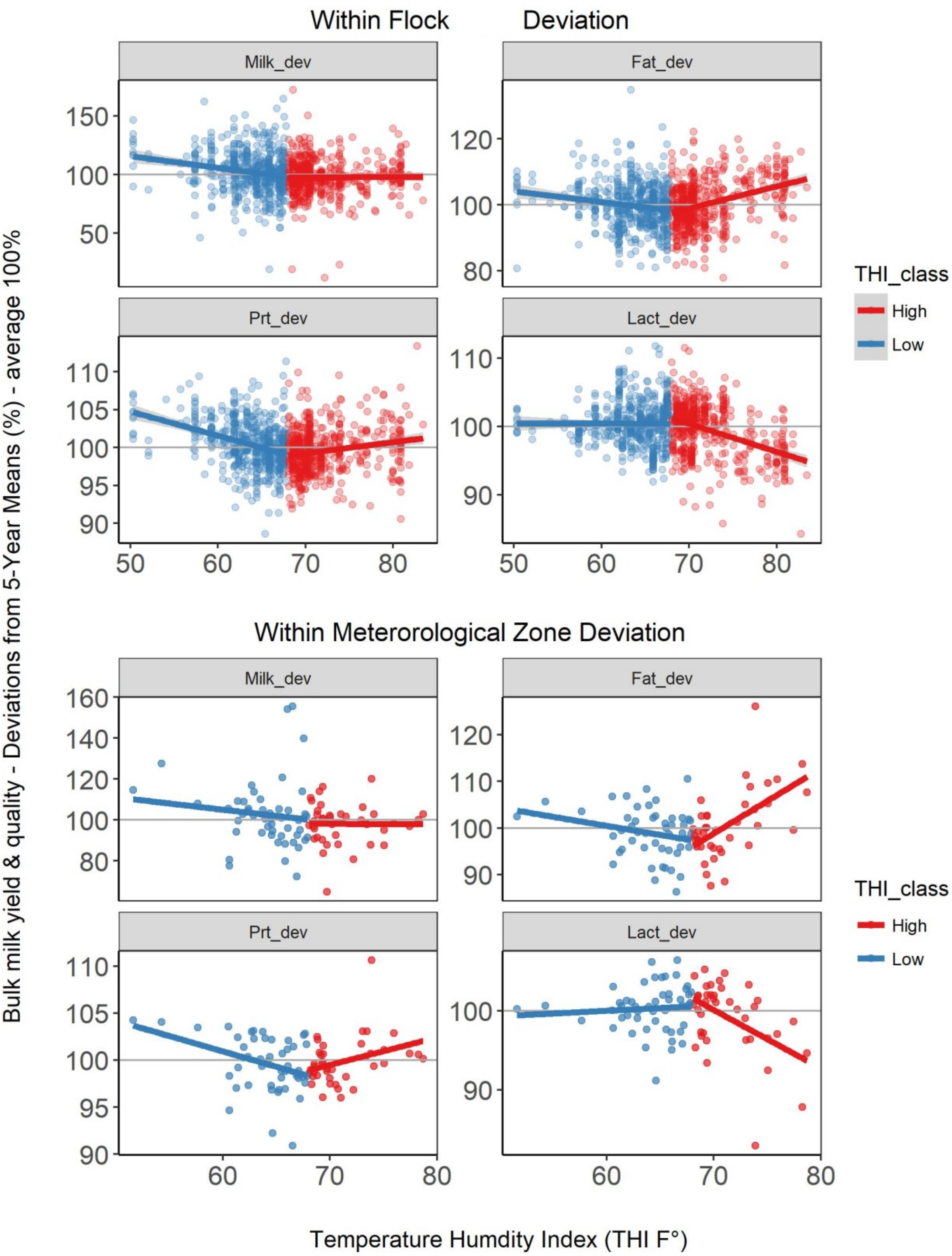

Figure 16 Regression of bulk milk and composition deviation on THI

Table 7 analyses how regression coefficients vary between the classes of THI, considering the days before sampling. The regression coefficients of milk quality traits vary across the THI scale. Each value represent the slope of variation in milk solid content. Each value is very low, because it is referred to the effect in one single day. Considering the average length of a month, the datum has to be multiplied for 30 and it means that, in a month, the higher class of THI loses approximately 0.36 percentage points of lactose. Even if the differences between the classes are small, all of them are significant. The highest variation (Δ_{LH}) between classes of THI was observed for fat percentage two/three days before milk sampling.

Table 7 Slopes of the linear regressions of daily average of bulk milk traits across farms on days of the year (from May to August) both in low (βL) and high (βH) THI class. THI values were recorded one, two or three days before milk sampling.

Days before Sampling[1]		THI Maximum				
		Low (THI<68) (n=184)		High (THI>68) (n=385)		
	Trait	β_L	se	β_H	se	Δ_{LH}[2]
1 day	Lactose (%)	–0.010***	0.001	–0.012***	0.0003	0.002
	Fat (%)	0.024***	0.001	0.015***	0.0005	0.009
	Protein (%)	0.007***	0.001	0.009***	0.0003	–0.002
	Casein (%)	0.005***	0.001	0.006***	0.0002	–0.001
	LogSCC	0.004***	0.001	0.003***	0.0003	0.001
2 days	Lactose (%)	–0.010***	0.001	–0.012***	0.0003	0.002
	Fat (%)	0.026***	0.001	0.015***	0.0004	0.011
	Protein (%)	0.008***	0.001	0.009***	0.0003	–0.001
	Casein (%)	0.005***	0.001	0.006***	0.0002	–0.001
	LogSCC	0.006***	0.001	0.003***	0.0003	0.003
3 days	Lactose (%)	–0.010***	0.001	–0.012***	0.0003	0.002
	Fat (%)	0.026***	0.001	0.015***	0.0004	0.011
	Protein (%)	0.008***	0.001	0.008***	0.0003	–0.000
	Casein (%)	0.005***	0.001	0.006***	0.0002	–0.001
	LogSCC	0.005***	0.001	0.003***	0.0003	0.002

In spite of slight differences in regression coefficients (Δ_{LH}) occurring among days of THI recording, they allow to hypothesize that the best evaluation of lagged effects of heat stress derives from the use of the THI referred to the meteorological conditions two days before sampling. These figures partially agree with results by Peana et al. (2017), who found that the weather conditions in the period included between the day of sampling and two days before milking was the most influencing for milk traits, and by Finocchiaro et al. (2005), who found that THI of the day before milk recording and the periods 0–2d and 0–3d before sampling produced the greatest losses in milk yield.

Bulk milk lactose content, averaged for sampling day across years (Figure 11), initially showed a nearly constant pattern and then decreased in mid and late lactation. Considering the different classes of THI, it is possible to identify two straight lines, one referred to the absence of heat stress and one referred to stressing conditions. The slope of each trait is slightly different.

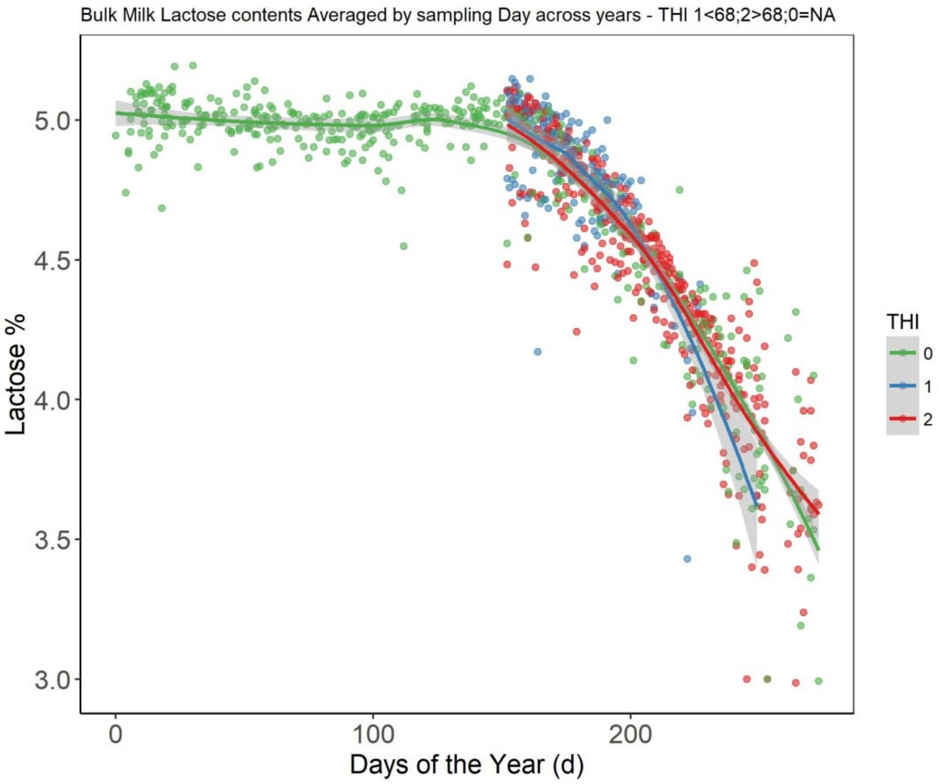

Figure 17 Bulk milk lactose behaviour at different THI across years

We analysed the change in milk composition among the years within THI classes (Figure 12 and Table 8). The slope of the regression line referred to heat stress should be higher than the other, due to a more pronounced reduction in milk yield. This behaviour is confirmed in 2003/2004, 2006/2007 and 2007/2008. In 2005/2006 the line referred to heat stress cannot be separated by the other, while in 2004/2005 the two had opposite trend than expected. This behaviour has been quantified by the analysis of regression coefficients between THI classes and milk traits over the years (Table 8). It is to underline that all regression coefficients were statistically significant.

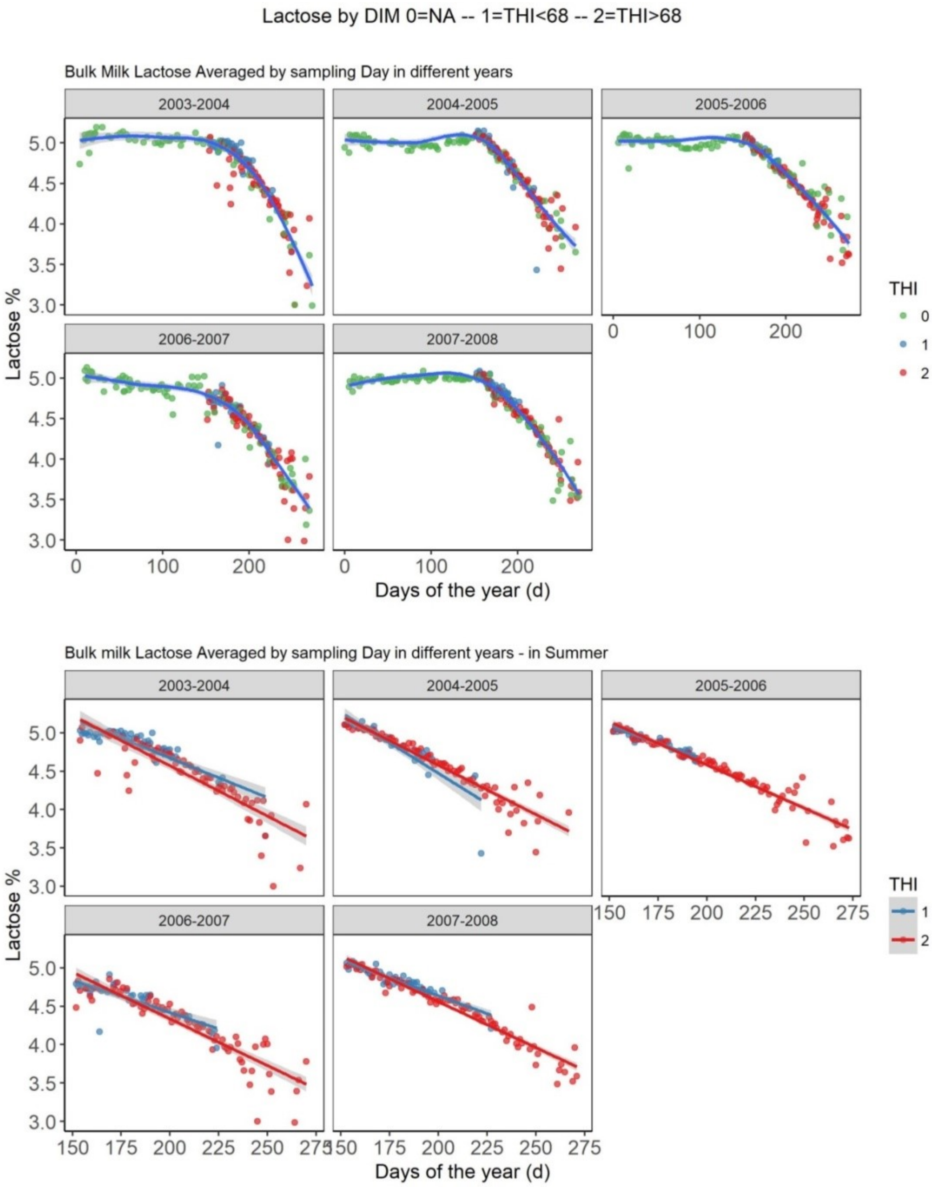

Figure 18 Bulk milk lactose in different years

Table 7 old Milk traits and years, regression coefficients:slopes of the linear regressions of daily average of bulk milk traits across farms on days of the year (from May to August) both in low (β_L) and high (β_H) THI class in different years.

		Lactose %		Fat %		Protein %		Casein %		Logcell	
Year	THI	β	se	β	se	β	se	B	se	β	se
2003-2004	Low (n=46)	-0.009***	0.001	0.022***	0.002	0.003***	0.001	0.002***	0.001	0.002NS	0.001
	High (n=70)	-0.014***	0.001	0.013***	0.001	0.005***	0.001	0.003***	0.001	0.003**	0.001
2004-2005	Low (n=35)	-0.016***	0.001	0.029***	0.002	0.014***	0.002	0.009***	0.002	0.008*	0.003
	High (n=76)	-0.013***	0.000	0.017***	0.001	0.010***	0.001	0.006***	0.000	0.004***	0.001
2005-2006	Low (n=27)	-0.010***	0.000	0.039***	0.002	0.010***	0.002	0.008***	0.001	0.006***	0.003
	High (n=81)	-0.011***	0.000	0.015***	0.001	0.010***	0.001	0.007***	0.000	0.002***	0.000
2006-2007	Low (n=36)	-0.007***	0.001	0.022***	0.003	0.007***	0.001	0.004***	0.001	0.006***	0.002
	High (n=77)	-0.012***	0.001	0.015***	0.001	0.008***	0.000	0.007***	0.000	0.003***	0.001
2007-2008	Low (n=40)	-0.010***	0.001	0.029***	0.003	0.006***	0.001	0.005***	0.001	0.006***	0.002
	High (n=81)	-0.012***	0.000	0.016***	0.001	0.008***	0.001	0.006***	0.000	0.005***	0.001

Figure 13 considers how milk traits are affected by the classes of THI. Fat percentage has an opposite trend than expected: the straight line referred to heat stress show a lower fat content than the line representing the absence of heat stress. On the other hand, proteins and caseins percentages behave according to expectation. Somatic cells have a wide dispersion. Generally, their count should rise as maximum temperature rises (Peana et al., 2017), but in our data it's not easy to detect the different pattern in the classes of THI, even if the rise in total number is confirmed.

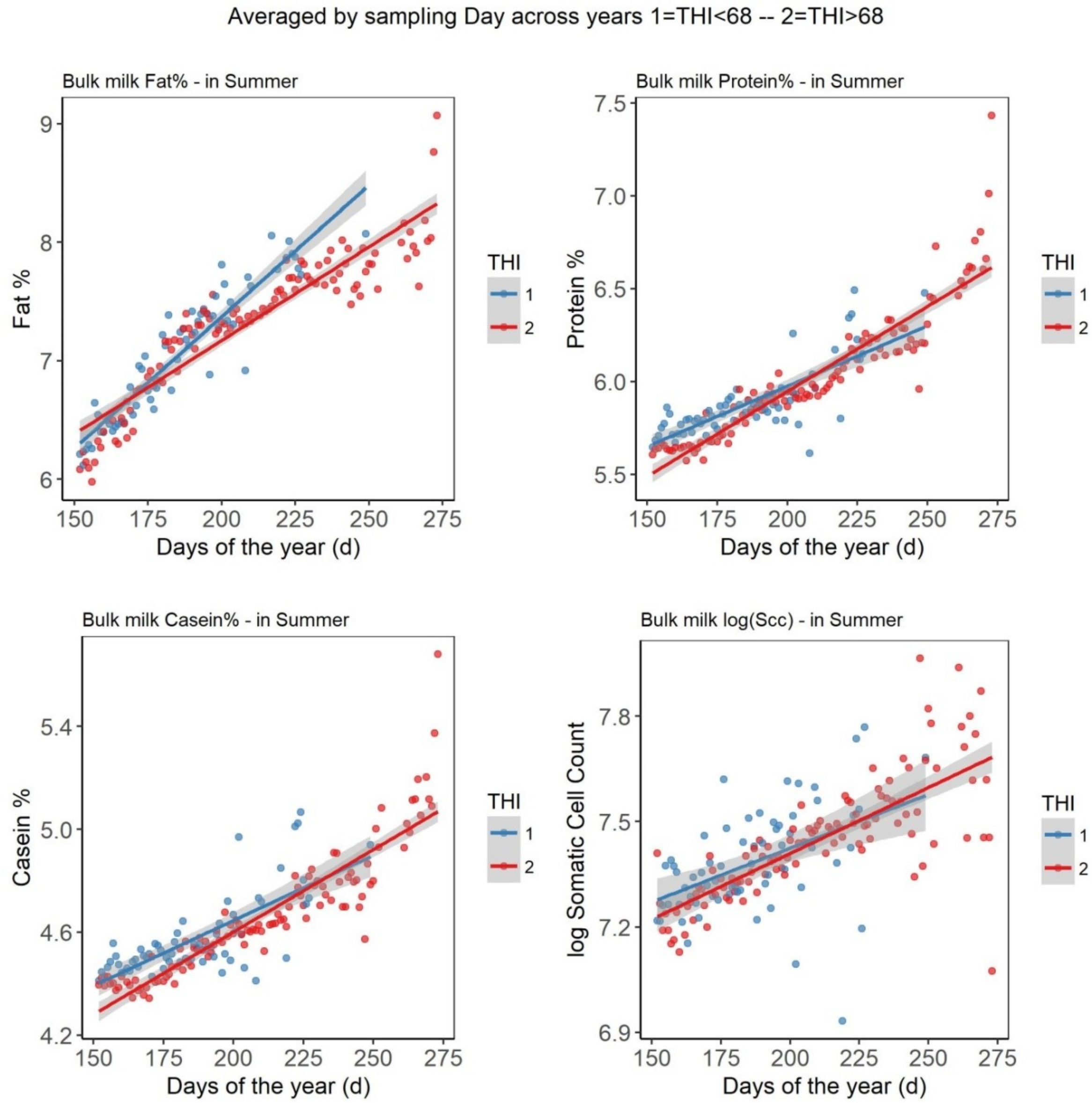

Figure 19 Milk quality traits as influenced by THI class

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Chapter 4: Assessing bulk milk composition variability in Sardinian farms as influenced by meteorological factors

Introduction

Climate and weather act on animals exerting a strong influence on farm production variations. Environmental stressors such as ambient temperature, relative humidity, solar radiation and wind can directly and adversely affect animal performance, leading to great economic losses (Al-Dawood, 2017). Sheep and goats have a wide geographical distribution and economic importance worldwide and knowledge on how their performances are influenced by heat stress is essential. To date, little attention has been given to complete and detailed information on unfavourable effects of heat stress and thresholds for dairy sheep (Finocchiaro et al., 2005; Peana et al., 2007a; 2007b; 2017; Ramón et al., 2016; Sevi et al., 2001). Furthermore, heat stress tolerance is negatively correlated with milk traits and levels of reduction are higher in high yielding selected animals both in sheep and goats (Finocchiaro et al., 2005; Menéndez-Buxadera et al., 2012).

Temperatures in the Mediterranean area can often exceed sheep thermoneutral zone, impairing sheep productivity. Beyond thermoneutral zone, production decays (Sevi and Caroprese, 2012). The heat tolerance seems to change across species and across breeds within species, and individual differences of heat effects on production are partially ascribable to genetic effects (Finocchiaro et al., 2005; Ramón et al., 2016; Sevi and Caroprese, 2012). Lactating Sarda sheep exposed to minimum environmental temperatures ranging between 9-12°C (Peana et al., 2007a; 2007b; 2017), maximum temperature between 15-24°C (Peana et al., 2017) and average temperature of 12-18 °C (Peana et al., 2017) showed the highest yield. Instead, Manchega sheep (Ramón et al., 2016) has an optimum in minimum temperature ranging from -1 to 10.2°C, maximum temperature of 19.1 – 29.6°C and average 11.5-21°C, respectively. But only a few studies exist on determining critical temperatures and effects on productivity in sheep. As told before, the index of heat stress generally used in farm animals is the THI (Temperature-humidity index), which combines the effects of both temperature and humidity in one index. In our study, on the basis of several studies on dairy cattle (Bertocchi et al., 2014; Brügemann et al., 2012; Ravagnolo et al., 2000) and on dairy sheep (Peana et al., 2007b; 2017; Finocchiaro et al., 2005), different THI were calculated: THImax uses daily maximum temperature and minimum humidity and it is representative of the worst environmental conditions registered in the day, because generally, in our environment, the highest temperature corresponds to the lowest humidity; THImin uses daily minimum temperature and maximum humidity and refers day-night conditions; and THIavg uses daily average temperature and humidity.

The objective of this work was to determine the effects of heat stress on bulk sheep milk composition in Sardinian farms. This effect, in spite of being small, is not negligible and reduces farm profit. In the current study two approaches were adopted. The first was aimed at estimating the effect of THI of the sampling day or of preceding days on milk composition, considering also the effects of other environmental factors. The second objective was to derive a bio-meteorological index able to take into account all the available meteorological variables (temperatures, wind speed, rainfall), combining multivariate statistics in the mixed model framework.

Materials and methods

Bio-meteorological and milk composition farm data

Bulk milk composition and meteorological variables were provided by ARAS and ARPAS, respectively (for a detailed description see chapters 2 and 3). Briefly, bulk milk composition refers to milk fat, protein, casein, lactose percentage, $\log_{10}$ total bacterial count (logTBC) and $\log_{10}$ somatic cell count (logSCC) recorded approximately at least one time at month in a 5 year period (from 2003 to 2008) in about 5000 farms spread in Sardinia. Meteorological variables were temperature (°C, minimum, max and average), wind speed (m/s. max and average), rainfall (mm) and temperature humidity index (computed as Kliber, 1964) referring to the same 5 year period and recorded by the closest meteorological station 1 or 2 days before bulk milk samplings were made. Meteorological variables have been obtained from the Sardinian meteorological network, including 60 stations evenly spread in Sardinian regional territory. (http://www.sar.sardegna.it/documentazione/strumenti/retestazioni.asp).

The dataset used in the current chapter differs from those used in the previous chapters for data editing only. In particular, data records were retained if productive and bio-climatic variables were both present for each sampling date. Data of August were discarded to avoid estimative issues because of the limited data density. Furthermore, farms with less than 4 milk samples per year were discarded. Yet, solar radiation information was rejected because of high frequency of missing values. Farms that met all abovementioned criteria were 2,898, for a total of 35,392 records spanning from May to July and covering 5 year period. Overall descriptive statistics of the dataset are provided in Table 1.

Table 1. Descriptive statistics for bulk milk composition traits and bio-meteorological indexes in May to July in 2003-2008.

Variable[1]	Mean	s.d.	Min	Max
Fat, g/100 ml	7.09	0.77	3.5	11.8
Protein, g/100 ml	5.89	0.36	4.2	8.0
Casein, g/100 ml	4.57	0.29	3.1	6.2
Lactose, g/100 ml	4.67	0.35	2.5	5.4
CMT, log_{10}CMT	2.48	0.73	0.3	4.2
SCC, log_{10}SCC	3.21	0.25	1.4	4.2
THI_{MAX-1d}	71.44	6.06	48.5	103.6
THI_{MAX-2d}	71.32	6.03	48.5	103.6
THI_{AVG-1d}	64.91	6.22	44.1	85.3
THI_{AVG-2d}	64.70	6.21	44.1	85.3

[1] n=35392 records in 2,898 farms; s.d. dtandard deviation

Based on the actual distribution of THI values and indication in literature (Peana et al., 2007a; 2007b; Macciotta et al., 2017), THI variables were discretized into 8 THI class. Temperature humidity index was categorized differently for THI_{MAX} (1 < 64; 2 = 64-66; 3 = 66-68; … ; 8 > 76) and THI_{AVG} (1 < 60; 2 = 60-62 ; 3 = 62-64 ; … ; 8 > 72) and their respective distribution are reported in Figure 1. Possible indicators of heat stress, thus detrimental for milk composition, were considered THI_{MAX} (THI_{AVG}) >70 (>66). Since the seasonal data availability, potential cold stressing effects on milk composition were not considered.

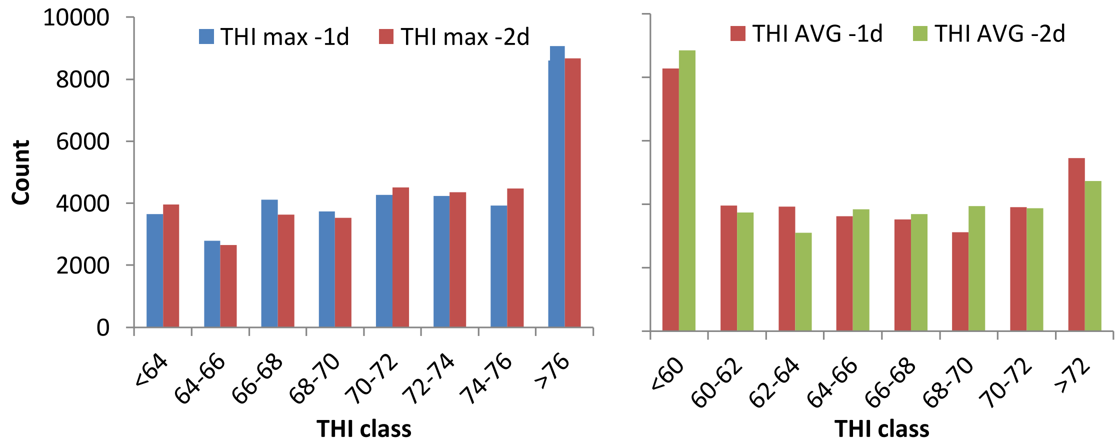

Figure 1. Distribution of THI records across the different class of THI identified.

Statistical analysis

Bulk milk composition traits were analysed with the following linear mixed model implemented with PROC MIXED of SAS (SAS Institute, 2008):

$$y_{ijkl} = mo(year)_{i(j)} + fs_k + fs \times THI_{(k \times l)} + THI(year)_{k(j)} + THI(mo)_{k(i)} + Flock(year)_{l(j)} + e_{ijkl} \qquad (1)$$

Where *y* is the analysed milk trait (see Table 1); *mo(year)* is the fixed effect of month (3 mo: May, June and July) nested within year (5 y: 2003-2008); *fs* and *fs*THI* are the fixed effects of the flock size (3 classes: <=100, 100-300,>300 heads) and their interaction with

THI (8 classes, see figure 1); *THI(year)* and *THI(mo)* are the fixed effects of THI nested within year and month, respectively; *Flock(year)* and *e* are the random effect of Flock nested within year (6,996 levels) and the random residuals distributed ~N(0, $\mathbf{I}\sigma^2_{Flock}$) and ~N(0, $\mathbf{I}\sigma^2_e$), respectively. The percentage of variance explained by Flock was calculated as the ratio between $\sigma^2_{Flock}/(\sigma^2_{Flock} + \sigma^2_e)$ where σ^2_{Flock} and σ^2_e are the estimates of flock and residual variances, each in order. Statistical model of equation (1) was run both using THI_{MAX} and THI_{AVG} (either -1 day or -2 days before milk sampling).

Least square mean of milk composition traits for all cross-classified fixed effect have been computed (Tukey adjustment was used to correct for post-hoc multiple comparison), even if, among the other source of variation, particular emphasis will be given in the discussion the different classes of THI (maximum and average) on bulk milk composition.

A second approach uses multivariate factor analysis (MFA). In our study the MFA is applied to decompose the correlation matrix of meteorological variables (temperature, relative humidity, wind speed and rainfall) and to identify potential interesting latent variables. The measure of sampling adequacy (MSA) of Kaiser was computed to assess the suitability of data to latent factor sought. The factorial model used was:

$$\mathbf{R}=\mathbf{BB'}+\mathbf{\Psi}$$

where **R** is the original correlation matrix and **B** contains the estimates of loadings coefficients (b_{ij}), i.e. the correlation between original variables and latent factors. The off-diagonal elements of **BB'** (b_{ij} for $i \neq j$) provide an approximation of the original correlation matrix, whereas the diagonal elements of **BB'** (b_{ij} for $i=j$) are the estimates of communality (h^2), or the variance shared by the original variable and explained by the common latent factors. The diagonal matrix **Ψ** brings on the diagonal the uniqueness of each variable, that is the part of the variance that each original variable does not share with all the others (Morrison, 1976; Krzanowsky, 2003). In our case, the estimates of b_{ij} allow thus to decompose set of meteorological variables (from y_1 to y_p) into a linear combination of latent factors (from f_1 to f_m with $m<<p$) plus a residual (e), i.e.:

$$y_1 = b_{11}f_1 + ... + b_{1m}f_m + e_1$$

……………………………….

$$y_p = b_{p1}f_1 + ... + b_{pm}f_m + e_p$$

To compute the matrix of factor scores (**F**) this system of equation is solved for **F** (Macciotta et al. 2004).

$$\mathbf{f'} = \mathbf{y'}(\mathbf{BB'}+\mathbf{\Psi})^{-1}\mathbf{B}$$

where

$\mathbf{f'} = [f_1 \; f_m]$,

$\mathbf{y'}$ = row vector of standardized meteorological records, and

$(\mathbf{BB'}+\mathbf{\Psi})^{-1}\mathbf{B}$ = scoring coefficients.

A number of factors explaining at least 70% of the original variance were retained. The *varimax* rotation of loading coefficients was chosen to simplify the interpretation of coefficients. The MFA was implemented with PROC FACTOR of SAS (SAS Institute, 2008).

The latent factors were then analysed aimed at interpreting their meanings. A correlation analysis of THI and latent factors was carried out. Comparisons with THI indexes were carried out after using the latent factors scores in the same linear mixed model used to analyse THI variables. The identified latent factors were categorized into 6 classes (**1**<=-2] , **2**=]-2:-1] , **3**=]-1:0] , **4**=]0:1] , **5**=]1:2] , **6**>2).

Results and discussion

Linear mixed modelling of THI

In Table 2 is reported the quota of trait variance explained by random flock effect (r^2Flock). Flock explains from 32% (Fat) to 64.4% (SCC) of trait variance. These estimates indicate that from moderate to large part of milk traits variation is explained by the flock. This agrees with results on milk composition in literature, ascribing a not negligible part of variance to differences between flocks. Results by Puledda et al. (2017) showed that flock explained the largest percentage of variance for test day milk yield, fat, protein and caseins percentages in dairy sheep. A similar amount of variance was also found in dairy sheep in other works (Bittante et al. 2012; Vacca et al., 2015). Performing a test of overall significance, all relationships are statistically significant for all characters and effects, for each THI considered.

Table 2. Variance Explained by Flock

Trait	r^2Flock %
Fat, g/100 ml	32.0
Protein, g/100 ml	41.1
Casein, g/100 ml	41.3
Lactose, g/100 ml	32.5
CMT, $\log_{10}$CMT	37.3
SCC, $\log_{10}$SCC	64.4

All the analysed effects are highly significant (P<0.001) for the whole set of milk composition traits, with the exception of log10 SCC (Table 3). In particular for log10 SCC, fixed effects of flock size and its interaction with THI are not significant, for each THI and Flock size class. In our dataset also small differences are significant due to the high number of records available.

Table 3 Significance of Fixed effect for log_{10}SCC using different measures of THI

	logSCC			
Effect	THI_{AVG1}	THI_{AVG2}	THI_{MAX1}	THI_{MAX2}
Month(Year)	<0.001	<0.001	<0.001	<0.001
FlockSize	0.14	0.22	0.16	0.19
FlockSize*THI	0.11	0.52	0.75	<0.001
THI(Year)	<0.001	<0.001	<0.001	<0.001
THI(Month)	<0.001	<0.001	<0.001	<0.001

When the effect of flock is removed, the effect month within year has a clear influence on milk traits. Passing from May to June, fat, protein and casein increase, even if the width of rise changes among years. On the other hand, lactose decreases. These trends are expected and due to the lactation stage of sheep milked in those months. Results by Abecia et al. (2017) showed that the relationship between milk yield and weather differed among the three phases of lactation (early, mid, late lactation). Somatic cell count have not a clear trend, keeping nearly constant, whereas and total bacterial count shows a higher inter annual variability in June and July (Figure 2).

Analysing the effect of THI classes within month of the year (Figure 3), milk traits show a similar behaviour considering the different THI (average and maximum, referred to -1d, -2d). Lactose experienced an evident reduction from May to July (5 in May; 4.7 in June; 4.3 in July), but the pattern in July is not as regular as the previous months, probably as a consequence of the reduced availability of data. Fat and protein percentages increase from May to July, passing from 6.5 to 8.1 and from 5.7 to 6.3 for fat and protein respectively.

Whereas in May fat and protein increase as THI rises, in June their percentages remains constant, and in July they experience a drop for higher values of THI. Slight different trends in milk composition are observed if, instead of average THI, the maximum THI is considered.

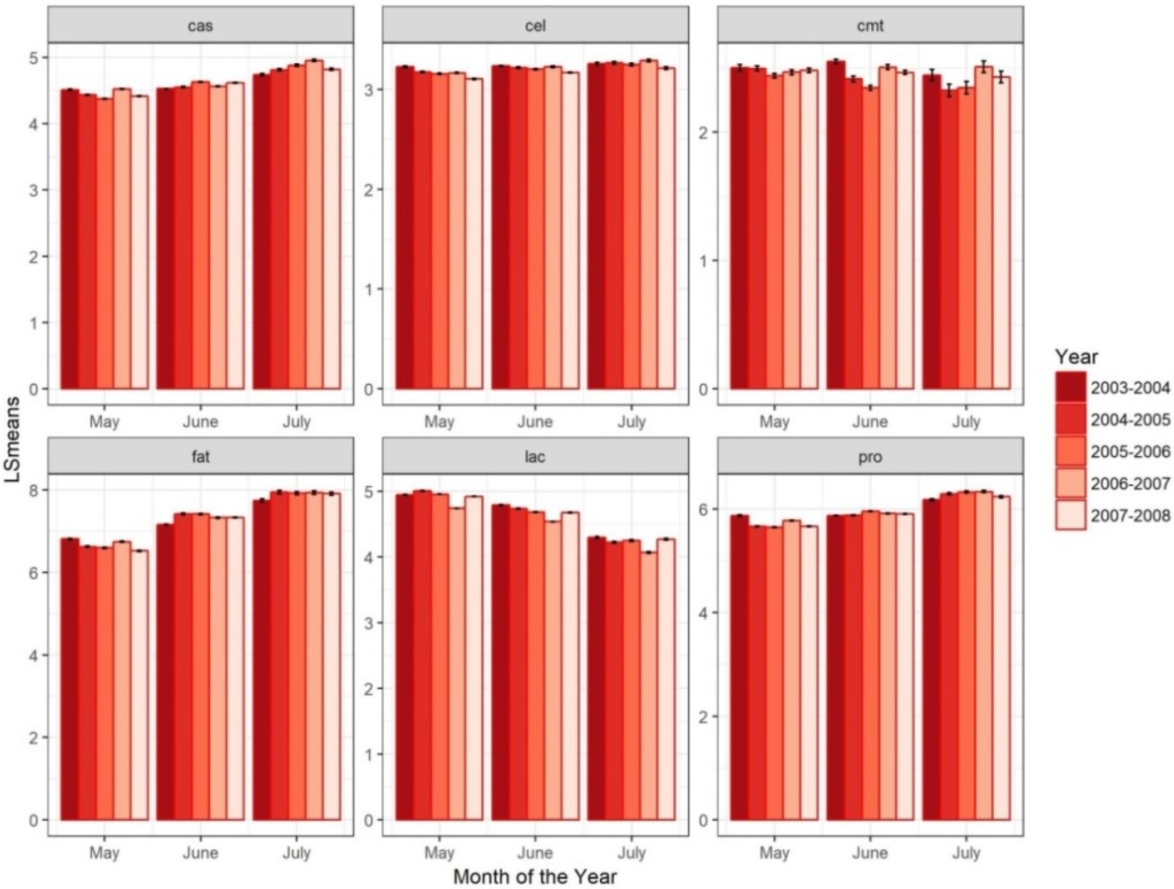

Figure 2. Effect of Month nested within year for the analysed trait

With regards to milk fat content in May, increases from +0.3% ($THI_{AVG\ -1-2d}$) to +0.4% ($THI_{MAX\ -2d}$) are observed for THI class spanning from 70 to >76. In June, no relevant differences are observed, keeping values almost constants. Whereas in July a decrease in fat percentage as THI values increase are found (-0.4% and -0.5% for $THI_{AVG\ -2d}$ and $THI_{MAX\ -2d}$ respectively). Same trend and magnitude of THI effect are registered for protein contents. Conversely, lactose on average decreases of about -0.2% passing from THI of 72 onward in May and June. Several studies exist underlying the effect of THI on milk traits, but effects vary within species and breed, but, as told before, only a few analysed the effect on sheep milk (Table 4). Furthermore, the systematic measurement of heat stress remains problematic, even if its quantification is needed to include it in breeding programs (Macciotta et al., 2017).

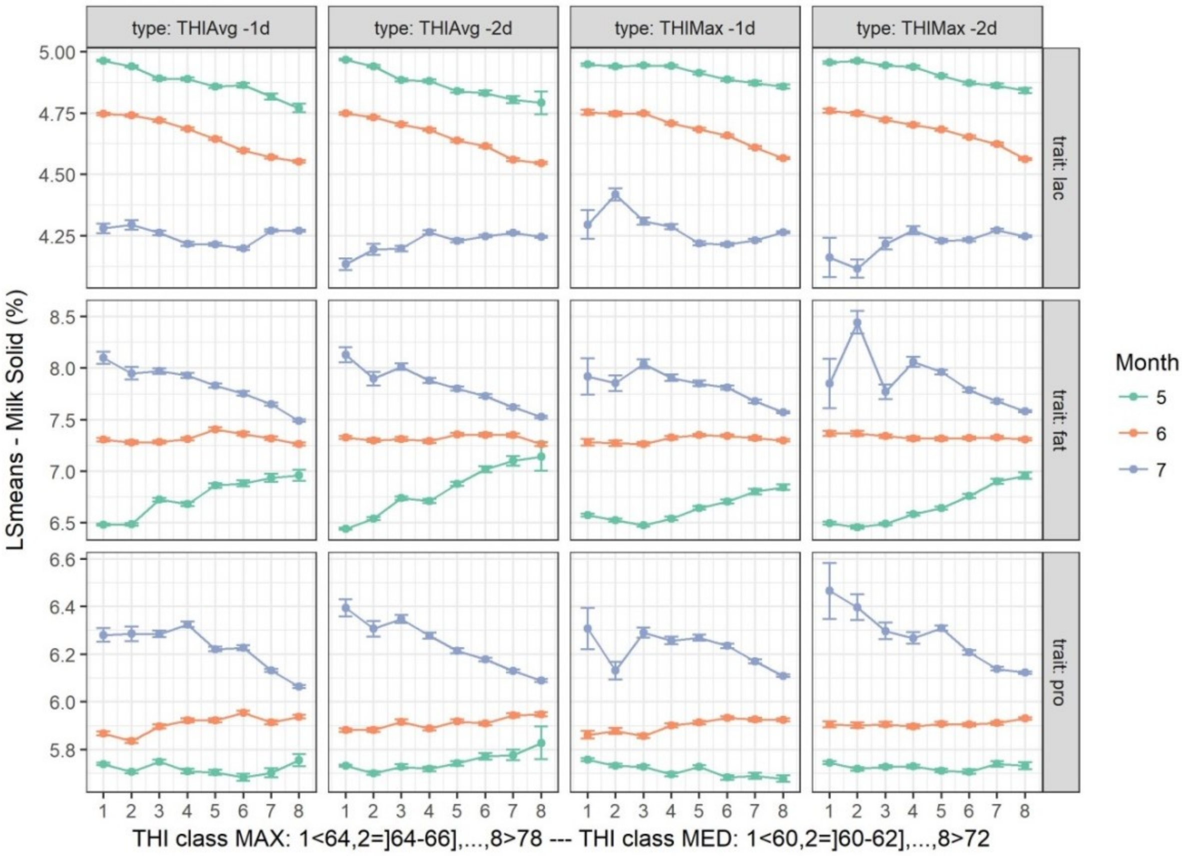

Figure 3. Effects of THI class nested with month of the year on milk fat composition (lactose, fat and protein %)

Table 4 Milk characteristics changes as a response to climatic variables

Milk characteristic	Behaviour of milk characteristic	Climatic parameter	Threshold/ range	Sheep Breed	References
Milk Yield	optimum	T_{min}	9-12°C	Sarda	PEANA ET AL., 2007A; 2007B; 2017
			-1 – 10.2°C	Manchega	RAMON ET AL., 2016
		T_{max}	15-24°C	Sarda	PEANA ET AL., 2017
			24-30°C		PEANA ET AL., 2007A
			19.1 – 29.6°C	Manchega	RAMON ET AL., 2016
		T_{avg}	12-18°C	Sarda	PEANA ET AL., 2017
			15-18 °C		PEANA ET AL., 2007A
			11.5-21 °C	MANCHEGA	RAMON ET AL., 2016
		THI_{max}	13.9 - 22		"
		THI_{avg}	60-65	Sarda	PEANA ET AL., 2007B
		THI_{avg}	10.3 - 18	Manchega	RAMON ET AL., 2016
		Wind speed $_{avg}$	1.5 – 4 m/s	Sarda	PEANA ET AL., 2017
		Wind speed $_{max}$	1.5 – 8 m/s		"
		RH	65-75%		PEANA ET AL., 2007A;
	Decrease	T_{min}	from 9-12 °C to 18-21 °C	Sarda	PEANA ET AL., 2007B
		T_{max}	≥21-24°C		"
			≥35°C	Comisana	SEVI ET AL., 2001
		T_{avg}	≥15-21 °C	Sarda	PEANA ET AL., 2007B;
		THI_{max}	≥68		PEANA ET AL., 2017
			≥23 ≈ 73	Valle del Belice	FINOCCHIARO ET AL., 2005
			≥72-75	Sarda	PEANA ET AL., 2007B
			≥80	Comisana	SEVI ET AL., 2001
		THI_{avg}	≥65	Sarda	PEANA ET AL., 2007B ; 2017
		Radiation	≥24MJ/m^2		PEANA ET AL., 2017
		Wind speed $_{avg}$	≥ 4m/s		"
		Effective rainfall	6 mm cumulative rain in two days		PEANA ET AL., 2007A
	Increase	Wind speed $_{avg}$	from 1.5-2.5 m/s to 2.5-4 m/s	Sarda	PEANA ET AL., 2007B
		RH $_{max}$	≥75-85%		"

		RH $_{avg}$	≥45-55%		
	Not affected/not clear	Radiation		Comisana	SEVI ET AL., 2001
		RH $_{min}$		Sarda	PEANA ET AL., 2017
		RH $_{avg}$			"
Fat + protein	Decrease	THI_{max}	≥23≈ 73	Valle del Belice	FINOCCHIARO ET AL., 2005
Fat (g/d)	Optimum	T_{min}	1.3 – 10.7 °C	Manchega	RAMON ET AL., 2016
		T_{avg}	10.2 – 19.5 °C		"
		T_{max}	19.3 –30.3 °C		"
		THI_{avg}	9.8 –19.3		"
		THI_{max}	11.7 – 20.4		"
	Not affected/not clear	THI_{max}		Valle del Belice	FINOCCHIARO ET AL., 2005
				Sarda	PEANA ET AL., 2007B
		Radiation		Comisana	SEVI ET AL., 2001
Protein (g/d)	Optimum	T_{min}	0.3 – 10.8°C	Manchega	RAMON ET AL., 2016
		T_{avg}	9.8 – 19.9 °C		"
		T_{max}	18.1 – 30.8 °C		"
		THI_{avg}	9.6 – 18.4		"
		THI_{max}	13.1 – 20.9		"
	Not affected/not clear	THI_{max}		Valle del Belice	FINOCCHIARO ET AL., 2005
				Sarda	PEANA ET AL., 2007B
		Radiation		Comisana	SEVI ET AL., 2001
SCC	Increase	T_{max}	from 21-24°C to 33-36°C	Sarda	PEANA ET AL., 2007B
	Not affected	Radiation		Comisana	SEVI ET AL., 2001

The interaction between flock size and THI (p-value <0.001) is analysed in Figure 4. Although a general significant effect of the number of animal per flock is observed, only slight differences among classes of flock dimension exist, especially for lactose. At animal level, environmental effects as herd size and management, justifies variances marginally higher in large herds compared with small herds (Brügemann et al., 2011). In our study, at farm level, higher fat, protein and lower lactose correspond to smaller flock size. Differences between in milk traits in different flock sizes can be ascribed to differences among farming systems (Pazzola et al., 2014). In fact in Sardinia, bigger flocks generally correspond to the use of modern facilities and feeding techniques, that allow a higher yield. Small flocks are generally kept by family operations and use traditional management and feeding techniques, rearing less selected animals (Pazzola et al., 2014). According to literature, we found that in some cases (protein and fat) THI exerted a slight different effect according to flock dimension class considered.

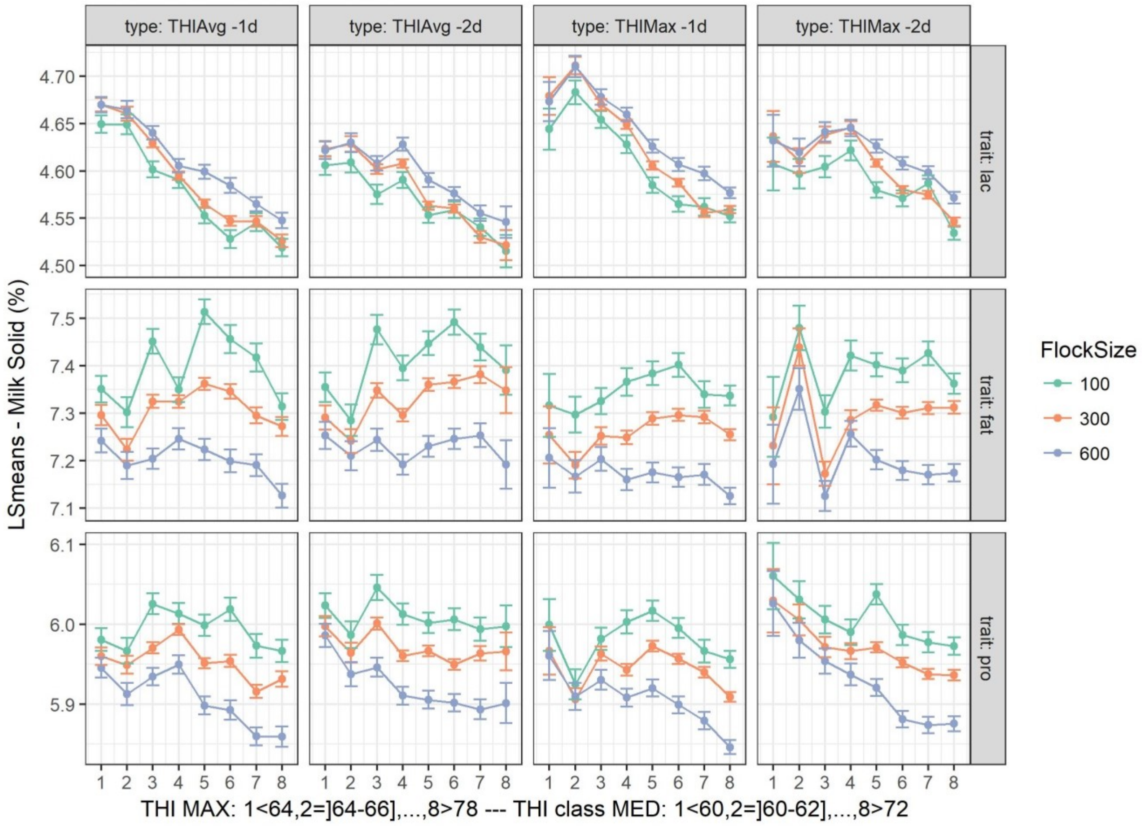

Figure 4. Least square mean of interaction THI*Flocksize form milk fat composition (lactose, fat and protein %)

Factor analysis on meteorological variables

The monthly averages of meteorological variables recorded one day before the milk samplings are reported in Table 5. Meteorological values are within ranges generally observed in Sardinia. Even if average values referred to two days before the milk sampling are available, they are not shown because slightly different from those reported in Table 5. Also wind speed and rainfall are provided.

Table 5. Mean and standard deviation (sd) of meteorological values the day of milk sampling and the day before from May to July.

	May		June		July	
Meteorological Variable	mean	sd	mean	sd	mean	sd
T_{AVG}, °C	16.1	3.4	20.6	4.0	23.8	3.1
T_{MAX}, °C	22.2	4.7	27.8	5.2	31.4	4.2
T_{MIN}, °C	10.1	3.6	13.7	4.1	16.6	3.5
RH_{AVG}, %	70.5	14.9	63.4	15.6	55.0	13.3

RH_{MAX}, %	91.1	10.4	88.2	13.0	83.9	13.2
RH_{MIN}, %	46.8	19.4	37.6	16.6	28.9	12.7
WS_{AVG}, m/s	3.1	1.9	2.7	1.3	3.1	1.9
WS_{MAX}, m/s	11.8	4.8	10.9	3.1	12.1	4.0
Rainfall, mm	2.1	6.0	0.6	2.7	0.2	1.7

Pairs of meteorological variables show correlations from moderate to high, with some exception (wind speed against all the others). In particular, variables clustered into different groups. Temperatures are negatively associated with relative humidity and rainfall. Moderate to high correlations were observed when the same variable is recorded the day before or 2 days before milk sampling (Table 6). All variables have the highest magnitude of correlation coefficients when coupling maximum and average values. Finally, partial correlations (below the diagonal) between the same pairs of variables experience a substantial drop in magnitude if compared with Pearson correlations (above the diagonal), often obtaining values close to zero. This is also confirmed by Kaiser MSA (MSA=0.71), that suggests possible latent variables explaining the actual correlation structure.

Table 6. Pearson correlation (above the diagonal) and partial correlation (below the diagonal) between pairs of meteorological variables recorded 1 or 2 days before milk sampling.

	T_{M1}	T_{M2}	T_{m1}	T_{m2}	T_{A1}	T_{A2}	RH_{M1}	RH_{M2}	RH_{A1}	RH_{A2}	RH_{m1}	RH_{m2}	WS_{A1}	WS_{A2}	WS_{M2}	WS_{M2}	R_{F1}	R_{F2}
T_{M1}		0.79	0.67	0.54	0.95	0.76	-0.42	-0.34	-0.70	-0.53	-0.71	-0.52	-0.22	-0.20	-0.09	-0.09	-0.34	-0.28
T_{M2}	0.33		0.67	0.66	0.81	0.95	-0.32	-0.41	-0.51	-0.70	-0.50	-0.70	-0.06	-0.20	0.05	-0.06	-0.21	-0.35
T_{m1}	-0.49	0.00		0.81	0.84	0.79	-0.47	-0.41	-0.44	-0.44	-0.30	-0.35	0.05	-0.07	0.09	-0.03	-0.09	-0.18
T_{m1}	0.02	-0.58	0.27		0.69	0.84	-0.34	-0.47	-0.34	-0.45	-0.24	-0.30	0.04	0.06	0.09	0.10	-0.08	-0.11
T_{A1}	0.87	-0.21	0.74	-0.14		0.84	-0.49	-0.40	-0.67	-0.54	-0.61	-0.50	-0.13	-0.17	-0.03	-0.08	-0.29	-0.27
T_{A1}	-0.22	0.88	-0.08	0.80	0.26		-0.36	-0.48	-0.50	-0.68	-0.45	-0.60	-0.03	-0.12	0.06	-0.01	-0.18	-0.30
RH_{M1}	-0.05	-0.01	-0.26	0.06	0.16	-0.03		0.55	0.75	0.47	0.43	0.29	-0.17	-0.06	-0.14	-0.06	0.19	0.14
RH_{M2}	-0.02	-0.12	0.04	-0.28	-0.03	0.22	0.23		0.47	0.75	0.29	0.42	-0.11	-0.18	-0.11	-0.15	0.12	0.19
RH_{m1}	0.21	-0.06	0.28	-0.09	-0.36	0.15	0.76	-0.09		0.64	0.87	0.58	-0.03	0.03	-0.05	0.00	0.39	0.25
RH_{m2}	-0.09	0.31	-0.05	0.36	0.15	-0.45	-0.10	0.76	0.21		0.56	0.86	-0.05	-0.05	-0.07	-0.07	0.18	0.39
RH_{a1}	-0.43	0.20	-0.10	0.00	0.33	-0.14	-0.47	-0.02	0.77	-0.14		0.63	0.12	0.12	0.08	0.08	0.45	0.27
RH_{a2}	0.23	-0.48	-0.05	-0.16	-0.12	0.37	-0.03	-0.48	-0.08	0.77	0.28		0.06	0.09	0.02	0.04	0.19	0.44
WS_{a1}	-0.21	0.15	0.04	0.01	0.09	-0.08	-0.08	0.06	-0.01	-0.05	-0.06	0.10		0.48	0.77	0.31	0.07	0.09
WS_{a2}	0.15	-0.16	0.03	0.07	-0.10	0.03	0.07	-0.03	-0.03	-0.06	0.06	-0.03	0.50		0.33	0.73	0.13	0.09
WS_{M1}	0.09	0.00	0.10	-0.07	-0.11	0.05	0.12	-0.04	-0.12	0.04	0.09	0.02	0.74	-0.32		0.41	0.18	0.12
WS_{M2}	-0.01	0.07	-0.09	0.10	0.02	-0.05	-0.06	0.04	0.01	-0.02	0.03	0.01	-0.35	0.70	0.43		0.17	0.18
R_{F1}	0.08	-0.05	0.13	0.00	-0.10	0.02	-0.04	0.09	0.02	-0.05	0.18	-0.07	-0.20	0.06	0.23	-0.01		0.19
R_{F2}	-0.04	0.05	-0.03	0.12	0.03	-0.09	0.03	-0.01	-0.02	0.01	-0.04	0.14	0.04	-0.15	0.01	0.19	0.10	

$T_{M1(2)}$,$T_{m1(2)}$ and $T_{a1(2)}$= Maximum(M), minimum(m) and average (a) temperature (°C) 1 or 2 (days) before milk sampling; RH=Relative Humidity (%); WS=wind Speed (m/s); RF=rainfall (mm).

The factor analysis highlighted two factors able to explain about 70% of the variability. Both of them were correlated with some of the original variables defining a simple pattern, as reported in Table 7. In particular, the first common factor, explaining half of the original variance, is positively correlated with temperature (corresponding higher coefficients to average and lower to minimum temperature), negatively correlated with rainfall and relative humidity, and it is not associated with wind speed. It is to underline that the impact of humidity on the thermal balance of the animal is species specific and varies from one head to another (Berman et al., 2016). In spite of it, in our climate, the effect of humidity is lower than how exerted in humid climate, because the hottest temperature generally corresponds to the lowest relative humidity, without being impeded evaporative heat losses. In this situation, the low relative humidity corresponds to decreases the thermal perception, as shown also by the formula of THI max, that combines maximum daily temperature and minimum relative humidity (Ramon et al., 2016).

Table 7. Factorial scheme. Loading coefficients for *varimax* rotation

Variable	Factor 1	Factor2	Communality
T_{AVG-1d}	0.90	-0.16	0.80
T_{AVG-2d}	0.89	-0.01	0.76
T_{MAX-2d}	0.87	-0.10	0.56
T_{MAX-1d}	0.86	-0.25	0.49
T_{MIN-1d}	0.75	0.05	0.84
T_{MIN-2d}	0.68	0.14	0.80
$R_{fall-1d}$	-0.29	0.22	0.36
$R_{fall-2d}$	-0.35	0.17	0.41
RH_{MAX-1d}	-0.57	-0.18	0.59
RH_{MAX-2d}	-0.59	-0.26	0.64
RH_{MIN-1d}	-0.68	0.19	0.49
RH_{MIN-2d}	-0.70	0.08	0.49
RH_{AVG-1d}	-0.77	0.01	0.53
RH_{AVG-2d}	-0.79	-0.11	0.51
WS_{AVG-1d}	0.00	0.73	0.48
WS_{AVG-2d}	-0.07	0.71	0.43
WS_{MAX-1d}	0.06	0.69	0.13
WS_{MAX-2d}	0.00	0.66	0.15

Var Explained %	51.9	17.4

The first factor (F1) can be described as a THI-like index. The second factor has moderate to high correlations with wind speed and negligible coefficients with all the other variables. In particular, the second factor will be neglected, since the low percentage of variance explained (see also the low communality) and association only with wind speed.

Heat stress is produced by a combination of several factors including temperature, relative humidity, solar radiation, air movement, and precipitation. Our study aimed at characterizing a thermal stress index able to integrate at the same time all meteorological data known into a single measure. The use of multivariate approaches allows it, synthesizing complex correlations without imposing specific constraints in the use of data, extracting new variables able to better describe the behaviour. On the contrary, the use of THI (maximum, average or minimum) requires the selection of data used (e.g. period of heat strain, temperatures and humidity to be used, etc.). The use of multivariate approaches has been recently applied to estimate or classify heat tolerance genotypes in dairy and beef cattle respectively (Macciotta et al., 2017; McManus et al., 2011). In our study quality milk data are merged with public weather information to develop, by means of multivariate techniques, a representative factor (F1) that reflects and assesses the potential impact of heat stress. This approach allows to neglect the determination of the most appropriate THI function, because simultaneously uses all available information. As expected, a strong relationship between F1 and THI values are observed for all kind of THI considered (Figure 5). Regardless the days of recording, the highest correlations were found with THI_{AVG} (Table 8).

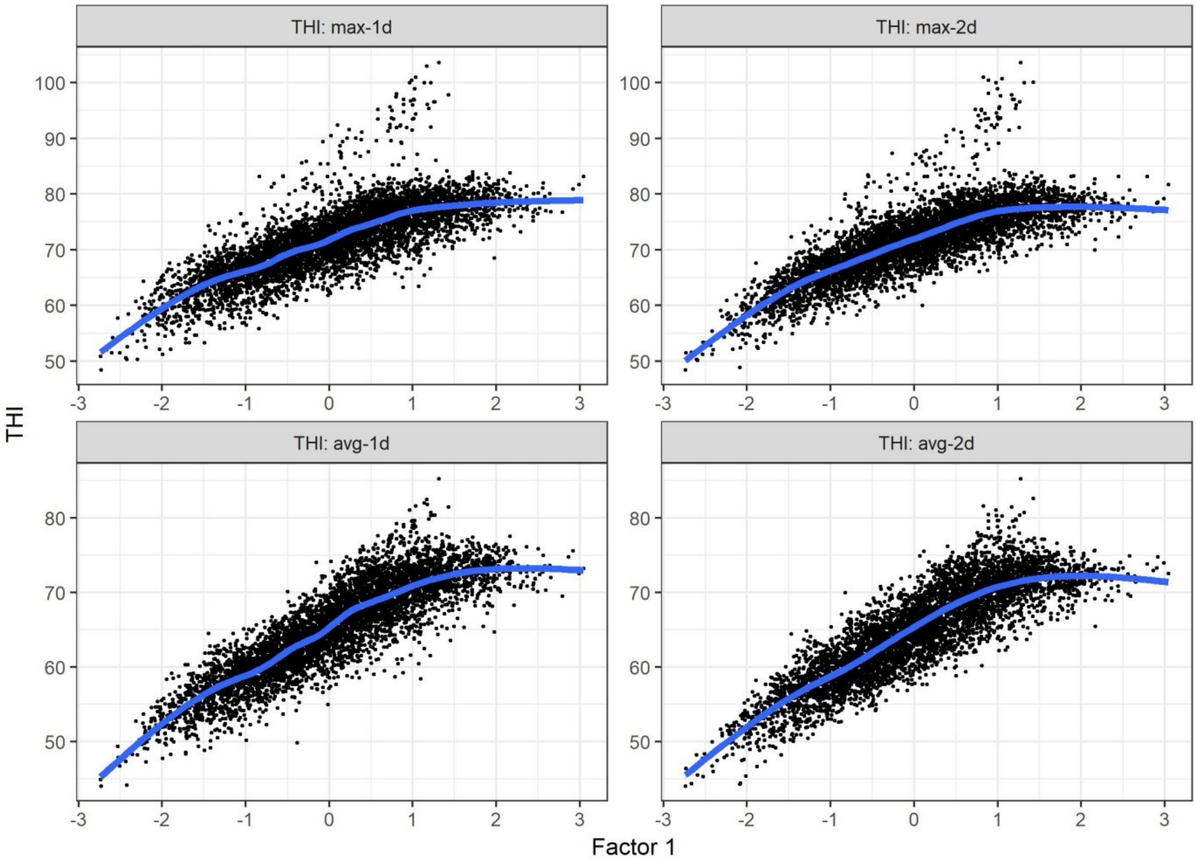

Figure 5. Relationship between Factor 1 and THI values (Max and Average) recorded 1 or 2 days before milk sampling

Table 8. Spearman Rank correlation among Factor 1 classes and THI values.

Classes[1]	THI_{MAX-1d}	THI_{MAX-2d}	THI_{AVG-1d}	THI_{AVG-2d}
Factor 1	0.75	0.75	0.81	0.80
THI_{MAX-1d}		0.79	0.93	0.77
THI_{MAX-2d}			0.81	0.94
THI_{MED-1d}				0.84

[1] THI_{MAX}: (1 < 64, 2 = 64-66, … , 8 > 76);THI_{AVG}: (1 < 60; 2 = 64-66; … ; 8 > 76); Factor 1 (6 classes <-2, -2-1, -1-0, 0-1, 1-2, >2).

The same linear mixed model applied for modelling THI was used to assess the effect of latent factor classes identified with multivariate analysis. At increasing values of F1, fat, protein and lactose show the same trend previously observed when analysing THI classes. All the effect analysed are highly significant (p-value <0.001). In particular, least square means for milk traits in different F1 classes are reported in figure 6. With regards to milk composition in May, changes of +0.6%, +0.1% and –0.3% are observed passing from F1 class 3 to class 6 in fat, protein and lactose, respectively. In June, no relevant differences are observed, as previously told. Conversely, in July a decrease in fat (-0.6%), protein (-0.3%) are found. Opposite trend is observed for lactose with average decreases of about -0.2% (-0.1%) passing from F1 class 3 onward in June and July respectively. Although most of least square means contrasts are significant, for some classes of F1 have large standard errors (Figure 6). Considering the effect of F1 on milk quality traits, similar behaviour to THI is obtained. It reflects the large weight of temperature in the THI, whose effect is predominant (0.82–0.95 of variance depending on the formula used) in the index (Berman et al., 2016).

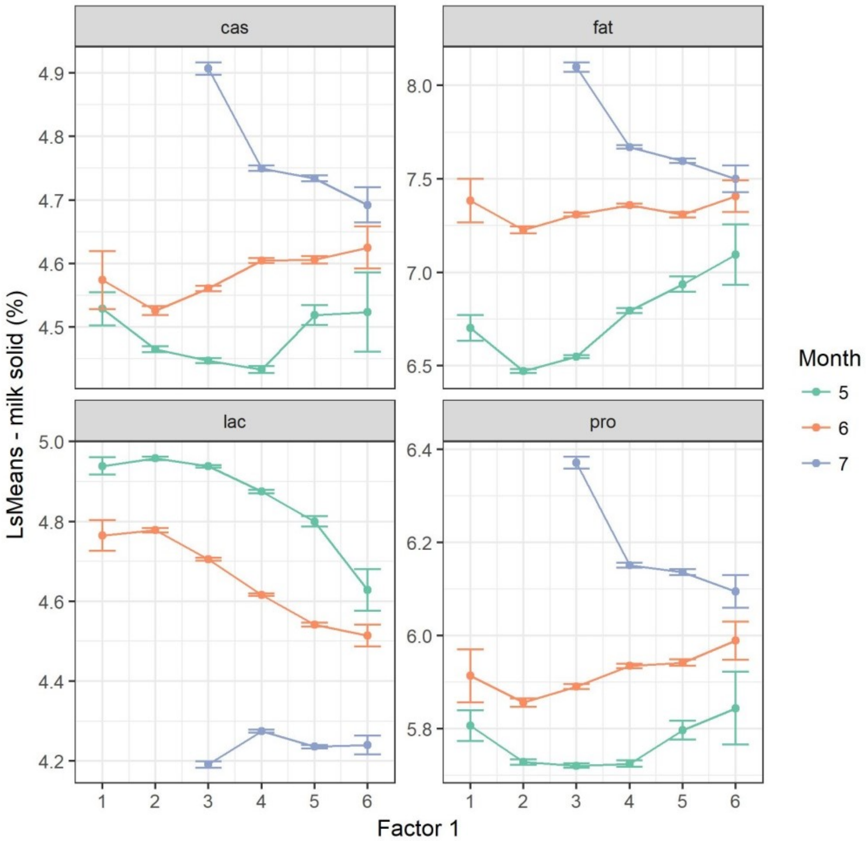

Figure 6. Effect of Factor1(Month) on milk composition traits (fat%, casein%, lactose%, protein%)

Conclusions

Climate and meteorological conditions are elements of great importance on milk production. The effects of heat stress can impair productivity of dairy ruminants in Mediterranean environment but the most of literature refers to dairy cattle, and only a few works exist on sheep and goats. Furthermore, most of studies focus on the effects of heat stress on milk yield, using individual milk production. Our work utilizes bulk milk composition and studies its changes occurring in late lactation, as related to meteorological data. Deeper analyses were hampered by the lack of information as milk yield per ewe, that partially prevented comparisons with results in literature. Due to this deficiency, we utilized changes in lactose percentage as an indirect measure of milk yield. In addition, the final phase of lactation and weather act in the same direction, making difficult to distinguish the magnitude exerted by each of them on milk quality traits, as previously observed by other authors (Finocchiaro et al., 2005; Ramon et al., 2016; Carabaño et al., 2016). Conversely to what observed in dairy cattle, dairy sheep farming is characterized by high seasonality of lambing. This limits the possibility to distinguish the effect of stage of lactation and hot weather. It was not possible to completely separate the two effects, also because of the lack of several information, as lambing period and phases of lactation (Abecia et al., 2017), since our data were referred to bulk milk. In statistical models, tolerance to heat stress and its effects on dairy traits might be fitted by means of test day repeatability model (Finocchiaro et al., 2005), reaction norm model (that expresses the phenotype as a linear function of an environmental variable), and the use of principal component analysis, to develop measures of tolerance to heat stress (Macciotta et al., 2017). The objectives of these studies were to measure tolerance to heat stress in a systematic way. Genetic variance components are estimated disentangling main features of trait deviations, to consider heat tolerance in breeding programs. In our work, following the approach proposed by Ravagnolo et al. (2000), climatological and meteorological information were provided by the nearest meteorological stations, whose daily weather records were assigned to the nearest farms. But, as pointed by the same Ravagnolo et al. (2000) and Gomes Da Silva (2006), sometimes the stations are not representative of farm conditions or do not adequately report temperature and humidity measures needed to calculate THI index values. We thought to carry on a more detailed study on how thermal effect of heat on animals vary depending on various climate variables as temperature, relative humidity, wind speed and rainfall. The use of multivariate techniques allowed to merge milk quality data with public weather information, developing the representative factor (F1). This factor reflects and assesses the potential impact of heat stress and it contemporary uses all available information. Temperature positively contributes to F1, while humidity is negatively correlated: this behaviour reflects the effect of humidity in decreasing the thermal perception at our climate.

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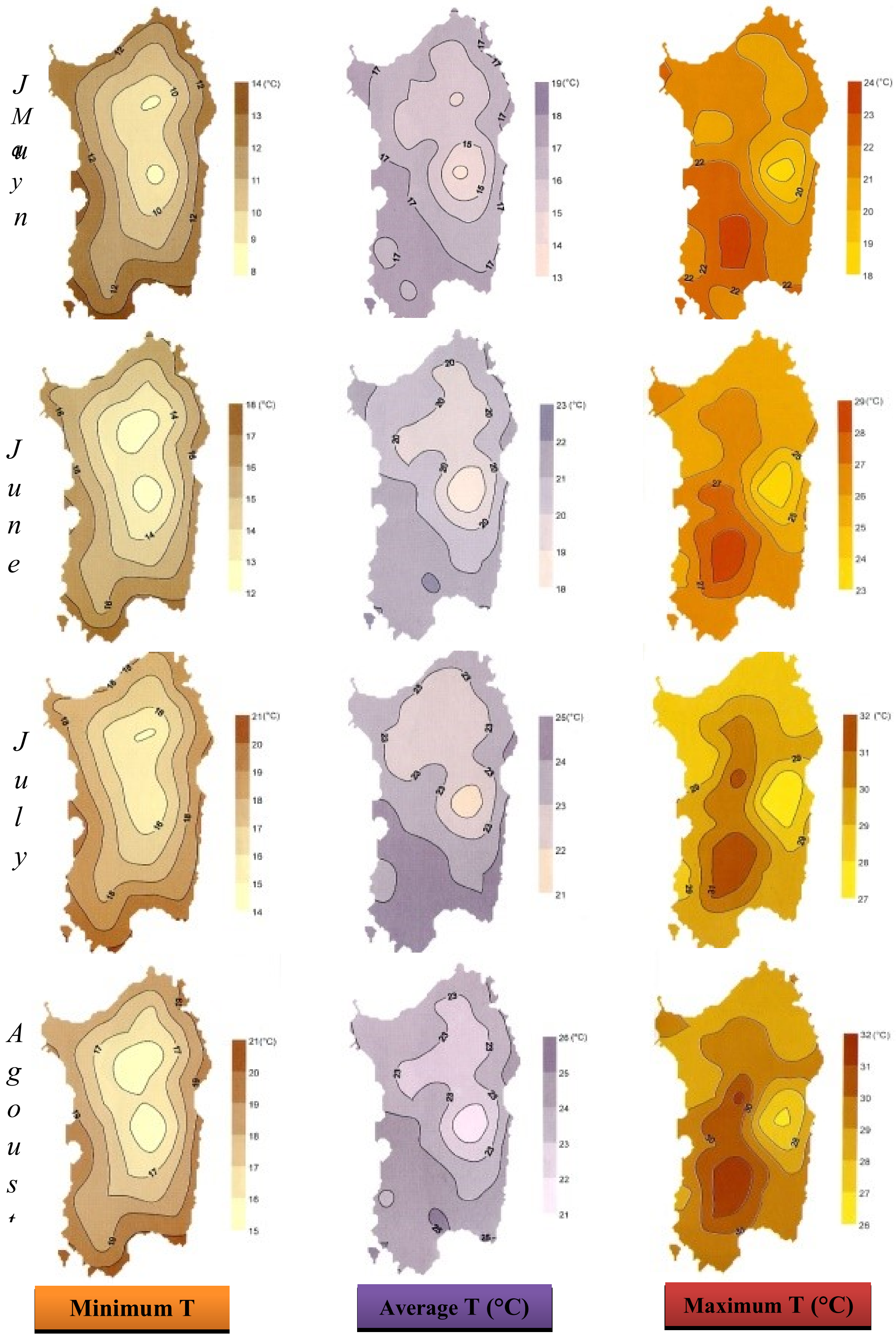
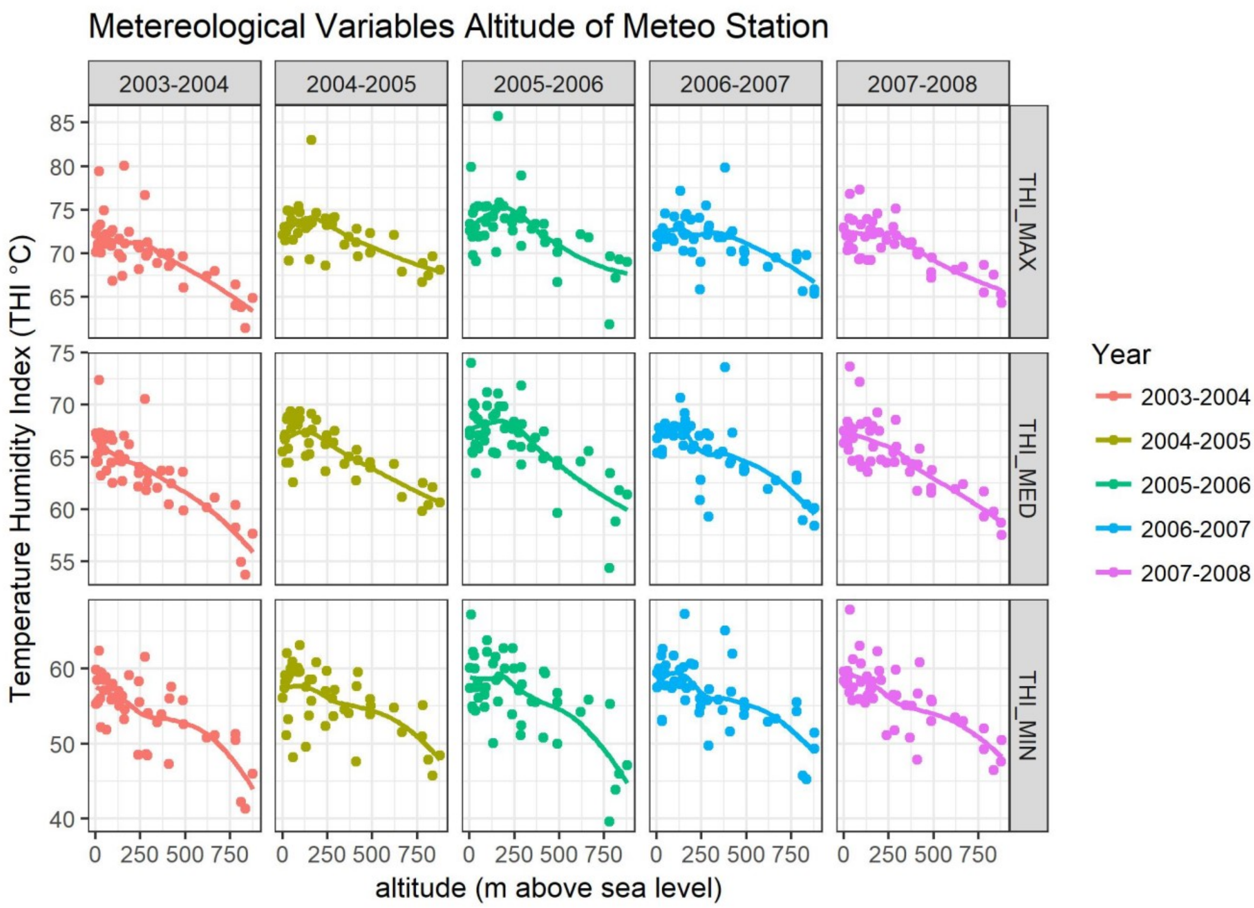
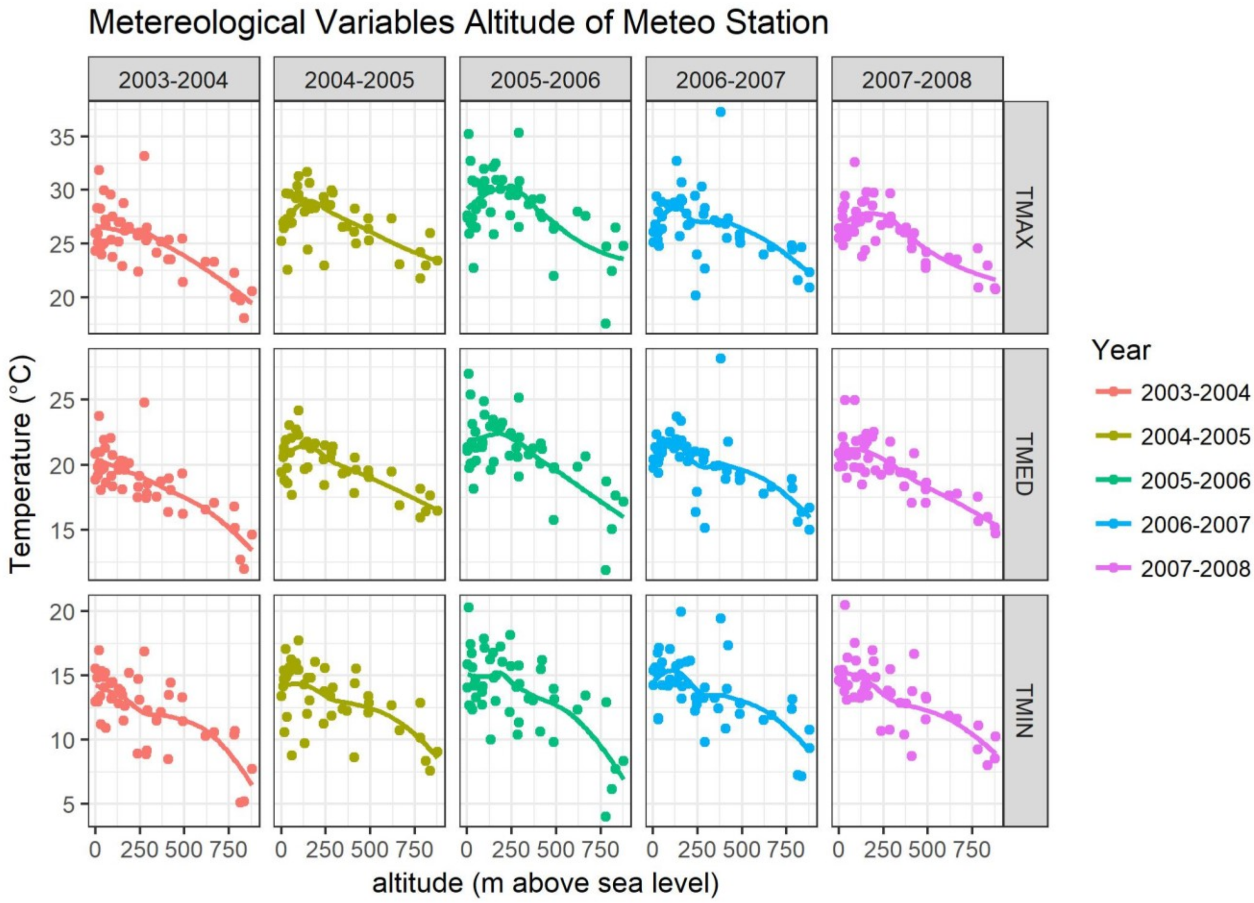
Appendix: supplemental material



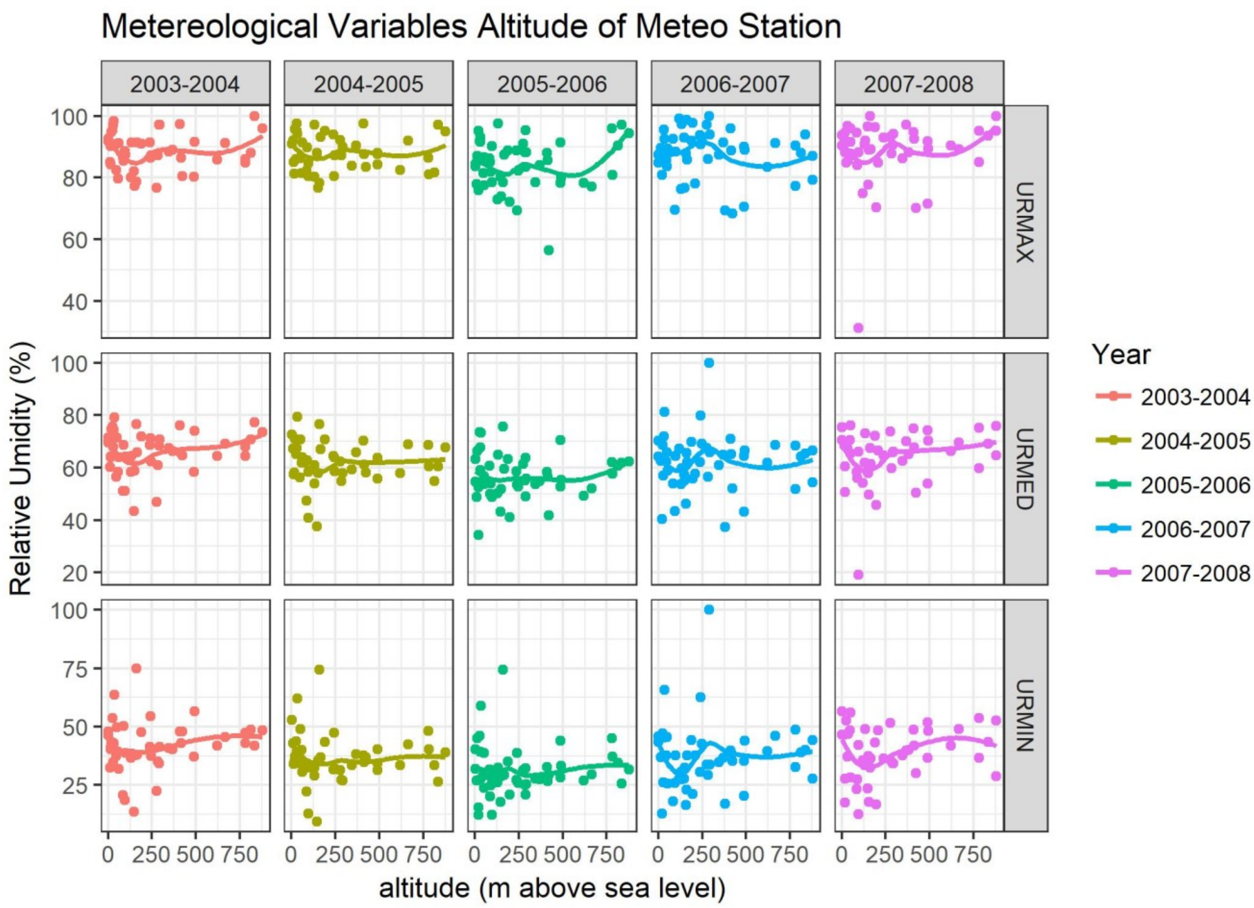
Supplemental Figure 1 Historical temperature elaborated by ARPAS which publish climatic map of Sardinia Island based on historical data (http://www.sar.sardegna.it/)



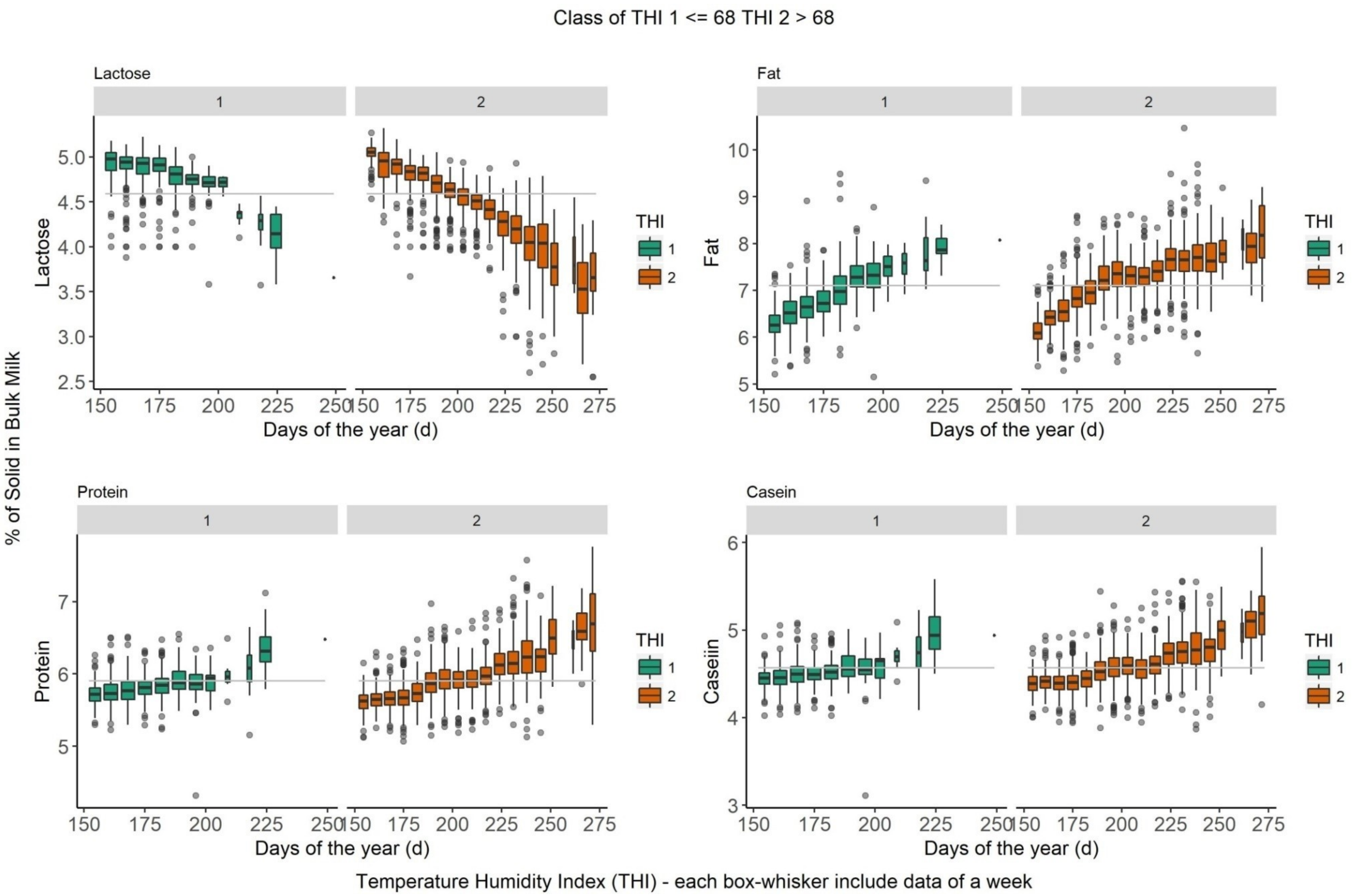
Supplemental Figure 2 Changes in THI depending on altitude and year



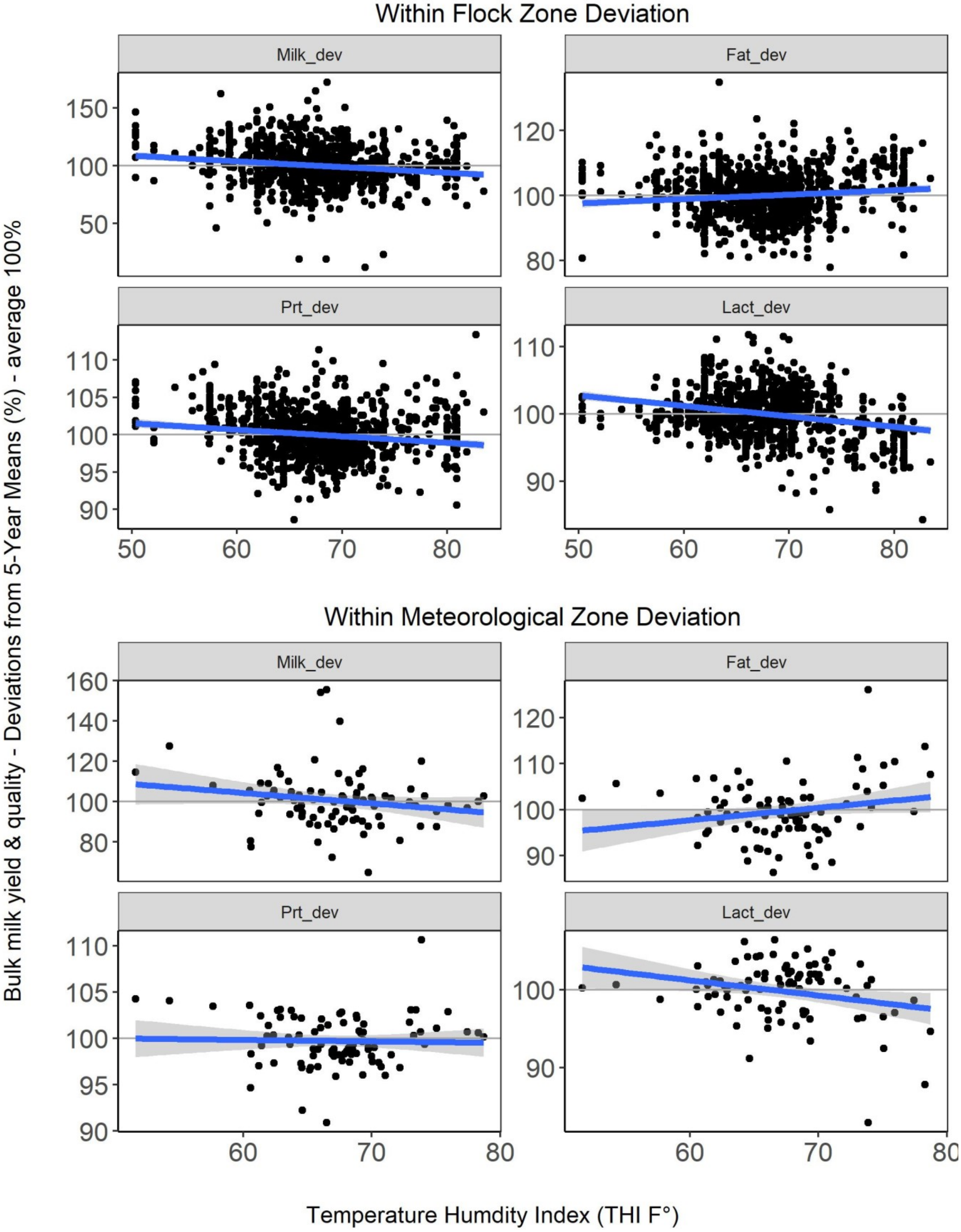
Supplemental Figure 3 Changes in maximum temperature depending on altitude and year



Supplemental Figure 4 Changes in relative humidity depending on altitude and year



Supplemental Figure 5 Changes in milk characteristics and heat stress classes



Supplemental Figure 6 Milk and composition as function of THI

Supplemental Table 1. Description of weather data on the test day and three days before

	N	Mean	Std	CV	Min	Max
T mean, °C	61,491	19.77	4.68	0.24	6.14	32.20
t-1	60,340	19.73	4.70	0.24	6.14	32.20
t-2	59,802	19.53	4.65	0.24	5.84	32.20
t-3	59,952	19.51	4.63	0.24	6.40	32.20
T max, °C	61,383	26.68	6.02	0.23	8.00	44.30
t-1	60,295	26.73	6.09	0.23	8.00	44.30
t-2	59,813	26.53	5.95	0.22	8.50	44.30
t-3	59,929	26.51	5.89	0.22	9.20	44.30
T min, °C	61,399	13.00	4.49	0.35	-2.00	26.60
t-1	60,237	12.90	4.51	0.35	-2.00	26.20
t-2	59,770	12.68	4.46	0.35	-0.60	26.60
t-3	59,885	12.64	4.45	0.35	-0.60	26.60
RH med, %	61,017	37.58	18.23	0.49	7.00	100.00
t-1	59,990	37.71	18.56	0.49	6.00	100.00
t-2	59,448	38.47	18.39	0.48	6.00	100.00
t-3	59,563	38.43	18.20	0.47	6.00	100.00
RH max, %	61,491	63.12	16.44	0.26	11.00	100.00
t-1	60,403	63.38	16.24	0.26	11.00	100.00
t-2	59,737	64.22	16.06	0.25	11.00	100.00
t-3	59,881	64.25	15.95	0.25	11.00	100.00
RH min, %	61,273	88.04	13.46	0.15	13.00	100.00
t-1	60,186	88.52	12.56	0.14	13.00	100.00
t-2	59,592	88.97	12.06	0.14	13.00	100.00
t-3	59,702	89.04	12.03	0.14	13.00	100.00
Effective rainfall, mm/day	59,719	1.00	4.23	4.23	0.00	53.20
t-1	58,899	1.03	4.16	4.04	0.00	76.60
t-2	58,646	1.07	4.03	3.77	0.00	76.60
t-3	58,666	1.01	4.01	3.97	0.00	76.60
Solar radiation, MJ/m^2	26,717	21.16	5.61	0.27	1.00	31.00
t-1	26,329	21.05	5.61	0.27	1.00	31.00
t-2	26,143	21.01	5.57	0.27	0.00	31.00
t-3	26,200	21.07	5.38	0.26	0.00	31.00
Wind Speed mean, m/s	56,936	3.07	1.87	0.61	0.00	14.40
t-1	56,062	2.96	1.73	0.58	0.00	18.48
t-2	55,832	2.88	1.66	0.58	0.00	18.48
t-3	55,952	2.85	1.63	0.57	0.00	18.48
Wind Speed max, m/s	56,489	11.86	4.24	0.36	0.00	48.15
t-1	55,663	11.61	4.18	0.36	0.00	48.15
t-2	55,435	11.43	3.97	0.35	0.00	48.15
t-3	55,557	11.36	4.00	0.35	0.00	48.15

Supplemental Table 2

Minimum, Average and Maximum Relative Humidity (%) for each Meteorological Station

Meteo Station		RHmin					RHavg					RHmax				
Location (Comune)	**Quote (m)**	**2003-2004**	**2004-2005**	**2005-2006**	**2006-2007**	**2007-2008**	**2003-2004**	**2004-2005**	**2005-2006**	**2006-2007**	**2007-2008**	**2003-2004**	**2004-2005**	**2005-2006**	**2006-2007**	**2007-2008**
MURAVERA	**2**	46.9	-	32.0	43.3	46.7	69.4	-	54.6	64.6	70.5	91.2	-	83.7	85.6	93.8
		12.8	-	*10.8*	*16.3*	*13.3*	*8.5*	-	*9.5*	*16.4*	*9.5*	*5.6*	-	*8.7*	*12.2*	*7.3*
VALLEDORIA	**2**	46.8	52.7	40.2	46.0	56.5	71.8	72.7	63.3	70.4	75.4	92.7	91.1	84.4	87.5	90.4
		15.5	*9.4*	*11.4*	*14.3*	*9.1*	*8.6*	*5.9*	*11.8*	*7.9*	*8.3*	*5.8*	*4.2*	*8.8*	*6.1*	*8.9*
SINISCOLA	**14**	40.7	34.0	45.0	36.8	46.5	64.4	57.5	66.4	62.7	68.5	90.5	85.0	87.0	89.5	91.9
		14.1	*8.6*	*17.7*	*7.2*	*14.4*	*11.4*	*10.6*	*15.3*	*10.8*	*12.7*	*7.7*	*13.3*	*10.9*	*14.0*	*9.7*
ARBOREA	**20**	36.9	37.1	-	-	-	61.1	71.0	-	-	-	83.7	95.8	-	-	-
		5.8	*11.8*	-	-	-	*10.9*	*6.7*	-	-	-	*8.9*	*2.9*	-	-	-
PALMAS ARBOREA	**20**	-	-	12.3	-	27.7	-	-	34.7	-	60.4	-	-	76.3	-	94.7
		-	-	*2.8*	-	*13.0*	-	-	*12.5*	-	*13.6*	-	-	*21.4*	-	*8.1*
UTA	**20**	-	-	17.1	12.8	17.2	-	-	53.4	40.3	49.8	-	-	95.2	80.9	87.9
		-	-	*7.0*	*4.3*	*8.0*	-	-	*11.1*	*10.2*	*12.3*	-	-	*8.7*	*18.5*	*11.4*
OROSEI	**26**	53.7	43.6	46.1	47.5	50.8	75.6	65.2	73.6	69.1	67.3	95.3	86.0	91.9	86.1	83.2
		12.6	*12.5*	*12.7*	*15.1*	*22.8*	*8.5*	*12.1*	*8.0*	*14.3*	*17.7*	*4.5*	*11.7*	*6.5*	*13.3*	*13.0*
DECIMOMANNU	**29**	34.9	34.1	27.5	26.1	-	67.9	67.5	59.1	57.0	-	97.2	95.2	91.5	87.3	-
		11.3	*10.5*	*9.0*	*4.8*	-	*8.9*	*7.9*	*7.2*	*6.6*	-	*2.8*	*4.8*	*5.8*	*4.7*	-
OLMEDO	**31**	43.0	42.1	39.0	45.3	37.2	74.5	71.4	67.5	72.0	67.2	98.4	97.1	93.1	95.6	96.7
		15.6	*13.6*	*11.4*	*13.0*	*14.5*	*10.1*	*8.2*	*7.7*	*9.7*	*12.4*	*3.4*	*3.8*	*4.7*	*4.3*	*6.9*
STINTINO	**35**	65.4	62.0	59.0	65.6	51.0	80.2	79.3	73.4	81.3	71.5	91.0	94.1	86.3	92.7	87.6
		15.7	*11.9*	*14.5*	*10.8*	*13.2*	*8.0*	*7.9*	*10.3*	*8.7*	*9.2*	*4.0*	*4.8*	*5.5*	*6.5*	*8.6*
JERZU	**47**	41.1	34.2	29.1	37.8	43.6	64.5	56.3	54.0	62.3	68.5	88.9	86.3	81.5	85.5	87.7
		13.3	*14.6*	*7.1*	*15.5*	*14.3*	*15.1*	*15.2*	*9.5*	*16.9*	*8.9*	*9.4*	*9.3*	*11.3*	*9.5*	*1.3*
SORSO	**50**	49.7	48.8	38.8	45.6	55.5	71.4	70.5	56.9	65.7	75.0	91.0	86.4	77.4	83.7	90.2
		14.6	*7.9*	*12.5*	*14.6*	*17.5*	*12.5*	*8.3*	*14.3*	*12.8*	*12.5*	*7.7*	*8.5*	*14.1*	*11.9*	*9.5*
DORGALI FILITTA	**85**	20.5	22.1	20.1	17.9	23.2	51.2	47.3	49.8	53.9	58.4	86.6	82.3	85.0	92.5	94.5
		11.9	*17.0*	*7.6*	*6.0*	*14.2*	*16.3*	*17.0*	*15.7*	*11.2*	*18.8*	*11.8*	*15.2*	*18.9*	*10.2*	*6.1*
SAMASSI	**89**	50.3	34.3	23.9	-	27.5	68.7	62.8	52.4	-	56.2	85.5	89.7	80.1	-	84.4
		20.8	*14.3*	*8.8*	-	*6.4*	*12.9*	*12.5*	*9.7*	-	*8.1*	*6.7*	*7.8*	*9.2*	-	*5.0*
MONASTIR MOBILE	**96**	18.4	14.1	12.1	25.9	12.5	51.0	42.9	49.2	43.3	19.1	88.6	81.1	90.7	69.6	31.3
		7.0	*4.3*	*0.3*	*24.8*	*0.8*	*13.7*	*9.5*	*7.6*	*26.6*	*4.8*	*6.3*	*7.5*	*5.0*	*19.8*	*8.0*
MILIS	**97**	38.6	32.3	27.0	37.7	42.2	64.3	58.3	47.8	64.5	66.2	87.5	84.7	76.3	88.4	88.2
		15.4	*7.9*	*10.4*	*13.5*	*21.0*	*11.8*	*8.0*	*11.7*	*12.3*	*16.1*	*4.5*	*6.9*	*12.0*	*7.9*	*10.6*
ARZACHENAMOBIL	**120**	-	-	-	27.8	34.2	-	-	-	67.2	56.0	-	-	-	99.2	78.8
		-	-	-	*11.4*	*18.2*	-	-	-	*9.8*	*27.0*	-	-	-	*3.5*	*30.0*
VILLACIDRO	**130**	35.7	29.0	26.2	30.9	34.2	57.8	54.0	50.3	53.8	61.8	79.9	80.3	73.0	76.2	90.7
		13.2	*8.2*	*9.6*	*9.4*	*10.1*	*11.6*	*6.8*	*8.5*	*8.3*	*8.9*	*9.1*	*6.5*	*7.9*	*6.8*	*7.3*
OLIENA	**132**	38.4	32.4	32.3	27.3	49.0	64.3	60.9	64.8	59.9	73.1	91.4	96.3	97.6	97.3	92.0
		17.2	*6.5*	*4.7*	*7.7*	*18.6*	*17.5*	*6.8*	*4.6*	*7.5*	*19.3*	*7.4*	*5.2*	*2.7*	*7.0*	*10.5*

GONNOSFANADIGA	**146**	13.4	9.4	17.9	27.7	23.4	43.0	35.7	44.3	57.4	57.7	82.0	83.5	81.9	92.9	96.8
		6.3	2.2	4.1	15.6	10.0	15.1	9.5	10.6	18.3	12.1	21.5	14.5	15.4	12.5	5.5
SASSARI S.A.R.	**150**	37.7	35.6	28.5	32.5	37.6	58.9	58.0	51.7	55.3	58.1	77.3	76.8	74.1	76.9	77.4
		13.6	8.7	7.5	8.9	13.2	10.9	8.7	9.5	8.7	11.9	6.8	7.3	8.6	6.5	9.1
DORGALI MOBILE	**156**	-	-	-	16.5	17.7	-	-	-	46.3	49.7	-	-	-	87.9	89.0
		-	-	-	2.0	9.8	-	-	-	22.6	22.0	-	-	-	6.3	21.5
OTTANA	**160**	74.8	74.5	74.3	22.8	32.3	76.7	76.5	75.8	65.6	67.4	78.6	78.4	78.5	98.7	99.9
		1.1	0.8	1.1	13.3	27.2	0.9	0.6	0.8	12.1	20.7	0.6	0.6	1.9	3.7	0.6
DOLIANOVA	**165**	37.9	36.9	29.3	37.7	36.2	65.9	67.1	59.6	62.2	61.4	91.4	93.1	87.4	85.9	89.1
		12.3	12.3	5.4	15.2	16.5	14.3	8.8	6.4	13.7	15.4	10.8	5.4	8.4	10.3	10.8
SARDARA	**189**	47.6	42.9	35.3	44.1	43.3	71.9	70.2	63.5	69.8	72.1	91.0	94.5	88.9	91.9	96.3
		18.9	14.1	11.1	15.8	15.7	12.6	11.1	12.1	14.2	14.0	7.0	7.5	9.6	9.3	7.5
USINI MOBILE	**197**	-	-	20.9	21.2	16.6	-	-	41.1	58.7	45.7	-	-	72.1	98.1	70.3
		-	-	9.2	11.2	11.9	-	-	19.0	13.8	26.1	-	-	21.9	5.5	30.8
IGLESIAS	**208**	-	-	-	37.6	48.3	-	-	-	57.6	66.5	-	-	-	78.1	84.9
		-	-	-	16.6	22.1	-	-	-	15.5	18.2	-	-	-	12.5	9.1
OZIERI	**238**	44.2	31.6	27.1	31.3	36.4	70.3	64.3	58.3	62.8	64.6	91.9	94.0	88.6	94.1	92.9
		20.0	12.5	10.9	11.2	15.4	12.5	10.2	10.6	9.2	15.1	2.8	3.8	6.7	3.5	12.8
MODOLO	**241**	54.3	49.7	38.8	62.6	-	71.2	65.8	53.4	80.0	-	86.3	81.6	69.2	90.8	-
		13.5	12.8	19.6	13.0	-	11.6	11.2	19.2	12.5	-	7.1	8.2	21.5	10.9	-
GUASILA	**246**	37.3	32.1	25.8	42.7	-	62.3	58.7	52.8	69.1	-	87.2	87.2	82.2	92.5	-
		15.3	14.3	7.2	15.3	-	14.0	13.7	10.6	13.4	-	8.1	8.6	12.8	8.3	-
BERCHIDDA	**276**	32.6	31.6	31.4	33.7	51.5	63.0	62.6	61.5	67.5	73.8	91.5	92.3	91.0	97.1	93.2
		14.8	11.2	8.1	10.9	25.9	18.5	11.9	10.7	10.6	18.8	13.1	10.4	10.8	5.0	15.0
BENETUTTI	**284**	35.0	27.3	25.1	30.0	35.1	60.9	54.8	54.9	56.2	59.8	87.1	87.2	87.9	85.8	87.8
		13.6	12.1	10.6	8.2	18.3	10.3	11.8	11.9	10.0	14.7	6.4	6.4	7.5	10.0	6.8
CHILIVANI (UCEA)	**290**	35.8	25.7	21.2	-	-	68.7	57.4	50.1	-	-	96.2	89.9	84.0	-	-
		14.0	6.8	7.4	-	-	11.4	5.6	7.5	-	-	4.8	6.3	9.6	-	-
ORGOSOLO OLA	**290**	-	-	31.4	100.0	34.3	-	-	62.2	100.0	65.8	-	-	93.5	100.0	90.5
		-	-	12.7	0.0	11.0	-	-	11.7	0.0	12.3	-	-	10.0	0.0	4.3
GHILARZA	**293**	40.9	-	24.7	35.1	34.2	70.2	-	54.7	68.2	66.0	96.9	-	87.6	94.6	94.2
		21.8	-	9.1	15.4	17.6	15.9	-	9.8	14.1	14.8	7.7	-	9.5	9.0	8.0
BONNANARO	**345**	40.7	33.5	27.8	34.7	37.6	67.4	59.4	53.6	61.4	62.6	88.5	83.8	78.5	87.8	86.2
		17.0	13.5	9.7	13.2	15.3	13.1	11.9	13.3	11.2	14.2	7.8	8.1	14.9	8.0	13.8
CHIARAMONTI	**368**	41.5	38.2	27.0	36.1	41.3	67.3	65.7	54.1	64.3	70.9	89.5	90.4	83.3	91.4	96.7
		16.0	11.6	7.7	13.3	18.7	11.7	10.2	10.0	11.6	11.2	6.0	6.6	8.9	7.2	3.6
NURALLAO	**380**	-	-	-	25.9	39.6	-	-	-	50.8	65.0	-	-	-	80.7	88.0
		-	-	-	7.9	18.9	-	-	-	13.4	17.6	-	-	-	11.8	10.2
GIAVE	**410**	47.8	37.7	32.5	40.1	48.7	76.0	70.3	58.2	71.3	74.9	97.3	97.6	88.2	96.1	94.7
		19.5	9.5	15.0	14.1	19.5	11.9	9.0	18.3	9.9	11.8	3.0	4.5	19.1	4.1	5.7
SIURGUS - DONIGAL	**414**	42.9	34.7	27.3	38.6	41.7	65.3	61.1	54.6	65.0	67.7	86.3	87.6	82.8	88.8	92.6
		22.2	16.2	8.3	15.5	21.4	18.8	17.7	13.1	15.8	18.7	12.7	12.1	13.1	9.3	10.3
PUTIFIGARI	**422**	47.8	36.9	29.2	35.6	30.1	64.7	58.2	41.8	52.1	50.5	80.5	83.5	56.5	68.5	70.1

		14.9	*14.0*	*14.2*	*11.1*	*13.8*	*16.3*	*13.4*	*19.1*	*16.4*	*17.8*	*12.4*	*11.3*	*21.8*	*16.9*	*20.3*
LURAS	**488**	37.1	31.5	27.4	20.5	36.5	58.5	55.7	51.7	43.6	54.0	80.3	87.6	78.1	71.1	71.5
		19.7	*18.7*	*11.5*	*7.6*	*20.7*	*21.8*	*17.1*	*15.0*	*8.4*	*19.3*	*17.9*	*11.4*	*16.2*	*10.7*	*16.3*
NUORO	**488**	-	34.2	43.9	35.4	51.3	-	63.8	70.5	64.5	73.8	-	88.1	91.4	90.0	91.6
		-	*13.1*	*15.7*	*11.2*	*21.2*	-	*11.4*	*12.6*	*10.2*	*14.5*	-	*6.5*	*5.2*	*6.0*	*6.4*
SCANO MONTIFERR	**491**	55.4	39.7	32.4	45.0	48.2	73.4	62.7	54.2	67.2	70.3	91.8	84.2	78.9	89.2	89.4
		24.2	*15.3*	*12.2*	*21.7*	*22.4*	*18.1*	*15.9*	*14.3*	*19.2*	*17.3*	*8.9*	*13.4*	*15.8*	*15.1*	*12.7*
ATZARA	**620**	39.5	33.3	26.7	39.5	41.9	62.7	57.9	48.7	61.8	66.3	85.4	82.5	77.4	83.5	90.4
		21.8	*10.8*	*5.4*	*17.6*	*20.4*	*18.4*	*14.0*	*9.5*	*18.0*	*17.8*	*11.8*	*13.0*	*12.5*	*13.0*	*11.0*
MACOMER	**665**	45.6	40.6	29.6	46.0	48.9	69.2	66.2	52.0	68.6	69.6	91.2	91.6	77.0	91.3	89.3
		20.1	*14.6*	*11.7*	*22.5*	*23.3*	*17.1*	*15.7*	*16.1*	*19.1*	*17.8*	*11.2*	*10.8*	*18.7*	*13.1*	*11.0*
SADALI	**780**	42.9	48.2	45.0	32.7	36.5	64.4	68.5	62.0	51.8	59.8	84.8	86.3	96.0	77.3	85.1
		17.6	*20.3*	*0.0*	*9.4*	*20.8*	*18.7*	*19.0*	*0.0*	*14.2*	*16.4*	*19.4*	*15.1*	*0.0*	*13.0*	*7.5*
BITTI	**782**	46.7	40.3	39.8	49.1	52.6	67.3	60.4	60.2	68.6	74.6	85.5	81.0	82.4	90.7	95.1
		19.3	*16.6*	*16.0*	*18.1*	*21.1*	*19.2*	*17.6*	*16.2*	*17.6*	*15.7*	*17.3*	*14.7*	*16.2*	*12.9*	*8.3*
VILLANOVASTRISA	**813**	48.5	33.3	34.4	38.8	-	70.7	54.8	60.6	64.2	-	87.9	81.7	90.4	88.0	-
		13.9	*12.1*	*5.7*	*17.9*	-	*9.2*	*13.2*	*4.2*	*19.6*	-	*2.4*	*11.8*	*3.4*	*8.6*	-
GAVOI	**835**	37.7	26.2	25.5	39.9	43.3	74.1	60.2	61.7	65.3	69.1	99.9	97.3	97.1	93.9	93.6
		19.1	*11.2*	*22.3*	*9.8*	*18.2*	*14.3*	*12.7*	*16.7*	*11.2*	*17.2*	*0.7*	*5.5*	*5.5*	*4.7*	*15.6*
ILLORAI	**878**	48.3	38.9	31.6	44.1	50.0	73.6	67.8	62.4	66.6	73.7	95.9	95.0	94.4	87.0	94.3
		18.2	*12.0*	*11.7*	*15.6*	*22.5*	*15.7*	*12.3*	*12.4*	*12.1*	*14.9*	*7.2*	*6.7*	*8.2*	*6.1*	*6.9*
ARITZO	**879**	-	-	-	27.6	28.6	-	-	-	54.3	64.7	-	-	-	79.2	100.0
		-	-	-	*14.8*	*12.7*	-	-	-	*23.5*	*25.1*	-	-	-	*25.9*	*0.0*
ALLAI	-	31.5	30.5	-	-	-	63.8	63.9	-	-	-	91.0	91.4	-	-	-
		12.0	*7.8*	-	-	-	*8.7*	*3.6*	-	-	-	*2.9*	*2.2*	-	-	-
ARZACHENA	-	-	-	31.7	-	-	-	-	60.7	-	-	-	-	86.2	-	-
		-	-	*4.7*	-	-	-	-	*9.3*	-	-	-	-	*9.0*	-	-
BARISARDO	-	-	-	23.7	25.6	28.1	-	-	53.6	58.8	65.4	-	-	86.6	89.3	96.0
		-	-	*9.8*	*11.7*	*9.0*	-	-	*10.0*	*18.5*	*8.1*	-	-	*11.5*	*13.9*	*4.7*
MASAINAS	-	39.3	40.0	-	-	-	58.4	61.9	-	-	-	79.6	81.5	-	-	-
		17.3	*9.6*	-	-	-	*16.6*	*7.6*	-	-	-	*12.8*	*5.2*	-	-	-
MEANA SARDO	-	37.1	34.9	16.3	40.0	23.7	55.8	67.3	27.9	83.9	46.7	76.5	90.9	55.2	100.0	76.5
		24.0	*16.2*	*2.3*	*0.0*	*5.2*	*23.4*	*20.7*	*5.0*	*0.0*	*8.3*	*20.4*	*20.1*	*9.6*	*0.0*	*6.9*
SAN TEODORO	-	40.9	34.6	-	-	-	64.2	58.0	-	-	-	84.2	81.3	-	-	-
		11.3	*10.1*	-	-	-	*10.2*	*11.7*	-	-	-	*10.5*	*11.0*	-	-	-
SILIQUA	-	36.5	-	-	-	-	64.6	-	-	-	-	90.9	-	-	-	-
		15.2	-	-	-	-	*12.1*	-	-	-	-	*5.1*	-	-	-	-
ZEDDIANI (UCEA)	-	32.9	39.5	28.0	-	-	60.1	65.3	51.7	-	-	84.9	91.6	81.0	-	-
	-	*11.3*	*8.2*	*9.1*	-	-	*7.4*	*5.3*	*9.4*	-	-	*4.0*	*4.7*	*9.6*	-	-
Average		**42.7**	**36.7**	**31.2**	**37.2**	**39.3**	**67.4**	**62.2**	**55.9**	**64.0**	**65.2**	**89.6**	**87.9**	**82.9**	**89.7**	**89.9**
Standard Deviation		*18.9*	*15.9*	*15.5*	*16.6*	*21.0*	*15.2*	*14.1*	*15.8*	*15.2*	*18.2*	*11.1*	*10.9*	*16.1*	*12.0*	*14.5*

Supplemental Table 3

Minimum, Average and Maximum Temperature (%) recorded in each Meteorological Station

Meteo Station		Tmin					Tmed					Tmed				
Place	**Quote (m)**	**2003-2004**	**2004-2005**	**2005-2006**	**2006-2007**	**2007-2008**	**2003-2004**	**2004-2005**	**2005-2006**	**2006-2007**	**2007-2008**	**2003-2004**	**2004-2005**	**2005-2006**	**2006-2007**	**2007-2008**
MURAVERA	2	15.5	-	15.9	15.6	15.4	20.7	-	21.3	19.9	20.9	25.9	-	27.7	25.2	26.5
		2.8	-	*4.5*	*1.9*	*2.8*	*3.5*	-	*4.2*	*2.4*	*3.0*	*4.1*	-	*4.7*	*2.9*	*3.4*
VALLEDORIA	2	12.9	13.4	14.1	14.2	14.6	18.8	19.4	21.1	20.4	19.8	24.2	25.2	27.4	26.1	25.5
		3.1	*2.9*	*3.7*	*3.1*	*4.2*	*3.8*	*1.9*	*3.6*	*2.9*	*3.5*	*4.4*	*2.1*	*4.0*	*4.4*	*3.5*
SINISCOLA	14	13.2	15.4	12.7	15.6	14.5	19.8	21.3	19.7	21.4	20.5	25.8	26.6	25.9	26.8	25.9
		4.0	*4.7*	*4.1*	*2.9*	*2.6*	*4.5*	*4.3*	*3.6*	*3.2*	*2.7*	*5.5*	*4.7*	*3.9*	*3.7*	*3.5*
ARBOREA	20	20.8	10.6	-	-	-	25.7	18.8	-	-	-	32.1	27.1	-	-	-
		3.2	*2.5*	-	-	-	*1.7*	*2.3*	-	-	-	*0.7*	*3.2*	-	-	-
PALMAS ARBOREA	20	-	-	17.2	-	15.4	-	-	25.2	-	22.1	-	-	32.6	-	28.1
		-	-	*5.3*	-	*2.6*	-	-	*4.5*	-	*3.2*	-	-	*5.4*	-	*4.7*
UTA	20	-	-	16.5	15.3	14.8	-	-	23.4	22.3	21.2	-	-	31.0	29.4	27.7
		-	-	*4.2*	*3.1*	*3.5*	-	-	*3.5*	*2.8*	*3.7*	-	-	*4.6*	*4.1*	*4.6*
OROSEI	26	15.3	17.0	16.7	16.9	15.3	20.2	21.8	21.7	21.4	19.9	24.9	26.8	26.8	25.8	24.8
		3.9	*3.9*	*4.6*	*2.4*	*2.1*	*3.8*	*3.5*	*4.2*	*2.2*	*3.0*	*3.9*	*3.7*	*4.4*	*2.7*	*3.8*
DECIMOMANNU	29	13.4	14.9	15.6	11.6	-	21.0	21.9	23.1	20.1	-	28.3	29.7	30.9	28.0	-
		4.0	*3.1*	*4.5*	*1.8*	-	*4.1*	*3.3*	*4.0*	*2.0*	-	*5.2*	*4.3*	*5.1*	*2.8*	-
OLMEDO	31	11.2	12.3	12.9	11.7	13.8	18.0	19.7	21.0	19.3	21.4	24.8	26.8	28.7	26.0	28.5
		3.0	*2.9*	*5.2*	*3.1*	*3.2*	*3.4*	*2.9*	*5.1*	*2.6*	*3.2*	*4.6*	*4.0*	*6.0*	*3.6*	*4.2*
STINTINO	35	15.0	15.1	14.2	17.1	20.1	19.2	18.6	18.2	20.5	24.5	23.5	22.6	22.7	24.8	28.9
		3.3	*3.2*	*1.8*	*2.9*	*1.7*	*3.8*	*3.1*	*2.3*	*2.3*	*2.4*	*4.7*	*3.9*	*3.5*	*3.4*	*3.6*
JERZU	47	12.3	15.6	14.7	15.3	14.6	20.9	23.0	22.5	21.8	20.8	28.3	29.6	30.7	28.9	27.6
		3.2	*2.9*	*4.1*	*3.2*	*3.7*	*4.4*	*3.8*	*3.5*	*3.4*	*4.1*	*5.4*	*5.1*	*4.4*	*4.6*	*5.0*
SORSO	50	14.9	15.3	14.4	16.0	16.2	19.8	20.9	20.5	20.9	20.6	25.0	26.8	26.6	26.4	26.2
		4.1	*2.9*	*3.3*	*2.4*	*1.7*	*4.4*	*2.4*	*3.1*	*2.7*	*2.3*	*5.4*	*2.8*	*3.9*	*4.5*	*3.8*
DORGALI FILITTA	85	14.4	15.9	12.7	14.2	13.3	22.0	22.7	21.3	21.7	19.8	29.6	29.2	28.7	28.7	26.1
		3.9	*4.3*	*4.7*	*3.0*	*2.8*	*5.3*	*4.2*	*4.5*	*2.9*	*3.8*	*6.8*	*5.1*	*4.7*	*3.4*	*5.3*
SAMASSI	89	13.2	15.4	14.4	-	17.5	19.2	22.4	22.1	-	25.0	25.3	30.4	30.3	-	32.8
		3.6	*3.7*	*4.7*	-	*1.4*	*4.8*	*4.7*	*4.5*	-	*1.4*	*6.6*	*6.1*	*5.7*	-	*2.0*
MONASTIR MOBILE	96	13.4	16.8	17.1	17.1	14.9	18.3	22.9	24.2	22.5	20.7	23.8	29.7	31.3	28.5	27.0
		3.6	*4.7*	*4.2*	*2.7*	*2.6*	*4.3*	*4.7*	*3.8*	*4.4*	*3.3*	*4.9*	*5.1*	*4.7*	*6.5*	*3.8*
MILIS	97	14.5	15.4	17.9	14.7	16.1	20.7	22.3	24.5	21.6	21.8	27.5	29.6	31.7	28.7	28.0
		3.5	*2.8*	*5.1*	*3.3*	*2.9*	*4.4*	*3.3*	*4.7*	*3.4*	*4.0*	*5.9*	*4.2*	*5.2*	*4.4*	*6.1*
ARZACHENA MOBI	120	-	-	-	14.5	12.9	-	-	-	21.2	19.6	-	-	-	28.8	26.8
		-	-	-	*2.0*	*2.7*	-	-	-	*1.6*	*3.7*	-	-	-	*3.5*	*4.9*
VILLACIDRO	130	14.0	14.3	16.3	15.8	15.1	19.8	21.6	23.0	22.0	21.8	25.5	28.9	30.1	28.5	28.5
		3.7	*3.6*	*4.5*	*3.4*	*3.1*	*4.3*	*3.2*	*4.3*	*3.3*	*3.5*	*5.2*	*3.5*	*5.1*	*4.1*	*4.6*
OLIENA	132	12.8	9.8	10.0	14.4	13.6	20.0	19.5	19.6	23.7	18.5	27.0	27.8	27.9	32.7	23.8
		3.1	*2.5*	*4.1*	*4.3*	*2.0*	*4.7*	*2.8*	*3.2*	*3.5*	*3.1*	*6.6*	*3.9*	*3.7*	*4.3*	*4.6*

GONNOSFANADIGA	146	13.9	12.7	15.4	14.9	14.1	20.5	22.4	22.2	21.6	21.8	27.2	32.4	30.8	28.5	29.4
		6.0	3.9	5.1	3.5	3.5	5.5	3.5	5.3	4.0	3.6	5.4	3.9	5.2	5.1	4.8
SASSARI S.A.R.	150	13.5	14.8	15.1	15.8	14.2	18.1	19.8	20.5	20.5	19.3	22.9	24.5	26.2	25.6	24.4
		3.8	2.8	4.0	2.6	3.1	4.3	3.0	4.5	2.9	3.3	4.9	3.5	5.2	4.2	4.2
DORGALI MOBILE	156	-	-	-	20.0	14.8	-	-	-	23.4	22.4	-	-	-	29.2	29.8
		-	-	-	0.5	2.9	-	-	-	0.2	4.6	-	-	-	1.5	6.9
OTTANA	160	11.5	13.0	13.5	13.4	14.2	20.2	21.6	23.4	22.1	21.8	28.8	30.7	33.1	31.0	29.8
		3.4	3.7	5.0	3.1	3.3	5.0	3.6	5.0	3.6	4.5	7.2	4.6	6.4	5.3	6.9
DOLIANOVA	165	12.6	14.8	15.0	14.1	13.4	19.6	21.2	23.0	21.5	20.5	26.2	28.2	30.9	29.0	27.4
		3.9	3.0	5.0	1.9	3.2	5.2	4.1	3.9	3.2	4.0	6.2	5.1	4.5	4.8	5.4
SARDARA	189	15.2	16.1	17.1	16.0	16.9	20.1	21.7	22.7	21.4	22.1	26.6	28.5	29.9	27.8	28.5
		3.3	3.3	4.4	2.8	2.8	4.2	3.6	4.5	3.7	4.1	5.7	4.6	5.5	5.0	5.8
USINI MOBILE	197	-	-	16.0	13.7	16.1	-	-	23.2	19.9	22.5	-	-	30.9	26.5	29.8
		-	-	5.0	2.8	4.2	-	-	6.3	2.2	4.0	-	-	7.5	3.1	4.7
IGLESIAS	208	-	-	-	16.1	14.8	-	-	-	20.8	19.5	-	-	-	26.9	25.2
		-	-	-	2.9	2.4	-	-	-	4.4	4.2	-	-	-	6.8	5.9
OZIERI	238	8.8	11.2	12.2	12.0	10.7	17.2	20.4	20.8	20.7	19.2	25.1	29.4	29.6	29.1	26.7
		3.0	3.9	5.1	3.7	4.5	4.0	4.0	5.4	3.8	4.0	5.9	5.5	6.9	5.3	5.6
MODOLO	241	14.7	14.2	18.1	13.3	-	18.3	18.6	22.3	16.4	-	22.4	23.1	27.6	20.2	-
		3.8	2.0	5.1	0.7	-	4.4	1.5	5.1	1.5	-	5.5	2.1	6.0	2.2	-
GUASILA	246	13.1	15.6	15.8	12.8	-	19.1	21.5	22.5	17.9	-	26.0	28.8	30.2	24.0	-
		3.9	4.0	4.5	2.3	-	4.9	4.8	4.4	3.4	-	6.4	6.1	5.3	5.2	-
BERCHIDDA	276	14.3	13.4	14.1	13.3	13.8	21.0	20.5	21.3	20.9	19.8	28.8	28.5	29.5	30.3	27.1
		3.0	3.8	5.4	2.7	3.0	5.2	4.2	5.1	3.4	4.8	6.7	5.5	5.5	5.1	7.1
BENETUTTI	284	8.9	11.9	10.6	12.9	10.8	17.4	20.9	20.7	19.9	19.5	25.3	30.0	30.3	27.1	26.9
		3.1	3.6	4.8	3.2	3.6	4.2	4.2	4.6	3.4	4.6	5.8	5.3	5.4	4.7	7.3
CHILIVANI (UCEA)	290	9.2	13.4	12.2	-	-	17.5	21.5	22.3	-	-	26.0	30.3	32.3	-	-
		3.6	3.2	4.9	-	-	4.3	2.8	4.4	-	-	5.9	3.6	5.3	-	-
ORGOSOLO OLAI	290	-	-	12.2	12.7	15.5	-	-	19.8	19.3	21.8	-	-	27.4	26.7	29.7
		-	-	5.9	4.3	1.4	-	-	5.6	5.4	1.5	-	-	6.7	6.0	2.0
GHILARZA	293	12.4	-	15.0	13.5	13.6	18.7	-	22.8	20.2	20.3	25.7	-	31.6	27.6	27.5
		3.8	-	5.3	3.2	3.0	5.0	-	5.1	4.1	3.9	6.7	-	5.8	5.3	5.6
BONNANARO	345	11.5	12.4	13.2	13.1	12.9	17.5	19.3	20.8	19.8	19.4	24.2	26.6	28.7	27.1	26.3
		2.8	3.5	4.9	2.8	3.6	4.1	4.6	5.3	3.7	3.8	5.8	6.1	6.2	5.6	5.2
CHIARAMONTI	368	12.0	12.2	13.3	12.1	10.8	18.5	19.5	21.5	19.8	18.2	24.9	26.6	29.4	26.8	25.6
		4.1	3.5	4.9	3.7	3.8	4.8	3.3	5.0	4.0	3.6	6.5	4.0	6.0	5.8	5.2
NURALLAO	380	-	-	-	14.3	12.9	-	-	-	22.5	19.5	-	-	-	30.6	26.6
		-	-	-	4.6	2.9	-	-	-	5.0	4.6	-	-	-	6.1	6.3
GIAVE	410	8.5	8.6	10.6	10.6	8.7	16.3	17.8	20.1	18.8	17.1	23.6	26.1	27.9	26.7	24.6
		3.7	3.8	5.0	3.6	4.7	4.6	3.5	5.4	3.8	3.9	6.4	4.2	6.1	5.3	5.4
SIURGUS - DONIGAL	414	13.5	14.4	14.9	13.9	13.8	18.9	20.5	21.5	19.5	19.2	25.3	28.3	29.0	25.5	25.3
		4.2	4.1	4.5	3.0	2.7	5.2	5.3	4.9	4.7	4.2	6.7	6.4	5.6	6.2	6.3
PUTIFIGARI	422	14.4	15.5	16.2	17.3	16.7	18.0	19.6	21.2	21.7	20.9	23.5	25.0	27.5	27.3	26.0

		4.4	4.1	5.8	3.8	3.4	4.9	4.8	6.5	3.5	3.8	5.6	5.8	7.0	3.9	4.2
LURAS	488	13.3	13.4	13.9	13.1	13.4	19.3	19.5	19.9	19.4	18.1	25.5	26.4	26.9	26.0	23.2
		4.0	5.1	4.6	1.7	3.5	5.7	5.9	5.5	2.2	4.4	6.9	7.4	6.7	3.4	5.2
NUORO	488	-	12.1	9.8	12.0	11.7	-	19.2	15.7	18.8	17.2	-	27.3	22.0	25.6	23.0
		-	4.9	3.7	2.9	2.3	-	3.9	3.9	3.4	3.6	-	4.0	5.4	4.8	5.7
SCANO MONTIFERR	491	11.5	13.3	13.6	12.8	13.2	16.4	19.4	20.3	18.8	18.6	21.7	25.7	27.2	24.8	24.2
		3.2	3.3	5.3	3.8	2.9	4.7	4.1	5.5	4.5	3.7	6.3	5.4	6.1	6.0	5.0
ATZARA	620	10.6	12.7	12.5	11.5	11.9	17.1	19.4	20.1	17.8	17.6	24.0	27.3	28.2	24.0	23.7
		3.5	3.5	5.3	3.1	2.6	5.3	4.1	5.2	4.6	4.2	7.1	4.7	5.8	6.2	6.2
MACOMER	665	10.6	11.6	13.4	11.9	11.6	17.0	18.0	20.6	18.3	17.8	23.3	24.4	27.6	24.7	23.5
		4.1	3.7	5.4	3.8	3.0	5.2	4.5	5.2	5.0	4.1	6.7	5.4	5.8	6.3	5.7
SADALI	780	10.4	10.2	4.0	12.4	9.2	16.8	16.0	11.9	18.9	17.5	22.3	21.8	17.6	24.9	24.6
		4.4	2.6	0.0	3.4	2.5	5.5	2.7	0.0	4.6	3.7	6.9	3.8	0.0	5.0	5.4
BITTI	782	11.1	12.9	12.6	12.9	11.4	15.6	18.1	18.2	17.9	15.9	20.6	24.2	24.1	24.2	21.2
		4.3	3.6	4.3	3.6	2.9	5.3	4.3	4.9	4.8	4.2	6.4	5.4	5.6	5.8	6.0
VILLANOVASTRISA	813	5.1	8.3	6.2	7.2	-	12.7	16.4	15.0	15.6	-	19.7	23.0	22.5	21.6	-
		3.8	4.2	4.8	3.6	-	4.8	3.8	4.4	5.4	-	7.0	3.8	5.0	7.0	-
GAVOI	835	5.5	7.6	7.7	7.2	8.0	13.1	17.7	17.6	16.4	16.0	19.8	26.1	26.4	24.7	23.0
		2.9	3.8	4.7	2.9	3.8	4.6	4.5	5.1	3.6	4.2	7.2	6.1	7.2	5.2	6.5
ILLORAI	878	7.7	9.0	8.3	9.3	8.5	14.6	16.4	17.2	15.0	15.6	20.6	23.4	24.8	20.9	21.6
		3.6	2.8	4.3	2.7	2.4	4.6	3.7	4.8	3.3	3.7	6.0	4.9	5.8	4.6	5.9
ARITZO	879	-	-	-	10.8	10.2	-	-	-	16.7	14.7	-	-	-	22.4	20.8
		-	-	-	4.2	2.2	-	-	-	5.1	3.7	-	-	-	5.2	3.9
ALLAI	-	10.9	8.7	-	-	-	18.7	17.7	-	-	-	26.9	26.9	-	-	-
		3.2	1.7	-	-	-	4.0	1.3	-	-	-	6.0	2.4	-	-	-
ARZACHENA	-	-	-	13.7	-	-	-	-	21.9	-	-	-	-	30.2	-	-
		-	-	4.0	-	-	-	-	4.6	-	-	-	-	6.2	-	-
BARISARDO	-	-	-	13.3	14.3	14.2	-	-	21.3	21.3	20.7	-	-	28.2	27.6	26.4
		-	-	3.8	3.7	4.3	-	-	3.1	3.8	3.7	-	-	4.1	3.8	4.2
MASAINAS	-	15.2	16.2	-	-	-	21.3	22.0	-	-	-	27.2	27.9	-	-	-
		3.8	2.4	-	-	-	4.9	3.0	-	-	-	6.3	3.9	-	-	-
MEANA SARDO	-	15.2	14.0	15.5	-	16.3	19.9	18.7	21.4	-	22.7	24.9	25.0	27.3	-	27.9
		4.8	4.6	3.7	-	0.9	5.9	5.3	4.8	-	3.3	7.5	5.7	5.0	-	3.9
SAN TEODORO	-	13.0	14.7	-	-	-	19.2	20.9	-	-	-	25.2	26.5	-	-	-
		3.7	3.6	-	-	-	4.0	3.3	-	-	-	4.6	4.0	-	-	-
SILIQUA	-	12.1	-	-	-	-	19.9	-	-	-	-	27.4	-	-	-	-
		3.4	-	-	-	-	4.6	-	-	-	-	6.4	-	-	-	-
ZEDDIANI (UCEA)	-	14.7	15.0	16.5	-	-	20.9	21.8	23.5	-	-	28.1	28.3	31.4	-	-
		2.6	3.0	5.5	-	-	4.1	3.1	4.6	-	-	5.5	4.1	5.1	-	-
Average		**11.8**	**13.1**	**13.7**	**13.4**	**13.1**	**18.3**	**20.0**	**21.2**	**19.9**	**19.6**	**24.8**	**27.1**	**28.9**	**26.7**	**26.2**
Standard Deviation		4.4	4.2	5.4	3.9	4.0	4.9	4.2	5.2	4.1	4.4	6.3	5.3	6.1	5.5	6.0

Supplemental Table 3

Minimum, Average and Maximum Temperature Humidity Index (THI) recorded in each Meteorological Station

Meteo Station		THImin					THImed					THImax				
Location	Quote (m)	2003-2004	2004-2005	2005-2006	2006-2007	2007-2008	2003-2004	2004-2005	2005-2006	2006-2007	2007-2008	2003-2004	2004-2005	2005-2006	2006-2007	2007-2008
MURAVERA	**2**	59.8	-	60.1	59.8	59.6	67.1	-	67.1	65.7	67.5	72.1	-	72.6	70.9	72.9
		4.8	*-*	*7.0*	*3.2*	*4.8*	*5.0*	*-*	*5.5*	*3.0*	*4.3*	*4.5*	*-*	*5.1*	*2.4*	*3.6*
VALLEDORIA	**2**	55.2	56.1	57.4	57.5	58.4	64.4	65.5	67.5	66.8	66.3	70.0	72.1	73.4	72.1	72.9
		5.5	*5.0*	*6.1*	*5.2*	*7.1*	*5.7*	*2.9*	*5.5*	*4.3*	*5.4*	*5.3*	*2.3*	*4.9*	*4.1*	*4.3*
SINISCOLA	**14**	55.7	59.1	54.9	60.0	58.0	65.3	67.1	65.5	67.8	66.8	71.0	71.6	71.9	72.3	72.1
		6.7	*7.2*	*6.9*	*5.1*	*4.5*	*5.9*	*5.2*	*5.3*	*4.5*	*3.8*	*5.3*	*4.8*	*4.3*	*4.2*	*3.8*
ARBOREA	**20**	68.1	51.1	-	-	-	73.6	64.4	-	-	-	78.5	72.5	-	-	-
		4.8	*4.4*	*-*	*-*	*-*	*1.5*	*3.1*	*-*	*-*	*-*	*1.4*	*2.9*	*-*	*-*	*-*
PALMAS ARBOREA	**20**	-	-	61.8	-	59.7	-	-	70.0	-	68.4	-	-	74.5	-	72.1
		-	*-*	*8.6*	*-*	*4.5*	*-*	*-*	*5.1*	*-*	*3.9*	*-*	*-*	*4.8*	*-*	*3.9*
UTA	**20**	-	-	54.0	59.1	58.3	-	-	65.0	67.4	66.3	-	-	69.7	71.7	70.5
		-	*-*	*6.4*	*5.1*	*5.8*	*-*	*-*	*3.3*	*3.4*	*4.1*	*-*	*-*	*3.6*	*3.7*	*3.7*
OROSEI	**26**	59.5	62.1	61.7	61.9	59.1	66.7	68.6	69.0	68.3	65.7	71.7	73.1	73.2	72.3	70.9
		6.9	*6.5*	*8.0*	*4.1*	*3.3*	*5.6*	*5.1*	*6.3*	*3.4*	*4.1*	*4.6*	*4.6*	*5.1*	*3.3*	*3.7*
DECIMOMANNU	**29**	56.0	58.7	60.0	53.1	-	67.3	68.8	69.9	65.7	-	73.3	74.9	75.2	72.2	-
		7.0	*5.5*	*7.8*	*3.1*	*-*	*5.8*	*4.5*	*5.5*	*2.6*	*-*	*5.0*	*3.7*	*4.8*	*2.7*	*-*
OLMEDO	**31**	52.2	54.2	54.5	53.0	56.8	63.2	65.8	66.3	65.2	68.0	70.1	72.6	73.1	72.1	74.0
		5.4	*5.2*	*9.3*	*5.5*	*5.7*	*4.8*	*4.3*	*7.0*	*3.5*	*4.4*	*4.5*	*4.2*	*5.6*	*3.2*	*4.1*
STINTINO	**35**	58.8	59.1	57.5	62.6	67.4	65.4	64.4	63.5	67.7	73.0	70.5	69.2	69.1	72.9	76.5
		5.7	*5.6*	*2.9*	*5.0*	*2.5*	*5.8*	*4.8*	*3.0*	*3.9*	*3.3*	*5.7*	*4.7*	*3.6*	*4.7*	*3.6*
JERZU	**47**	54.1	60.0	58.2	59.4	57.8	66.7	69.3	68.6	68.2	66.5	74.0	74.8	75.4	74.5	73.2
		5.5	*4.9*	*6.3*	*5.4*	*6.3*	*5.0*	*4.5*	*4.3*	*4.7*	*5.4*	*4.2*	*4.4*	*4.2*	*4.4*	*4.2*
SORSO	**50**	58.5	59.5	58.0	60.4	61.0	65.6	67.6	66.1	67.2	67.4	71.1	73.8	72.1	72.4	73.3
		6.9	*4.9*	*5.4*	*4.1*	*3.0*	*6.0*	*3.5*	*4.4*	*3.7*	*3.3*	*5.9*	*3.1*	*4.3*	*4.2*	*4.0*
DORGALI FILITTA	**85**	57.6	60.1	54.8	57.4	55.9	67.1	68.1	66.6	67.6	64.7	72.4	72.4	72.0	71.8	69.3
		6.5	*6.8*	*8.1*	*5.2*	*4.8*	*5.9*	*4.6*	*5.5*	*3.9*	*4.5*	*5.7*	*4.0*	*4.5*	*3.5*	*4.2*
SAMASSI	**89**	55.9	59.5	57.9	-	63.0	64.4	68.9	67.8	-	72.3	70.9	75.4	74.0	-	77.5
		6.1	*6.2*	*7.7*	*-*	*2.4*	*6.8*	*5.8*	*5.7*	*-*	*1.7*	*7.0*	*5.2*	*4.9*	*-*	*1.5*
MONASTIR MOBILE	**96**	56.0	61.7	62.4	61.8	58.2	62.5	68.0	70.3	67.0	64.0	66.8	72.1	73.4	71.4	69.5
		6.2	*7.6*	*7.2*	*4.3*	*2.7*	*5.0*	*5.1*	*4.6*	*4.5*	*2.9*	*4.3*	*4.5*	*4.3*	*5.0*	*3.4*
MILIS	**97**	58.0	59.6	62.7	58.3	60.6	66.6	68.7	70.2	68.0	68.1	72.7	74.7	75.7	74.2	73.3
		5.9	*4.5*	*7.6*	*5.5*	*4.8*	*5.9*	*4.3*	*5.5*	*4.3*	*5.0*	*5.7*	*4.0*	*4.7*	*4.0*	*5.3*
ARZACHENAMOBIL	**120**	-	-	-	58.2	54.9	-	-	-	67.9	64.1	-	-	-	73.1	71.5
		-	*-*	*-*	*3.7*	*4.5*	*-*	*-*	*-*	*2.1*	*3.7*	*-*	*-*	*-*	*2.7*	*4.5*
VILLACIDRO	**130**	57.1	57.6	60.7	59.8	59.0	64.9	67.3	69.0	67.8	68.1	70.1	73.4	74.3	73.1	73.6
		6.0	*5.8*	*6.9*	*5.4*	*5.3*	*5.4*	*3.8*	*5.4*	*4.2*	*4.7*	*5.1*	*3.0*	*4.9*	*4.1*	*4.4*
OLIENA	**132**	55.1	49.8	50.1	57.9	56.5	65.2	64.9	65.4	70.7	63.6	71.7	72.7	73.0	77.2	69.2
		5.3	*4.5*	*7.2*	*7.6*	*3.3*	*5.7*	*3.5*	*4.7*	*4.2*	*4.0*	*6.0*	*3.7*	*4.1*	*3.2*	*4.2*

GONNOSFANADIGA	**146**	56.1	54.9	59.2	58.6	57.4	64.7	66.9	67.6	67.1	67.8	69.6	73.9	73.8	72.4	73.0
		9.4	*6.6*	*8.4*	*6.0*	*6.1*	*5.8*	*3.8*	*6.6*	*4.7*	*4.5*	*4.8*	*3.4*	*4.9*	*4.2*	*4.1*
SASSARI S.A.R.	**150**	56.3	58.6	58.8	60.0	57.7	62.7	65.2	65.6	65.9	64.5	67.4	69.3	70.4	70.2	69.2
		6.0	4.3	6.2	4.1	4.8	5.9	4.1	5.5	3.7	4.3	5.1	3.7	5.2	4.0	4.2
DORGALI MOBILE	**156**	-	-	-	67.3	58.3	-	-	-	69.2	67.8	-	-	-	72.1	72.6
		-	-	-	*1.2*	*4.9*	-	-	-	*2.3*	*5.8*	-	-	-	*1.8*	*6.2*
OTTANA	**160**	53.3	55.7	55.4	56.2	57.5	67.0	69.1	70.8	68.9	68.0	80.1	83.0	85.5	74.5	73.7
		5.3	*5.9*	*7.7*	*5.5*	*6.0*	*7.7*	*5.6*	*7.4*	*4.9*	*5.5*	*11.0*	*6.9*	*9.6*	*4.6*	*5.4*
DOLIANOVA	**165**	54.6	58.6	59.0	57.4	56.0	64.8	67.6	69.8	67.6	65.9	71.1	73.5	75.9	74.5	72.2
		6.7	*5.2*	*8.4*	*3.2*	*5.4*	*6.8*	*5.5*	*5.5*	*4.1*	*5.0*	*6.2*	*4.6*	*4.8*	*4.7*	*4.9*
SARDARA	**189**	59.1	60.9	62.4	60.6	62.3	66.2	68.7	69.6	67.9	69.2	72.5	74.7	75.3	73.8	74.5
		5.7	*5.9*	*7.6*	*4.8*	*4.8*	*6.0*	*4.9*	*6.1*	*4.9*	*5.6*	*5.9*	*4.3*	*5.3*	*4.8*	*5.6*
USINI MOBILE	**197**	-	-	60.0	56.5	59.7	-	-	67.7	65.4	67.5	-	-	74.0	69.9	72.4
		-	-	*7.8*	*5.0*	*5.6*	-	-	*6.9*	*2.9*	*4.2*	-	-	*6.8*	*2.9*	*4.0*
IGLESIAS	**208**	-	-	-	60.5	58.5	-	-	-	66.1	64.7	-	-	-	71.6	70.6
		-	-	-	*4.5*	*4.0*	-	-	-	*5.3*	*5.3*	-	-	-	*6.1*	*5.5*
OZIERI	**238**	48.3	52.3	54.0	53.7	51.1	61.7	66.2	66.2	66.7	64.4	70.1	74.0	73.6	73.7	71.6
		5.2	*6.8*	*8.8*	*6.5*	*7.6*	*5.7*	*5.3*	*7.0*	*5.0*	*5.1*	*5.8*	*4.8*	*6.4*	*4.9*	*5.5*
MODOLO	**241**	58.3	57.6	62.7	56.0	-	63.4	64.1	67.6	60.9	-	68.2	69.0	72.6	65.9	-
		6.2	*3.2*	*6.6*	*1.2*	-	*6.4*	*2.3*	*5.5*	*2.0*	-	*6.7*	*2.3*	*4.9*	*2.1*	-
GUASILA	**246**	55.5	59.7	60.0	55.0	-	64.0	67.1	68.4	62.8	-	70.6	73.2	74.2	69.0	-
		6.6	*6.5*	*7.2*	*3.9*	-	*6.4*	*5.7*	*5.6*	*4.5*	-	*6.2*	*5.2*	*5.0*	*5.2*	-
BERCHIDDA	**276**	57.4	56.0	57.1	55.9	56.5	66.5	66.3	67.3	67.2	65.4	73.1	73.2	74.4	75.5	73.0
		5.2	*6.5*	*9.3*	*4.8*	*4.5*	*6.4*	*5.4*	*6.9*	*4.7*	*6.2*	*5.6*	*5.1*	*5.6*	*4.9*	*7.3*
BENETUTTI	**284**	48.6	53.6	51.2	55.3	51.8	61.8	66.4	66.1	65.1	64.5	69.7	74.1	74.1	71.5	71.0
		5.4	*6.0*	*8.2*	*5.4*	*6.1*	*5.5*	*5.0*	*6.0*	*4.2*	*5.6*	*5.5*	*4.6*	*5.0*	*4.5*	*6.9*
CHILIVANI (UCEA)	**290**	48.7	56.0	54.8	-	-	62.1	67.5	68.1	-	-	70.6	74.5	75.7	-	-
		6.5	*5.6*	*8.5*	-	-	*6.0*	*3.5*	*5.9*	-	-	*5.8*	*3.1*	*5.2*	-	-
ORGOSOLO OLAI	**290**	-	-	53.8	49.6	59.8	-	-	65.1	59.7	68.5	-	-	71.7	72.8	75.1
		-	-	*10.1*	*5.2*	*2.4*	-	-	*7.6*	*6.7*	*1.5*	-	-	*6.6*	*10.5*	*1.5*
GHILARZA	**293**	54.2	-	58.3	56.3	56.4	63.6	-	68.4	66.0	66.0	70.2	-	75.1	72.4	72.0
		6.7	-	*8.9*	*5.7*	*5.2*	*6.9*	-	*6.5*	*5.7*	*5.1*	*6.5*	-	*5.3*	*4.9*	*4.9*
BONNANARO	**345**	52.9	54.6	55.6	55.7	55.1	62.0	64.3	65.9	65.2	64.7	68.9	71.0	72.8	71.8	71.2
		4.7	*5.6*	*7.6*	*4.7*	*5.7*	*5.6*	*5.7*	*6.6*	*4.9*	*4.9*	*5.8*	*5.6*	*6.0*	*5.4*	*5.2*
CHIARAMONTI	**368**	53.6	54.1	55.9	53.9	51.5	63.5	65.0	67.1	65.3	63.4	69.9	71.9	73.6	71.7	70.7
		7.0	*6.1*	*8.2*	*6.3*	*6.7*	*6.7*	*4.3*	*6.4*	*5.2*	*5.1*	*6.8*	*3.8*	*5.9*	*5.5*	*5.1*
NURALLAO	**380**	-	-	-	57.3	55.1	-	-	-	67.9	64.5	-	-	-	74.5	71.4
		-	-	-	*6.9*	*4.8*	-	-	-	*4.9*	*5.7*	-	-	-	*4.9*	*5.8*
GIAVE	**410**	47.3	47.6	50.8	51.2	47.9	60.5	62.8	64.9	64.2	61.7	68.5	71.3	72.3	72.0	70.0
		6.7	*6.7*	*8.3*	*6.4*	*8.2*	*6.9*	*4.6*	*6.1*	*5.3*	*5.6*	*6.6*	*3.9*	*5.2*	*5.3*	*5.5*
SIURGUS - DONIGAL	**414**	56.0	57.6	58.5	56.9	56.6	63.7	65.7	67.0	64.6	64.3	70.0	72.8	73.1	70.2	69.9
		6.9	*6.5*	*7.5*	*5.1*	*4.6*	*6.7*	*6.1*	*6.3*	*5.9*	*5.2*	*6.5*	*5.4*	*5.4*	*6.0*	*5.6*
PUTIFIGARI	**422**	57.5	59.5	59.3	61.9	60.9	62.5	64.7	65.0	67.2	65.8	68.9	69.6	71.3	72.5	70.1

		6.6	6.6	7.6	5.3	4.5	5.9	6.3	6.9	3.9	4.0	5.4	6.4	6.1	3.6	3.6
LURAS	**488**	55.7	56.0	56.4	55.9	55.8	63.5	64.0	64.2	63.9	62.1	69.6	70.1	70.3	69.2	67.2
		6.7	8.7	7.0	2.7	5.1	7.2	7.0	6.5	2.5	5.2	6.7	6.2	6.1	2.7	5.0
NUORO	**488**	-	53.9	50.0	53.8	53.1	-	64.5	59.6	64.0	61.8	-	72.3	66.7	70.4	68.0
		-	8.3	6.2	4.9	4.0	-	5.0	5.2	4.7	4.9	-	3.7	5.2	4.8	5.7
SCANO MONTIFERR	**491**	52.8	55.7	56.1	54.8	55.6	60.3	64.4	65.1	63.5	63.7	66.4	70.6	71.6	69.7	69.5
		5.4	5.3	8.2	6.2	4.8	6.7	5.1	6.8	5.7	5.1	7.1	5.0	5.8	5.9	5.1
ATZARA	**620**	51.3	54.8	54.4	52.9	53.5	60.8	64.3	64.8	61.9	61.9	68.1	72.1	72.4	68.4	68.1
		6.0	5.6	8.4	5.3	4.5	7.3	5.0	6.3	6.0	5.8	7.5	4.1	5.7	6.0	6.2
MACOMER	**665**	51.1	53.1	55.8	53.3	53.0	61.1	62.5	65.5	62.8	62.4	67.9	69.2	71.9	69.5	68.5
		7.0	6.3	8.5	6.4	5.1	7.4	5.9	6.4	6.6	5.7	7.3	5.6	5.5	6.4	5.9
SADALI	**780**	50.5	50.9	39.6	54.3	49.2	60.4	59.8	54.3	63.2	61.7	66.4	66.7	61.9	69.3	68.7
		7.5	4.0	0.0	5.3	4.3	7.4	3.7	0.0	4.9	4.6	7.4	3.6	0.0	4.5	4.8
BITTI	**782**	51.6	55.1	54.6	55.1	52.5	58.5	62.5	62.7	62.5	59.7	64.3	68.9	68.9	69.6	65.8
		6.9	5.7	7.2	6.2	5.1	7.2	5.5	6.6	6.7	6.2	6.8	5.5	6.0	6.6	6.8
VILLANOVASTRISA	**813**	42.2	47.9	43.8	45.7	-	54.9	60.4	58.8	58.9	-	63.8	67.5	67.2	65.7	-
		6.4	6.8	8.3	6.0	-	6.9	4.8	6.2	6.8	-	7.6	4.0	5.7	7.0	-
GAVOI	**835**	41.9	45.8	45.9	45.3	46.5	55.4	62.2	61.8	60.5	59.8	63.2	69.7	69.3	69.8	67.5
		5.3	6.7	8.6	5.1	6.4	7.1	5.9	7.0	5.2	5.9	7.8	5.5	6.4	5.8	6.9
ILLORAI	**878**	46.0	48.4	47.1	49.4	47.6	57.6	60.6	61.4	58.4	59.3	64.9	68.1	69.0	65.4	66.1
		6.3	5.1	7.6	4.4	4.1	6.6	5.2	6.5	4.4	5.6	6.6	5.0	5.8	4.8	6.7
ARITZO	**879**	-	-	-	51.4	50.4	-	-	-	60.1	57.5	-	-	-	65.9	64.3
		-	-	-	7.0	4.0	-	-	-	5.8	5.1	-	-	-	4.4	3.5
ALLAI	-	51.9	48.2	-	-	-	63.8	62.6	-	-	-	71.2	71.6	-	-	-
		5.6	3.0	-	-	-	5.4	1.8	-	-	-	5.7	2.0	-	-	-
ARZACHENA	-	-	-	56.5	-	-	-	-	68.1	-	-	-	-	75.3	-	-
		-	-	6.8	-	-	-	-	5.9	-	-	-	-	6.4	-	-
BARISARDO	-	-	-	56.1	57.7	57.5	-	-	67.1	67.2	66.8	-	-	72.0	71.6	70.5
		-	-	6.0	6.3	7.7	-	-	4.1	5.1	5.0	-	-	3.9	3.6	3.6
MASAINAS	-	58.8	60.9	-	-	-	66.7	68.6	-	-	-	72.2	73.7	-	-	-
		5.8	3.9	-	-	-	5.7	4.1	-	-	-	5.9	3.8	-	-	-
MEANA SARDO	-	58.2	56.3	59.2	-	60.8	64.0	63.2	65.2	-	68.1	68.5	69.3	70.1	-	71.7
		7.8	6.5	4.9	-	1.3	7.9	6.2	4.9	-	3.4	8.1	5.2	4.5	-	3.2
SAN TEODORO	-	55.4	58.3	-	-	-	64.6	66.7	-	-	-	70.5	71.5	-	-	-
		5.9	5.9	-	-	-	5.4	4.2	-	-	-	4.8	3.8	-	-	-
SILIQUA	-	53.8	-	-	-	-	65.3	-	-	-	-	72.2	-	-	-	-
		5.7	-	-	-	-	6.1	-	-	-	-	6.1	-	-	-	-
ZEDDIANI (UCEA)	-	58.3	58.9	61.1	-	-	66.7	68.5	69.7	-	-	72.8	74.3	76.0	-	-
		4.3	5.1	9.2	-	-	5.4	4.4	5.9	-	-	5.1	4.2	5.0	-	-
Average		**53.2**	**55.5**	**56.3**	**56.0**	**55.5**	**63.0**	**65.5**	**66.6**	**65.4**	**64.9**	**69.8**	**72.2**	**73.3**	**71.6**	**71.0**
Standard Deviation		7.5	7.1	8.9	6.6	6.8	6.8	5.4	6.6	5.5	5.7	6.8	5.4	6.2	5.3	5.7