UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO in

SCIENZE VETERINARIE

INDIRIZZO: **Riproduzione, produzione e benessere animale (XXVIII CICLO)**

Investigating the genetic and productive characteristics of autochthonous Sarda goat

Docente Guida

Prof. Giuseppe Massimo Vacca

Il Direttore

Prof. Sergio Ledda

Tesi di dottorato del

Dott. Pietro Paschino

ANNO ACCADEMICO 2014 – 2015

Index

Preface

Goat farming plays, in Sardinia, a key role for local economy, expecially in those areas where for environmental conditions it is not possible to rear other animals. In fact, while in the lowlands and hills there are several intensive farms in which cosmopolitan breed are reared, in mountainous areas the extensive rearing system is the most spread, and the autochthonous breed, the Sarda, is widely present.

The growing global market and the loss of economic power for local products could be prevented through the enhancement of those typical products that are still well appreciated by consumers, but that still don't own a trademark of designation of origin. Such recognition would allow a good profit margin for their work to farmers, and high quality products for consumers.

Goat products, in Sardinia, are for the best part related to dairy productions, while there is only one meat product, represented by the suckling goat kids.

With the aim of an increase of incomes from those products, a strategy of improvements and preservations of the local breed should be applied. In order to do this, the best knowledge about the health status of animals, milk traits, its hygienic and coagulation properties and meat characteristics is needed.

This should help for preservation of an interesting biodiversity heritage and for a traditional rearing system that, without the proper valorisation, is going to disappear.

1. Introduction

1.1 The Goat

Goat (*Capra hircus*) is a mammal belonging to Artiodactyla, Bovidae, and it is probably the first reared species, as it is known about its breeding since 10,000 years ago in the Middle East (Zeder & Hesse, 2000), from which it rapidly spread all over the Mediterranean area, giving birth to several hundreds of breeds (Scherf, 2000). The main characteristic of this species is its great adaptability to the environment, being capable of use to the best the poor grazing of mountainous and hill areas (Simon, 1990; Gall, 1996; Vacca et al., 2014). The first and most important Country where goat farming had an increase was Greece, thanks to its orographic characteristics and to the fact that it was not possibile to rear different species than goat on its mountains. In Greece goat farming became one of the most important income for farmers, and several classical authors described it in their poems, such as Omero in the epic poem Odissea. By the way, no one described in detail goat rearing system until Catone, during the II century B.C., wrote a review on this animal, describing in detail the farming system and milk characteristics. In the same period, another Roman writer, Polibio, described goat farming in Corse, emphasising the fact that, due to the impossibility for farmers to reach the mountainous areas where goats browsed, they were used to call them back for milking by the use of a horn (Durante, 1958).

The enlarging Roman Empire started to remote island of the Mediterranean Sea as a meat reserve, abandoning goats on the islands and

hunting them in case of necessity during travels. Those island where commonly named whit names related to goats, just like the islands of Capri in Campany and Caprera, in Sardinia. Furthermore, during to the Roman Empire were stated the first regulations for rearing this animal. As goats browse and eat the best part of the edible plants they found on their pasture, it was forbidden to farmers to rear these animals near olives and vines, two of the most important cultivars for the Empire. Due to these limitations, sheep farming became the most important farming system during the Empire, and goat farming rapidly decreased. Mac Kinnon (1999) and Santillo Frizell (2004) reported a goat:sheep ratio of 1:4 during the late Empire. In this period, the first attempts of a semi-intensive rearing system were described, with animals conducted to browsing only during the day by slaves and closed on fences at night.

The goat rearing system didn't change much in the centuries, until the Middle Age, when goat farming moved from common grazing places to the woodlands, in order to gain space on plans for cultivars (Delatouche, 1968). During the Middle Age, for the first time, goat milk was described as a standalone product, both for the direct consumption and for transformation in dairy products. Goat meat assumed a good importance too, and it was common to eat suckling kids and castrated adult meat, although these products where related to the lower incomes people, while the richer preferred sheep or pork meat. With the beginning of the industrial era, also woodlands started to be used for industrial purpose,

both for the buildings and for wood use, and goat farming had sudden stop. It was only in the first decades of last century that farmers started again to rear this species, both in extensive and intensive methods, and, like the Phoenix, goat farming raised again from its ashes.

Nowadays, there are several hundreds of breeds in the world, but it is possible to group them in few groups according of their origin or to the main productive attitude.

As for what concern productions, breeds with a high milk production are usually related to the Alpine group, such for example Saanen, Toggenburg, Camosciata and so on. Breeds with a midrange production of both meat and milk belongs to the African (or Mediterranean) group, the larger one, on which is possible to classify breeds as Maltese, Nubian, Egyptian and our Sarda. Latter, breeds reared for their hair, such as Angora or Kashmir, belongs to the Asian group. These breeds are reared for the best part only in their Countries of origin, and this is one of the because of high costs of derivated products.

Furthermore, lately a new concept of goat farming started: the one related to small sized breeds, as the Pigmy Goat for example, reared in Europe as company pets in city houses too (agriculture.com).

In 2013, 1.005.603.003 goats were reared all over the World (Fig.1), with more than 59% reared in Asia, followed by Africa with 35% of heads and Americas, with 3.5%.

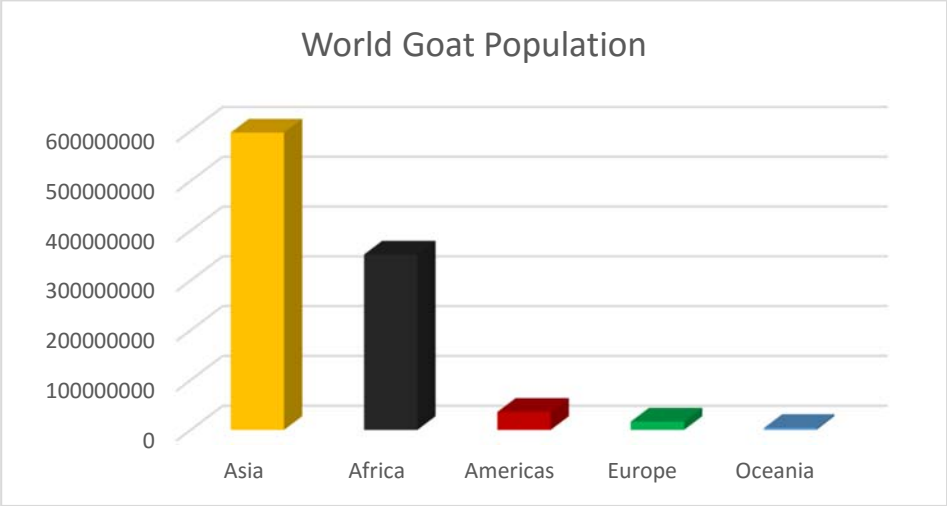

Fig.1 World Goat Population (FAOSTAT 2013)

Europe, with 16.487.290 of heads, occupy a marginal place for the number of animals, but if we consider milk productions, the main product for which this species is reared, it gains several percentage points, placing at more than 14% of milk produced all over the world (Fig.2). In this Continent, in the past decades, rearing of goats had an increase, establishing itself as a real farming system instead of the role of association with the sheep farming it was usually related. Goat farming had also an increase for the unique characteristics of its milk, which is a perfect replacer for infant feeding, providing a low amount of as1caseins and smaller fat globules, responsible of allergenic reactions and digestibility respectively.

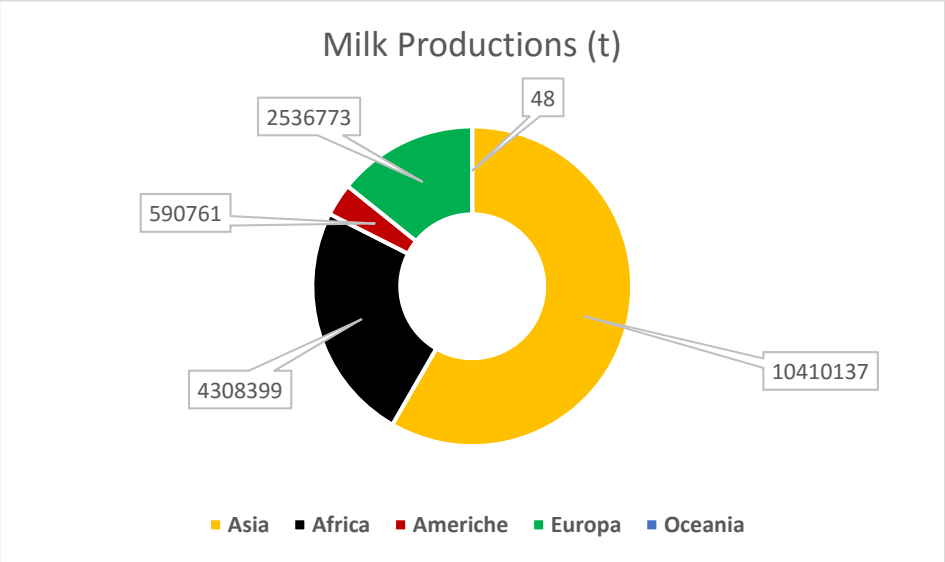

Fig.2 World Goat Milk Production in tonnes (FAOSTAT 2013)

Italian situation is vary, and concerning the amount of reared animals, it appears quite different from the European one. According to FAOSTAT (Tab.1), in Italy there is less than 5,5% of the European goat population, with 891.604 goats in 2013. In 2003 there were 988.000 heads. These data clearly define the descending trend for goat farming in this Country, but if we look to milk productions it's possible to notice that they're at same level, due to the fact that the efficiency in productions increased. Greece leads goat rearing in Europe, followed by Spain and France. These three Countries are in the first positions for milk productions too, providing about the 60% of fresh milk, where Italy produces only a 1%.

In our Country, the first attempts of improving goat rearing system started on '50s, with the introduction of males from several cosmopolitan breeds in order to increase milk productions (Rubino, 1990). Due to the

environmental conditions of southern parts of Italy, where goat farming is historically located, it was not possible to exploit the crossbred animals to the best, and as concerning to new technologies, it was difficult to apply them in those rural systems.

Country	Goats	Milk (t)
Greece	4.250.000	407.000
Spain	2.609.990	443.625
Russia	2.118.697	248.001
France	1.291.028	624.016
Romania	1.265.676	-
Italy	**891.604**	**27.944**
Albania	810.000	67.741
Ukraine	664.800	227.700
Nederland	412.550	217.330
Others	*2.172.945*	*273.416*
Total	16.487.290	2.536.773

Tab.1 UE 27 Goat Population and Milk production in tonnes (FAOSTAT 2013)

As in the rest of Italy, in Sardinia farmers tried to increase productions by crossbreeding dairy breeds with the local one, the Sarda. This practice, as it happened in other parts of the world (Dubeuf et al., 2009; Marshall, 2014), was at first conducted without planning crossbreeding schemes. This led to a first increase of the milk amount produced, but had negative effects on the native population, that had a loss of genetic heritage that is really difficult to estimate today. Nowadays, in order to recover the original breed, plans of selection within the breed have

been set up, with the creation of a genealogic book and an association for the breed.

1.2 Goat farming in Sardinia

In Sardinia more than 272.000 goats in 3.661 farms are reared (Laore 2013), representing more than 25% of the entire Italian population. Intensive farms (on which dairy breeds are reared) are widely present in the Island and their number sudden increase in the earlies 2000, but the best part of farms are extensive-managed farms, on which Sarda goats (or crossbred) are reared. According to the Italian Regulation (Decreto del Ministero dell'Agricoltura e delle Foreste del 13/06/1985, "Regolamento per lo svolgimento dei controlli funzionali del latte nella specie caprina"), Sarda breed belongs to the group of mediterranean breeds, as Garganica, Jonica, Girgentana and Maltese ones. Only two breeds belong to the alpine group, the Saanen and the Camosciata delle Alpi. These two latest breeds are widely distributed and reared in intensive systems, because of their high milk productivity. Thirtythree local breeds are reared in Italy, and their population is regulated by the institution of a genealogic book, on late 1998 (Registro anagrafico delle popolazioni ovine e caprine autoctone a limitata diffusione).

Goat farming in Sardinia, as in the rest of Europe, had a marginal role during the past centuries, with the use for pastures of the most marginal areas, as the lowlands were associated with the more remunerative sheep farming. Although this, Sardinia is the first goat milk producer in Italy, with more than 36% of the entire Italian production (Tab.2).

Goat farming elected places were those zones, such as mountains of Sulcis–Iglesiente, Sarrabus–Gerrei, Barbagia, Ogliastra and Gallura, where it was possible only for this species to exploit the shrubs. Sarda goats, thanks to their rusticity, were (and are) the best ones to rear, perfectly adapting to the orography and to the scarce feed opportunity of these areas (Casu et al., 1981).

Region	Goats	Milk (t)
Abruzzo	20.378	1
Campania	44.322	10
Umbria	5.044	41
Toscana	15.538	80
Liguria	15.319	91
Calabria	115.185	92
Emilia Romagna	18.118	127
Trentino Alto Adige	13.495	237
Valle d'Aosta	4.934	371
Basilicata	58.491	424
Puglia	35.710	584
Sicilia	125.573	964
Friuli Venezia Giulia	4.467	1.348
Veneto	7.126	1.376
Lazio	38.139	2.561
Piemonte	66.018	4.309
Lombardia	75.596	5.097
Sardegna	**216.536**	**10.139**
Molise	7.037	-
Marche	4.578	-
Totale	891.604	27.852

Tab.2 Goat Population and Milk production in Italy (ISTAT 2012)

Starting from the last decade of 1900, with the increasing of numbers of intensive farms and the improvements of semi-extensive and extensive systems, goat farming took back its place in the economic and cultural substrate of Sardinia (Boi, 2010). Cosmopolitan dairy breed are reared in the first type of farms, while the Sarda, or its common crossbred with Maltese is reared under the extensive farming systems.

In 2013, according to Laore, there were 3.661 goats farms in Sardinia, best part of which were mixed farms with more than one species reared (Tab.3).

	Farms	%	Goats
	3.661		272.959
< 50 goats	2.322	63	173.056
Goat only	798	22	74.535

Tab.3 Number of Farm and Goat distribution in Sardinia (Laore 2013)

Extensive farms, that are the ones studied for this PhD work, are the most commons in Sardinia. Traditional management system conducted, they represent the large majority of goat farms, and are often associated to other activities, such as sheep or pig farming (78% on total of goat farms). Small farms with two or three heads are widely present, with the aim to provide milk and meat for the family subsistence (Fig.3).

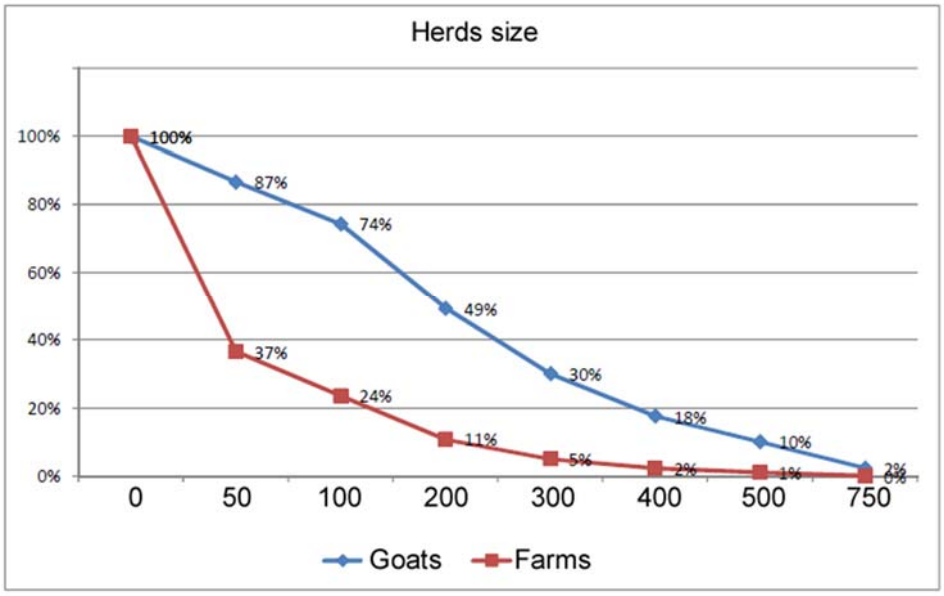

Fig.3 Herd size and farms percentage distribution (Laore 2013)

Over 2.300 farms on which goats are present have a little number of heads (less than 50), and goats were considered by farmers as a secondary activity. Only 798 farms were single-species farms. In these single-species farms, less than 28% of the total number of goats were reared, with 74.535 heads.

1.3 The local market development in Sardinia

As in other parts of the world, even in Sardinia farming is undergoing profound changes, mainly due to the need to increase production by reducing costs. This necessity, which on one hand can be curbed with the genetic improvement of animals, clashes with another issue of great importance, which is related to the environmental impact of livestock. The loss in biodiversity, the waste-emissions in the environment, the cutting of forests to make way for crops and pastures (Dubeuf et al., 2014) are just some of the experiencing problems, and on which even the average consumer is giving now more and more attention.

In our Region, thanks to the low number of cattle and pig intensive farms, and the fact that sheep and goat farms are still conducted predominantly in extensive or semi-intensive systems, these problems are not very prominent, although in some particularly productive areas (eg Arborea, Oristano, for dairy cattle farms) they can be observed, and the rationalization of resources is necessary.

In this contest, the goat breeding should be a good alternative to the global distribution of the products, thanks to a short-chain system, unofficially adopted during the years by the large part of the farmers, but that only in recent years has seen the involvement of authorities such as Confagricoltura and Coldiretti, that, by installing zero-km markets, started to establish a two-way direct relationship between farmers and consumers.

In addition to the direct sale of products, the development of the local market also needs the convergence of farms the “urban” consumer (Sayadi and Calatrava, 2001). In this optic, even in Sardinia more and more farms open their doors to visitors, showing them how animals are raised and how the products they buy in markets are processed, creating a relationship of trust and knowledge that is positive both for producers and consumers.

This process needs to be further developed, giving to the consumers not only the information about the animal welfare and farm rearing conditions, but also the ones related to the characteristics of the products be it meat or milk.

1.4 The Sarda goat breed

Sarda Goat is autochtonous of Sardinia and belongs to the Mediterranean group, but it's usually considered as a dairy goat. It's mainly reared in extensive and semi-extensive systems, in herds of medium or high dimension with farms often located in mountainous and difficult to access areas (Figg.4-5). There are three main populations, according to the altitude they are usually reared (lowloands, hills, mountains), with a high range of body size. Females are usually in a average of 45 kg and males about 60, but there are severe variations related to the altitude zone and within the same herd too. The season is a factor that affect the weight of animals too, especially in those farms where a low or none feed integration is given to the goats.

Fig.4 Sarda goat herd

Fig.5 Sarda goat in a mountainous area

Milk production is high variable, averaging on less than 1 l per day during the middle of lactation, but with huge variations depending by several factors, both genetic and management related (Vacca et a., 2006; Balia et al., 2009; Dettori et al., 2015a; Dettori et al., 2015b). These factors also affects fat and protein percentage, which are usually high if compared with other breeds, but with a high difference depending within the herds.

Productivity season is highly conditioned both by the annual pasture cycle and the strong reproductive season. Young goats become fertile approximately at the 7^{th} - 9^{th} month, usually from September to November, while pluriparous goats, after an eventual pause due to the end of lactation, have a longer reproductive period, usually from June to December. Mating is free and males per females ratio is in a range of 1:20 - 1:40. Parturitions are concentrated in autumn for the pluriparous and during the spring for young goats. Depending on farms, the percentage of females reared for replacement vary from 20 to 30%. Animals are usually hand-milked, with

a ratio of 1:150 operator:animals, and lactation last for 5-6 months after kids weaning. Kids are reared with traditional system, usually locked for the entire day in buildings named “Caprettile” (Fig.6), with natural feeding until 5-6 weeks if destined to meat production or until 10 weeks with gradual weaning if destined to the replacement quota. In latest years, artificial feeding has been proposed in order to fight those viral disease such as SRLVs that are strongly present in goat and sheep farms in the Mediterranean area (Peris et al., 1997), as it has been widely proofed that the main cause for infection in kids is dams milk (Reina et al., 2009).

Despite goat kids meat and goat milk price are usually higher than lambs meat and sheep milk ones, there are none or very few companies that pay farmers for milk quality, although it has been advocated by the same region Sardinia, in particular for milk, the development of a payment system based on the quality (Pirisi et al., 2007).

Fig.6 A “Caprettile”, the typical building where goat kids are locked during the day

As concerns purposes of selection and the characteristics of the breed, they have been set in 2009 by Asso.Na.Pa and can be briefly summarized.

Milk production increase is the main objective of selection, both for quantity and quality traits. The average production of this breed is lower than dairy specialized breed, but it can be considered as satisfactory if related to the environmental and management conditions (Bittante et al., 2005).

In order to be included in the genealogic book, goat must have ancestors already included in it, or, if the herd is requiring inscription for the first time, animals of first registration must be under one year of age and offspring of registered animals.

Characteristics of registered animals must be:

- Body of medium size, ranging from 68 to 78 cm of height and from 40 to 60 kg of weight for males; 64 to 70 of height and from 30 to 45 kilos of weights for females.
- Head: straight front-nose profile, large and heavy in males, small and light in females. Middle size and horizontal ears. Horned or polled.
- Neck: long and thin, with or without laciniae.
- Trunk: deep chest, wide abdomen, straight back, sloping croup.
- Udders: rounded udders with big and well spaced teats; additional teats are considered as defective, but tolerated.

- Legs: strong, tough hoof.
- Coat: on white or grey base or totally coloured (Figg. 7-8).
- First parturition: 18 month.
- Fertility 0,93; prolificity 1,3.

Selection, as intended by Asso.Na.Pa., started just few years ago, but farmers' one has its root in the beginning of the last century, and led to the three different sizes of Sarda Goat, reared in different geographical areas. Brandano and Piras (1978) gave an association for size and geographical areas as follows:

- Small size animals are mainly reared on Ogliastra, Gerrei and Montalbo areas, and it counts about a quarter of the entire population.
- Medium size animals are about the 60% of the population, and their rearing is concentrated on Barbagia, Sarrabus and Iglesiente.
- Big size animals are usually reared on coastal areas, such as Baronie, Sulcis and Planargia and it is the smallest part of the population.

Small and medium size population are usually characterized by small ears goats, and in the usual classification, these animals are considered the most similar to the native breed, while in the big size animals long ears are often present, fact that identifies those animals of crossbred population with Maltese blood. Horned and polled animals are present in all population with a ratio of 4:1 and a great variety of horn shapes and sizes.

Fig.7 Sarda goats colour variety is well evidenced in this photo

Fig.8 Sarda goat with white base coat

Starting from the beginning of the century, several researches (Macciotta et al., 2002; Pazzola et al., 2002) pointed out that animals reared in coastal areas have the bigger variety and more rustic characteristics (presence of horns, small size and ears), while the ones of mountains are the most similar each others, and on some spot areas there are more crossbred animals than native one (Sulcis, for example). The most used breed for crossbreeding is Maltese (Fig.9), thanks to its

adaptability to the environment, its good prolificacy and longevity (Vacca et al., 2005).

This is basically due to the fact that farmers choices are often related to functional needing, such for udder shape, or to personal preferences, for example for coat colour or horns presence.

Fig.9 Sarda goat herd with evidence of Maltese breed

As Sarda breed has is strength point in the rusticity, selection is also intended on improving those trait related to the ability of animals to survive in the scarcity of the habitat they are reared. So the preferred traits for selection are related to the morphological characteristics that allow to animals to better exploit the environment. Selection schemes are set up by a technical commission named Commissione Tecnica Centrale (Central Technical Commission) which list the best animals to be chosen by farmers for the best performance in the desired trait. This list change year

by year, and each farmer has free access to it and the opportunity to register animals if they reach the minimum performances required by the Commission. For males, in order to be admitted for AI, the minimum requirements must be proved for the selected trait (fertility, for example), and a series of anomalies, such as cryptorchidism or umbilical hernia, for example, automatically excludes animals from selection.

In 2013 11.524 animals, that is less than 5% of goats reared in Sardinia, were registered on genealogic book (ICAR), although the best part of the goat population presents the phenotypical traits of the breed.

1.5 The Goat milk and the casein polymorphisms

Goat milk is a good replacer of meat in those areas where, for religious or environmental reasons, is not possible to rear meat animals (Cristofori, 1994), and about half of the human world population use ita s food. Its chemical composition is not so different from the bovine one, with fat percentage in a average of 4,5% and protein of 3,5%. Depending on the breed, these values can assume important differencies and it's well known that alpine breeds produce a milk more similar to the bovine milk, while mediterranean breeds, including the Sarda, produce a milk similar to the sheep one (Trujillo et al., 1997; Awad et al., 1998).

Goat milk gain the consumer attention as in it β-lactoglobulin and caseins are present in a lower amount than in bovine milk, giving to goat milk a high digestibility and a lower risk of intolerance for infants. Furthermore, fat globules have a smaller diameter (Attaie & Richter, 2000), and selenium (Debski et al., 1987) and taurine (Mehaia et al., 1992) contents are similar to the human milk.

Casein fraction constitutes about the 80% of the total proteic content of milk, and althought the casein structure is almost the same trought the different species, there are several differences in the casein ratio from species to species and within the breeds.

In the latest years researchers that studied goat milk pointed the focus on caseins, as they are the principal responsible of coagulation, giving to the curd a particular consistence different from all the other dairy

animals milk, althoug you consider similar casein concentrations (Park, 2007).

As in the other species, in goat milk caseins are present in 4 different types (αs_1, αs_2, β and κ), clustered with calcium. The α- and β-caseins, are calcium sensitive caseins and precipitate in the presence of calcium. The k-casein are not calcium sensitive, and contain on the surface of micelles a hydrofil C-terminal that give stability to the micelle and prevents the association with other micelles. The introduction of rennet split this hydrofil C-terminal and micelles are free to aggregate, causing the formation of the curd. The strenght and the speed of formation of the curd depends on several factors, such as pH, temperature, amount of rennet and so on. This mechanism is still under investigation, as several components look like they are involved in it (Lucey, 2004).

Goat displays a high variable composition regarding the genetic casein cluster, that affect the possibility of using the milk for cheese making or as drinking milk (Grosclaude et al. 1994; Barillet 2007). Genetic information about casein production are written on 6 in goat and are organized as a cluster with this succession: αs_1, β, αs_2, and κ (Rijnkels, 2002).

The CSN1S1 (αs_1), CSN2 (β) and CSN1S2 (αs_2) genes encode for the so named calcium-sensitive caseins, whereas CSN3 (κ) is a gene that have the role of stabilizing the casein micelle (Rijnkels et al. 2003). The αs1-casein is the one most studied and shows at least 18 alleles. It's

possibile to classify those alleleas as strong: A, B1, B2, B3, B4, B0, C, H, L, M; intermediate: E, I; weak: D, F, G; null: 01, 02, N, depending on the gene expression levels (Grosclaude & Martin 1997; Martin et al. 2002). The β -casein gene includes at least 8 alleles: A, A1, B, C, D, E, 0 and 01 (Marletta et al. 2007). The αs2-casein gene includes 7 alleles classified as: strong (CSN1S2 A, B, C, E, F), intermediate (CSN1S2 D) and null (CSN1S2 0) (Ramunno et al. 2001). The k-casein, encoded by the CSN3 gene, has at least 22 alleles: A, B, B', B'', C, C', D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R and the lately, first described in Sarda goat, S (Prinzenberg et al. 2005; Gupta et al. 2009; Vacca et al., 2014).

Sarda goat milk has been association with casein genotypes have been widely invistagated during the past years by (Vacca et al. 2009; Balia et al. 2013; Vacca et al., 2014), and it has been possibile to describe a huge variation in the casein expression.

Due to the high variability of the Sarda goat further studies are needed in order to characterize the genetic heritage of this breed.

2. Aim

Biodiversity, intended as the degree of variation of life, is constantly endangered by human activities, especially by those that concern farming systems and animal rearing. Also, markets going day by day more global evidenced the necessity of the introduction of more productive breeds in order to compete with the foreign products, often by complete replacing local breeds. This practice happened also in Sardinia (Italy) for the goat farming system, in which the local breed, Sarda, has been crossbred with other dairy goat breeds in order to increase productivity, causing a loss in the genetic heritage of this Mediterranean breed.

In this scenario very few opportunities for an income increase are offered to the single farmer, so Farmers Associations should try to push in order to obtain a PDO or PGI product, just like the ones already present for Sarda sheep (three for cheese and one for meat).

At the moment, the knowledge consumers have about caprine products is really scarce, and only a few part of them ask for products to farmers, usually directly or by the intermediation of small factories family conducted, and there is no or organized market for the distribution of extensive reared goat products. In the latest years, short chain markets are emerging, trying to close the consumer to farmers and viceversa. This practice of direct buying from farmers in a zero-km market, if from a point of view consents to maintain the caprine products market active, on the other side is the weak ring of the chain, closing the possibility to

distribute goat products on supermarkets, because of the lack of an official health certificate.

In order to create a market for these products it is necessary to guarantee to farmers the possibility of an adequate income, and the best way to do this is to widen the portfolio of products and/or to define a disciplinary of production for PDO products.

In this PhD work, the focus has been set on the two caprine products of Sarda breed: kids meat and goat milk. The increasing in knowledge about these two products must be considered as the basis for the further development of this extensive farming system. Furtehrmore, it should help farmer's association interested in the appliance for PDO products requests, assuring them the proper income and helping to the preservation of the breed and its uniqueness.

3. Research plan

Preparing a research plan for this PhD course the main problem I encountered it was to attribute a different grade of importance to each part. In an extensive system, the first production we have is the one of goat kids meat, as milking starts after weaning of kids, so the first two works listed in this thesis are related to goat kids meat production, its characteristics and the possibility of rearing kids by the use of a milk replacer. After that, papers related to milk composition and caseins polymorphisms in Sarda goat are listed.

As far as concern meat productions, I worked in traditionally managed farms, where goat kids are usually slaughtered at 42 days of age. Object of my study was to better know the productive traits of goat kids carcasses and meat and to find out if it's possible to set up an artificial system for feeding them instead of natural feeding.

As regards the productive traits, 40 kids where used, 20 males and 20 females. They were fed by their dams, and weighed at birth and weekly until slaughter. Several body measurements where recorded during weighing sessions, such as height at withers, height at rump, body length, etc. After slaughtering, the percentage of the different components in relation with body weight has been recorded, and after a 24 hours refrigeration, measurements on the most commercial cuts have been performed. Furthermore, moisture, ashes, crude protein, total lipids,

cholesterol content were determined. Fatty acid composition of the meat has been registered too.

Concerning the set up of an artificial feeding system, a research has been conducted on 32 goat kids, born from SRLVs-free dams, half of which were naturally fed and half were artificial fed. After the initial four days from birth, on which colostrum and mixed colostrum+replacer were used for feeding, the artificially fed animals where fed by using an acid replacer for milk, using for each one a single box. Milk of dams and the commercial replacer were compared. Before slaughtering, a blood sample was taken from kids, and biochemical, enzymatic and mineral parameters were registered and compared between the two feeding groups. After slaughtering, carcass measurements and indexes of kids were recorded, and right half of carcasses was divided into commercial cuts. Components of the cuts had been separated and their percentage had been registered. Data were later submitted to statistical analysis performed by using SAS software.

Regarding milk traits, renneting properties and their association with genetic, our research mainly focused on casein gene cluster.

In our first research we investigated the influence of casein gene polymorphisms on renneting properties of milk from Sarda goats in 200 animals from 3 commercial farms. Milk yield and composition, renneting properties (rennet coagulation time, firming time and curd firmness) were evaluated at monthly intervals from March to July. Furthermore, animals

were genotyped at CSN1S1, CSN2, CSN1S2 and CSN3. A general linear model has been used in order to analyse the recorded data.

In the second research, genetic variants at the CSN1S1, CSN2, CSN1S2 and CSN3 gene loci were investigated by PCR-based methods, cloning and sequencing. In this research a new found allele for CSN3, variant S (GenBank KF644565) has been described here for the first time in Capra hircus.

3.1 Paper I

Productive traits and carcass characteristics of Sarda suckling kids

G.M. VACCA, M.L. DETTORI, P. PASCHINO, F. MANCA, O. PUGGIONI, M. PAZZOLA

Dipartimento di Medicina Veterinaria, Università di Sassari, via Vienna, 07100 Sassari, Italy

SUMMARY

Italy is known for its tradition in goat farming and the Sarda autochthonous goat from Sardinia is one of the most important breeds of the Mediterranean Basin. The suckling kid from this breed is a traditional meat product and, despite the good acceptance by the consumer, it is not recognised with a protected geographical indication or designation of origin mark. In order to give a first cognitive insight into the main productive and quality characteristics of carcass and meat, a total of 40 kids (20 males and 20 females) milk fed by their dams and slaughtered at 42 days of age were studied. *In vitam* and *post mortem* performance traits were registered. These included the sectioning in commercial parts and their dissection into the different tissue components: muscle, separable fat and the bone + tendon. Chemical composition, fatty acid and cholesterol content were determined from the *longissimus dorsi* muscle. Student's t test was applied to evaluate the differences between the sexes. *In vivo* parameters were similar between sexes, and differences between sexes were registered for height at withers, rump length and body length, with higher values in males. Males showed better carcass yields which were longer and with higher hip width. Carcasses, classified as first quality in both sexes, were characterized by a low content of adipose tissue and a good percentage of muscle, and showed the tissue composition similar between sexes, except for the lower content in muscle tissue of the leg from males. The low lipid deposit suggested the possibility to increase the age and weight at slaughter, in order to improve the commercial value of this product. The chemical composition of the *longissimus dorsi* muscle was similar between the sexes, while cholesterol content, oleic acid and MUFA (Monounsaturated Fatty Acids) were lower in males than females. In conclusion, meat had low cholesterol and lipids content, confirming that the Sarda suckling kid has an excellent nutritional value.

KEY WORDS

Sarda breed, kids, carcass traits, meat composition.

INTRODUCTION

Among the Italian regions, Sardinia has a predominant role in goat farming both for the animal heritage and for the amount of dedicated farms[1]. The Sarda, autochthonous goat from Sardinia, is one of the most important breeds of the Mediterranean Basin area[2]. The Sarda goat is an excellent user of pastures of high hills and mountains and it has been selected for centuries by farmers, in order to increase the amount of milk produced[3], which is characterized by high fat and protein content[4], a good content of essential amino acids[5] and a fairly good cheese making attitude[6]. The most popular goat meat product is the suckling kid. It has been estimated that about 100,000 kids per year are slaughtered, in the period from November to April. Kids are mainly held in a goat hut and fed by their dams coming back from the pasture for milking; they are slaughtered when they are 40 days old and sold at a price constantly higher than that fixed for lambs[7], contributing for a significant percentage to the total income of the farm. This income, however, does not correspond to a real profit, since milk feeding implies high production costs which are not fully rewarded by the market. Best revenues could result from the recognition and protection on the markets of this product: for the lamb produced in Sardinia, a protected geographical indication (PGI) has been established ("Agnello di Sardegna", European Union, Commission Regulation no. 138/2001), but nothing has been done in this direction for the Sarda goat kids. Considering the lack of information on Sarda goat kids, the aim of this study was to provide a preliminary framework on the productive performance and meat quality of Sarda suckling kid.

MATERIALS AND METHODS

The research was performed on 40 Sarda kids (20 males and 20 females), milk-fed by their dams and slaughtered at 42 ± 1 days of age. Animals were all born from single parturition and were reared in a farm located in Central Sardinia (40.15° N; 9.25° E); according to traditional extensive techniques, the herd was allowed to browse about 200 hectares with an average elevation of about 650 m above sea level. Kids were weighed at birth and later at weekly intervals until slaughter; furthermore, during each of the weighing sessions, the following somatic measurements and indexes were also detected: height at withers, height at rump, body length, chest height, chest width, chest girth, rump length, rump width at hips, rump width at pin bones, metacarpus girth, head length, head width, leg and carcass compactness. After slaughter, carried out in accordance with the European Community Regulation 86/609, percentage of the different components

Autore per la corrispondenza:
Giuseppe Massimo Vacca (gmvacca@uniss.it).

in relation to net live weight was recorded for each kid. Carcasses were classified after a 24-hour refrigeration period at 4° C, in accordance to the scheme proposed by the Council Regulation EEC n. 2137/92, and measured to achieve body length and width at loin and chest. Carcasses were later divided in two symmetric halves along the midline and each one of these was measured for internal length, chest depth and leg length. Right halves of all carcasses were dissected into commercial anatomical parts: neck, shoulder, ribs, chest, leg, loins and flank; from each of these parts muscle tissue (M), separable fat tissue (SF) and the bone and tendon components (BT) were separated. Measurements, indexes and dissections were performed according to the scheme reported for Sarda lambs[8].

The *longissimus dorsi* muscle (LD) was cut off from left halves to determine moisture, ashes and crude protein, by Association of Official Analytical Chemists methods, total lipids and cholesterol content[9]. Fatty acid composition from LD total lipids was determined after derivatization with trimethylclorosilane and analyzed on a gas chromatograph, with flame ionization detector. Separations were performed using a Varian WCOT polar capillary column (50 mx 0.25 mm id). Milk samples from the dams were taken on the day of weaning and analyzed for fat, protein, casein and lactose content using an infrared spectrophotometer (Milko-Scan 133B; Foss Electric, DK-3400 Hillerød, Denmark). Energy of milk was calculated using the values proposed by the National Research Council. Data were subject to Student's t-test to evaluate differences between the sexes, using the statistical software Minitab™ release 13.32 (Minitab Inc. 2000, State College, PA).

RESULTS AND DISCUSSION

The composition of milk, collected from the dams, on the day of weaning of kids showed that protein (3.90±0.31%), fat (5.23±0.76%) and lactose content (4.93±0.13%) were similar or higher than the range reported for the Sarda goat breed[10,11] in a stage of lactation similar to the present study; energy was on average 3.76±0.42 MJ/kg. Figure 1 shows live weights recorded at weekly intervals. Weights at birth (3.42 kg for males and 2.95 kg for females) and at 42 days of age, which corresponds to the day of slaughter (9.34 kg for males and 8.55 kg in females), were similar in both sexes as those reported for suckling kids of Balkans[12], Florida[13] and Serrana and Brava breeds and their crossbreeds[14]. Weights at slaughter for Sarda kids were higher than those reported for the Garganica breed[15] slaughtered at the same age and for some Spanish breeds slaughtered between 42 and 46 days[16]. As regards somatic measures (Table 1), differences between sexes were registered for height at withers, rump length ($P<0.01$) and body length ($P<0.05$), with higher values in males.

Figure 2 shows, for both sexes, the trends of average daily weight gains. No statistical difference was found between sexes and throughout the whole period males showed a mean value of 140.9 g/d and females 133.3 g/d. Average daily weight gains were, as expected, gradually increasing.

Table 2 shows the results for measurements at slaughter. The percentage of the carcass on the net live weight was higher in males, while the other traits, as well as the pH of the *longissimus dorsi* registered on the hot muscle, showed similar values in both sexes. Different incidences of the carcasses between sexes have also been reported in Balkan goat kids[12], but those animals have been slaughtered at a live weight more than twofold, if compared to the present research and with females showing the highest carcass yield. The incidence of

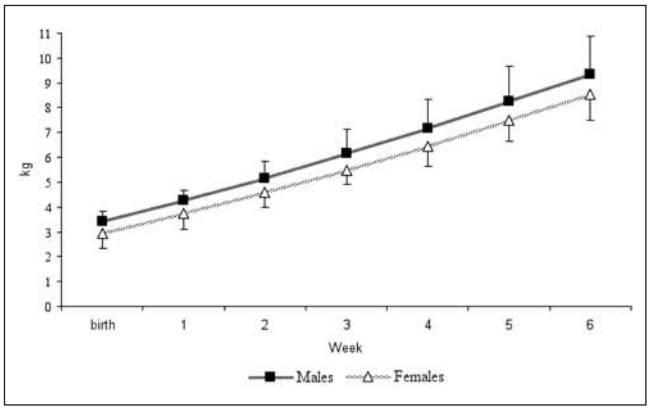

Figure 1 - Live weights (kg) and standard deviations of kids according to age and sex. Differences between sexes are not significant.

Table 1 - Somatic measurements (cm) of kids at 42 days of age according to sex.

Measurement (cm)	Males Mean S.D.	Females Mean S.D.	P-value
Height at withers	44.2 ± 2.36	39.6 ± 1.14	**
Height at rump	45.7 ± 3.33	42.2 ± 1.52	ns
Body length	43.1 ± 2.13	39.6 ± 2.22	*
Chest height	16.6 ± 1.27	14.8 ± 1.57	ns
Chest width	10.1 ± 1.08	9.3 ± 0.88	ns
Chest girth	49.0 ± 3.26	46.9 ± 2.53	ns
Rump length	14.6 ± 0.65	13.1 ± 0.65	**
Rump width hips	7.8 ± 0.97	7.1 ± 0.54	ns
Rump width pin bones	5.4 ± 0.65	4.9 ± 0.74	ns
Metacarpus girth	6.7 ± 0.33	6.3 ± 0.27	ns
Head length	14.8 ± 0.83	14.4 ± 0.42	ns
Head width	8.7 ± 0.56	8.7 ± 0.27	ns

** = P<0.01; * = P<0.05; ns = not significant.

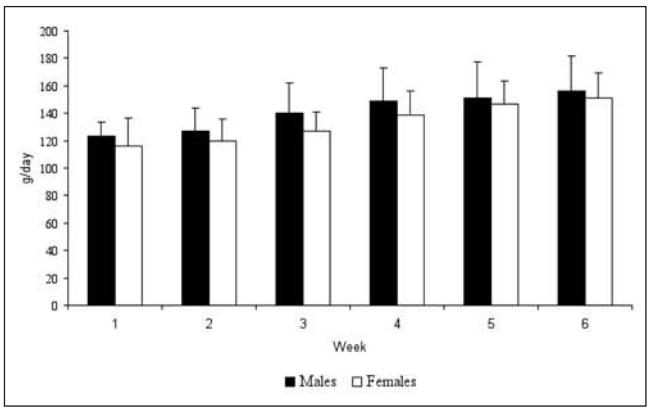

Figure 2 - Average daily weight gain (g/day) and standard deviations of kids according to the sex and week of age. Differences between sexes are not significant.

Table 2 - Slaughtering performance of kids according to the sex.

Parameters	Males Mean S.D.	Females Mean S.D.	P-value
EBW (g)	8,830 ± 987	7,780 ± 940	*
Proportion (%) on EBW of:			
Hot Carcass	53.12 ± 1.52	51.53 ± 2.37	*
Head	6.98 ± 0.46	7.08 ± 0.41	ns
Skin and limbs	12.89 ± 0.34	12.97 ± 0.23	ns
Internal depots fat	3.44 ± 1.14	3.43 ± 0.97	ns
Heart, lungs and liver	7.28 ± 0.91	7.74 ± 0.88	ns
Empty gastrointestinal tract	7.51 ± 0.50	7.92 ± 0.45	ns
Blood	5.02 ± 0.37	5.43 ± 0.62	ns
Urogenital organs and weight loss	3.76 ± 1.11	3.90 ± 1.33	ns
pH *longissimus dorsi* muscle (hot)	6.40 ± 0.26	6.34 ± 0.19	ns

EBW: empty body weight; * = P<0.05; ns = not significant.

the carcass was in accordance with the range of values measured in Canary[17] and in Spanish breeds[16].

As regards slaughter yield, the incidence of head and offals (heart, lungs and liver) should be carefully considered as in the tradition of many European countries[14] they are sold together with the carcass. The incidence of head was lower than those described in Girgentana[15] and Canary kids[17] and higher than that reported in Florida kids[18], while the incidence of offals was higher than those of most of the breeds[13,15,17]. The incidence of internal fat (perirenal, pelvic, mesenteric and omental) was similar or lower than data regarding kids of several Mediterranean breeds[13,14,15] and it can be considered positive, due to the fact that in goats fat is early deposited as internal fat[18].

Table 3 shows values of the measurements carried out on the carcasses and the compactness values of carcass and leg. Half carcasses of male kids were longer (P<0.01), with higher rump width and length of leg (P<0.05). Carcasses were classified according to the weight (A), meat color (pink) and adiposity class (3rd class), and were ranked, for both sexes, as first quality. This can be considered a very positive feature in relation to the possibility of obtaining a product with a recognized mark.

Table 3 - Carcass measurements and compactness indexes of kids according to the sex.

Parameters	Males Mean S.D.	Females Mean S.D.	P-value
Carcass length (cm)	50.87 ± 3.81	48.73 ± 5.09	ns
Loin width (cm)	15.53 ± 1.61	14.22 ± 1.14	*
Chest width (cm)	11.71 ± 1.63	11.25 ± 2.37	ns
Half carcass internal length (cm)	40.89 ± 2.54	37.53 ± 1.85	*
Chest depth (cm)	10.82 ± 0.80	9.13 ± 1.47	ns
Leg length (cm)	24.21 ± 1.65	22.02 ± 1.03	**
Leg compactness (g/cm)	3.03 ± 0.84	2.74 ± 0.93	ns
Carcass compactness (g/cm)	118.2 ± 26.2	135.9 ± 33.1	ns

** = P<0.01; * = P<0.05; ns = not significant.

Table 4 - Proportion of parts and pH of cold half carcass according to the sex.

Parameters	Males Mean S.D.	Females Mean S.D.	P-value
CHCW (g)	2,345 ± 542	2,005 ± 672	*
Proportion of parts (%):			
Neck	8.11 ± 0.75	8.26 ± 0.54	ns
Shoulder	23.62 ± 1.11	23.01 ± 1.32	ns
Breast	11.31 ± 0.78	11.64 ± 0.89	ns
Rib	14.54 ± 0.91	14.83 ± 0.95	ns
Loin	7.56 ± 0.37	7.69 ± 0.47	ns
Flank	2.86 ± 0.61	2.93 ± 0.60	ns
Leg	32.00 ± 1.72	31.64 ± 1.94	ns
pH *longissimus dorsi* muscle (cold)	5.89 ±0.19	5.92 ± 0.12	ns

CHCW: cold half carcass weight; * = P<0.05; ns = not significant.

Table 4 shows weights of the right cold half-carcasses and the percentage of the different anatomical parts. Although kids with the present light-weight are sold on the market as a whole carcass on the basis of local tradition, it was considered useful to make the dissection to better understand how the parts are distributed in view of a commercial production of subjects of a higher weight, for which it could be possible marketing of the different parts. The production of heavier kids is based on the extension of the interval of milk-feeding and should be consequently supported by the improvement and profitability of meat price or the production of animals fed with milk replacer. Half carcasses of males showed higher weight values than females (P<0.05), while the percentage of the different commercial parts and the pH of the *longissimus dorsi* muscle were not significantly influenced by sex. The incidence of commercial parts were similar in both sexes also in kids of "Cabrito de Barroso" PGI[14]. Percentage of the main marketable parts are similar to those recorded in kids of similar weight of different breeds reared in Spain[17]. It is significant that the sum of shoulder and leg represented, in both sexes, more than half of the entire half-carcass weight (55.62% in males and 54.65% in females). Percentages of these parts detected in Sarda kids are similar to those reported in Florida kids[13].

Table 5 shows the results after separation of muscle, adipose and bone tissues. In both sexes, parts with high muscle percentages were the flank, leg and shoulder, while high adipose percentage was recorded for the chest. Half carcasses were characterized by a low lipid content (10.63 in males and 21.11% in females) and a good percentage of muscle (60.43 in males and 58.87% in females), which was intermediate between the one found in kids of similar weight of Florida kids[13] and that reported in "Cabrito de Barroso" kids[14]. Tissue composition of anatomical parts did not differ significantly between the sexes, except for the percentage in muscle tissue of the leg which was higher (P<0.05) in females (69.17% vs. 64.08). Similar results are reported in "Cabrito

Table 5 - Tissue proportion obtained after dissection of left half carcass according to the sex.

Parameters		Males Mean S.D.	Females Mean S.D.	P-value
Neck	M	56.34 ± 5.51	59.42 ± 5.36	ns
	IF	12.28 ± 2.91	11.84 ± 2.40	ns
	BT	31.38 ± 3.22	28.74 ± 2.97	ns
Shoulder	M	61.24 ± 2.23	62.83 ± 1.21	ns
	IF	9.55 ± 1.54	8.12 ± 1.14	ns
	BT	29.21 ± 1.64	29.05 ± 1.26	ns
Breast	M	48.98 ± 6.23	50.15 ± 5.98	ns
	IF	17.61 ± 5.76	15.78 ± 6.06	ns
	BT	33.41 ± 4.32	34.07 ± 4.57	ns
Rib	M	59.37 ± 5.70	59.23 ± 6.26	ns
	IF	9.15 ± 6.97	8.65 ± 7.19	ns
	BT	31.48 ± 4.02	32.12 ± 4.44	ns
Loin	M	54.74 ± 7.14	58.08 ± 6.91	ns
	IF	13.81 ± 3.33	13.41 ± 3.24	ns
	BT	31.45 ± 8.41	28.51 ± 9.00	ns
Flank	M	69.41 ± 6.34	71.11 ± 7.45	ns
	IF	30.59 ± 6.34	28.89 ± 7.45	ns
Leg	M	64.08 ± 1.81	69.17 ± 1.03	*
	IF	6.47 ± 1.71	5.71 ± 1.68	ns
	BT	29.45 ± 1.84	25.12 ± 1.55	ns
Half carcass	M	60.43 ±11.38	58.87 ±14.86	ns
	IF	10.63 ± 2.96	11.21 ± 3.17	ns
	BT	28.94 ± 9.99	29.92 ± 8.45	ns

M: muscle; IF: intermuscolar fat; BT: bone + tendons; * = P<0.05; ns = not significant.

de Barroso"[14] as no differences has been found in the composition of the half-carcasses between the sexes, while higher percentages of fat were found in females kids slaughtered at a higher weight[13]. This could be due to a higher precocity of females for fat deposition, but this characteristic usually occurs in kids older than the Sarda of the present study. As regards percentage of separable fat, Sarda kids carcasses had lower values than those found in "Cabrito de Barroso"[14] and similar to those found in Florida kids[13].

Data about chemical composition and cholesterol content of LD muscle are shown in Table 6. Chemical composition was similar between the sexes, while cholesterol content was significantly higher in females (45.39 vs. 36.69 mg/100 g). Both sexes evidenced good contents of proteins and low levels of lipids. This latter finding may be due to a lower capacity of goats, compared to sheep, for the deposition of intramuscular fat[18] and is in accordance with data regarding the "Agnello di Sardegna PGI" (Sarda lamb) slaughtered at 30-40 days of age[19] which shows in the muscle about a twofold percentage of lipids and cholesterol if compared to Sarda kids of the present study. Furthermore, comparison with other goat breeds evidence that chemical composition is similar to that described for Murciano-Granadina kids[20] but with a higher protein and lower fat content than kids of equal weight of Garganica[21]. Papers reporting cholesterol content allow some interesting comparisons with kids of other goat breeds. Sarda kids had a cholesterol content half of that registered for Murciano-Granadina[20] and lower than Criollo Cordobes and Anglonubian[13]. This can be considered a very favourable result because the levels of cholesterol in meat are negatively correlated to weight and age of the animals[8].

Table 6 - Chemical composition and cholesterol content in *longissimus dorsi* (LD) muscle according to the sex.

Parameters	(unit)	Males Mean S.D.	Females Mean S.D.	P-value
Moisture	(%)	75.64 ± 0.85	75.92 ± 0.91	ns
Crude protein	(%)	22.22 ± 0.66	21.89 ± 1.18	ns
Total lipids	(%)	0.99 ± 0.56	1.03 ± 0.52	ns
Ashes	(%)	1.15 ± 0.05	1.16 ± 0.07	ns
Cholesterol	(mg/100 g)	36.69 ±12.99	45.39 ± 19.32	*

* = P<0.05; ns = not significant.

Table 7 shows fatty acid composition of the *longissimus dorsi* muscle. No difference was found between sexes, with the exception of C18:1 and MUFA (Monounsaturated Fatty Acids) higher in females (P<0.05). Among the different fatty acids, the highest percentages were recorded for C18:1, C16:0 and C18:0, with higher values to that reported in other breeds[16,21]. This characteristics could be explained by the digestive functionality of kids which are monogastric in this stage and the noticeable influence of the fatty acid composition of milk. In particular, milk from extensively reared Sarda goats in the first stage of lactation show s the prevalence of C16:0, C18:1, C10:0 and C18:0[5]. If compared to Sarda suckling lambs[19], Sarda kids of the present study had a similar content of essential fatty acids C18:1, C18:2 and stearic

Table 7 - Fatty acid composition of intramuscular fat of *longissimus dorsi* muscle according to the sex.

Fatty acids		Males Mean S.D.	Females Mean S.D.	P-value
Lauric	C12:0	1.13 ± 0.91	1.20 ± 0.64	ns
Miristic	C14:0	8.20 ± 3.38	8.33 ± 1.68	ns
Palmitic	C16:0	30.19 ± 4.38	29.15 ± 5.35	ns
Palmitoleic	C16:1	2.26 ± 1.35	2.34 ± 0.99	ns
Stearic	C18:0	15.26 ± 1.49	13.94 ± 3.22	ns
Oleic	C18:1	30.30 ± 3.74	32.53 ± 4.41	*
Linoleic	C18:2	8.77 ± 2.38	8.92 ± 2.30	ns
Eicosatrienoic	C20:3	1.06 ± 0.43	0.91 ± 0.53	ns
Arachidonic	C20:4	2.71 ± 1.16	2.56 ± 1.07	ns
Docaseinoic	C22:6	0.12 ± 0.03	0.12 ± 0.06	ns
Sums and ratio				
SFA		54.78 ± 5.38	52.62 ± 6.80	ns
MUFA		32.56 ± 2.89	34.87 ± 3.57	ns
PUFA		12.66 ± 3.96	12.51 ± 3.48	ns
SFA/UFA		1.21 ± 0.34	1.11 ± 0.31	*

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = sum of MUFA and PUFA; * = P<0.05; ns = not significant.

(C18:0); on the contrary, C12:0, C14:0 and C16:0, which are considered responsible for rising of human blood cholesterol, and contents of SFA (Saturated Fatty Acids), were characterized by higher concentrations. On the other hand, Sarda kids showed leaner carcasses, lower intramuscular lipid and cholesterol content than Sarda lambs[8,19]. As regard the comparison with other caprine breeds, values of SFA of Sarda suckling kids were lower than those recorded in other dairy goat breeds[20]. Overall, the fatty acid composition was less favourable than that registered for some Spanish meat specialized breeds and this result has been explicated by an earlier deposit of lipids in skeletal muscles which occurs in dairy breeds[1]. These results are apparently negative but they should be considered together with the low lipid content of the meat. Indeed, the total amount of saturated fatty acids per kg of meat from Sarda kids is very low when compared with other goat breeds. On the basis of these results, it could be suggested an increase of slaughtering age, but attention should be paid to the fact that weight has a significant effect on fat deposit, as like the increase of intermuscular fat which has been recorded in kids with a live weight of 25 kg[17].

CONCLUSIONS

The results of the present study showed that *in vitam* and *post mortem* performances of Sarda kids at 42 days of age are little influenced by the sex, except for the fact that males had better carcass yields and meat with a lower cholesterol content. Overall, Sarda suckling kids had excellent lean carcasses and were characterized by low levels of cholesterol and intramuscular fat. The low lipid deposit also suggested the possibility of a slightly increase for age and weight at slaughter, in order to improve the commercial value of this product. An increase of the marketing price, on the basis of its quality, could better pay back the high costs which derive from natural milk-feeding. Further studies may help to better characterize this product also at different slaughtering ages and facilitate the recognition with an official mark which can surely certificate the origin and uniqueness and contribute to the preservation of this animal genetic resource.

ACKNOWLEDGEMENT

This study was supported by FAR funds.

References

1. Pazzola M., Dettori M.L., Pira E., Noce A., Paschino P., Vacca G.M. (2014) Effect of polymorphisms at the casein gene cluster on milk renneting properties of the Sarda goat. Small Ruminant Res, 117: 124-130.
2. Vacca G.M., Dettori M.L., Carcangiu V., Rocchigiani A.M., Pazzola M. (2010) Relationships between milk characteristics and somatic cell score in milk from primiparous browsing goats. Anim Sci J, 81: 594-599.
3. Carcangiu V., Vacca G.M., Mura M.C., Dettori M.L., Pazzola M., Luridiana S., Bini P.P. (2009) Relationship between MTRN1A melatonin receptor gene polymorphism and seasonal reproduction in different goat breeds. Anim Reprod Sci, 110: 71-78.
4. Dettori M.L., Rocchigiani A.M., Luridiana S., Mura M.C., Carcangiu V., Pazzola M., Vacca G.M. (2013) Growth Hormone gene variability and its effects on milk traits in primiparous Sarda goats. J Dairy Res, 80: 255-262.
5. Balia F., Pazzola M., Dettori M.L., Mura M.C., Luridiana S., Carcangiu V., Piras G., Vacca G.M. (2013) Effect of CSN1S1 gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. J Dairy Res, 80: 129-137.
6. Pazzola M., Balia F., Carcangiu V., Dettori M.L., Piras G., Vacca G.M. (2012) Higher somatic cell counted by the electronic counter method do not influence renneting properties of goat milk. Small Ruminant Res, 102: 32-36.
7. Vacca G.M., Daga C., Pazzola M., Carcangiu V., Dettori M.L., Cozzi M.C. (2010) D-loop sequence mitochondrial DNA variability of Sarda goat and other goat breeds and populations reared in the Mediterranean area. J Anim Breed Genet, 127: 352-360.
8. Vacca G.M., Carcangiu V., Dettori M.L., Pazzola M., Mura M.C., Luridiana S., Tilloca G. (2008) Productive performance and meat quality of Mouflon x Sarda and Sarda x Sarda suckling lambs. Meat Sci, 80: 326-334.
9. Christie W.W., Dobson G. (1999) Thin-layer chromatography revisited. Lipid Technol, 11: 64-66.
10. Daga C., Paludo M., Luridiana S., Mura M.C., Bodano S., Pazzola M., Dettori M.L., Vacca G.M., Carcangiu V. (2013) Identification of novel SNPs in the Sarda breed goats POU1F1gene and their association with milk productive performance. Mol Biol Rep, 40: 2829-2835.
11. Pazzola M., Balia F., Dettori M.L., Mura M.C., Carcangiu V., Vacca G.M. (2011) Effect of different storage conditions, the farm and the stage of lactation on renneting parameters of goat milk investigated using the Formagraph method. J Dairy Res, 78: 343-348.
12. Marković B., Marković M., Josipović S. (2011) The growth during suckling period and carcass traits of kids of the Balkan goat breed. Maced J Anim Sci, 1: 61-66.
13. Peña F., Perea J., García A., Acero R. (2007) Effects of weight at slaughter and sex on the carcass characteristics of Florida suckling kids. Meat Sci, 75: 543-550.
14. Santos V.A.C., Silva A.O., Cardoso J.V.F., Silvestre A.J.D., Silva S.R., Martins C., Azevedo J.M.T. (2007) Genotype and sex effects on carcass and meat quality of suckling kids protected by the PGI "Cabrito de Barroso". Meat Sci, 75: 725-736.
15. Todaro M., Corrao A., Barone C.M.A., Schinelli R., Occidente M., Giaccone P. (2002) The influence of age at slaughter and litter size on some quality traits of kid meat. Small Ruminant Res, 44: 75-80.
16. Horcada A., Ripoll G., Alcalde M.J., Sañudo C., Teixeira A., Panea B. (2012) Fatty acid profile of three adipose depots in seven Spanish breeds of suckling kids. Meat Sci, 92: 89-96.
17. Marichal A., Castro N., Capote J., Zamorano M.J., Argüello A. (2003) Effects of live weight at slaughter (6, 10 and 25 kg) on kid carcass and meat quality. Livest Prod Sci, 83: 247-256.
18. Banskalieva V., Sahlu T., Goetsch A.L. (2000) Fatty acid composition of goat muscles and fat depots: A review. Small Ruminant Res, 37: 255-268.
19. Addis M., Fiori M., Manca C., Riu G., Scintu M.F. (2013) Muscle colour and chemical and fatty acid composition of "Agnello di Sardegna" PGI suckling lamb. Small Ruminant Res, 115: 51-55.
20. Bañón S., Vila R., Price A., Ferrandini E., Garrido M.D. (2006) Effects of goat milk or milk replacer diet on meat quality and fat composition of suckling goat kids. Meat Sci, 72: 216-221.
21. Longobardi F., Sacco D., Casiello G., Ventrella A., Contessa A., Sacco A. (2012) Garganica kid goat meat: Physico-chemical characterization and nutritional impacts. J Food Compos Anal, 28: 107-113.

3.2 Paper II

Small Ruminant Research 121 (2014) 161–167

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

The effect of cold acidified milk replacer on productive performance of suckling kids reared in an extensive farming system

G.M. Vacca*, M. Pazzola, G. Piras, E. Pira, P. Paschino, M.L. Dettori

Department of Veterinary Medicine, University of Sassari, via Vienna 2, Sassari, 07100, Italy

ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form 31 July 2014
Accepted 1 August 2014
Available online 12 August 2014

Keywords:
Sarda goat
Kids
Milk replacer
Productive performance.

ABSTRACT

This study was conducted, in an extensive farm, to assess the effects of artificial feeding with an acidified milk replacer on productive performance of goat kids. Thirty-two Sarda kids, randomly divided into two feeding groups of 16 kids, (NM, naturally milk fed by their dams, and AR, artificially bottle-fed with an acidified milk replacer) were weighed at weekly intervals until slaughtering, at 42 days of age. A blood sample was collected from each goat kid before slaughter for the assessment of the metabolic status. Post-mortem data were registered: carcass characteristics; percentages of commercial cuts; muscle, separable fat and bone + tendons percentage. Results were analysed by a General Linear Model procedure. The two feeding groups showed similar live weights, except for the period between 7 and 21 days of age. As regards slaughter data, AR kids had a reduced fat deposition and carcasses with shorter diameters and a longer leg. Measurements after dissection showed that the muscle/fat ratio was more favourable in AR kids. Although there were some differences between the groups, haematochemical parameters of both NM and AR were in the range reported for goat kids. On the basis of the results of this trial, artificial feeding with an acidified milk replacer can provide carcasses similar to those obtained with natural milk feeding and can be used both for goat kid meat production and eradication schemes of diseases transmitted by milk-feeding.

1. Introduction

Extensive goat farming has an important economic and social role in many marginal areas of the world (Bertaglia et al., 2007). This is due to the good adaptability of the goat, which can exploit pastures not easily accessible to other livestock species, and provide meat, milk and dairy products which are well appreciated by consumers (Vacca et al., 2009a; Pazzola et al., 2011a) both for nutritional characteristics (Haenlein, 2004; Peña et al., 2009) and organoleptic qualities (Longobardi et al., 2012; Balia et al., 2013). These products have an additional value if they are obtained in particular ecosystems and in compliance with animal welfare requirements (Pirisi et al., 2007; Koknaroglu and Akunal, 2013). A typical farming system which fits this scheme is the one conducted in Sardinia (Italy). Goat farming in this island is mainly based on traditional techniques, extensive pasture on Mediterranean shrub (Vacca et al., 2010b) and the exploitation of the autochthonous Sarda goat. This breed is the most consistent in Italy on the basis of total heads (Vacca et al.,

* Corresponding author. Tel.: +39 079 229442; fax: +39 079 229592.
E-mail addresses: gmvacca@uniss.it (G.M. Vacca), pazzola@uniss.it (M. Pazzola), gppiras@hotmail.com (G. Piras), emanuelapira@hotmail.com (E. Pira), ppaschino@uniss.it (P. Paschino), mldettori@uniss.it (M.L. Dettori).

http://dx.doi.org/10.1016/j.smallrumres.2014.08.002
0921-4488/

2009b) and milk production, which is about 20% of the entire amount of goat milk produced in Italy (Pazzola et al., 2011b). Milk from Sarda goats is characterized by a good cheese-making aptitude (Pazzola et al., 2012) which mainly depends on the high protein and fat contents (Dettori et al., 2013) and on the prevalence of "strong" casein genotypes (Dettori et al., 2009; Pazzola et al., 2014). The genetic variability of the Sarda is high (Vacca et al., 2010a) and breeding programs and selective pressure are not particularly severe (Daga et al., 2013) and mainly directed to increase milk yield (Carcangiu et al., 2009). Breeding is based on natural mating and kids are separated from the flock for most of the day and milk fed by their dams when they come back from grazing. Artificial milk replacers have been proposed as an alternative feeding method to improve the economic balance of kid meat production (Peris et al., 1997), but it is also an essential method of prevention for diseases transmitted by ingestion of infected milk or colostrum. In the Mediterranean Basin, small ruminant lentiviruses (SRLVs), specifically the caprine arthritis-encephalitis virus (CAEV), have a very high incidence (Reina et al., 2010; Bertolotti et al., 2011). Among the possible methods for eradication, the culling of infected animals would result in a dramatic reduction of animal heritage. A suitable strategy is based on the early separation of kids from dams after kidding, the administration of colostrum from healthy goats and the successive feeding with milk replacers (Reina et al., 2009; Konishi et al., 2011). The average level of facilities of traditional and extensive farms often represent a limit for the utilization of milk replacers, which should be warmed during administration. An alternative feeding system is based on acidified milk replacers, which can be administered at ambient temperature and maintain a low bacterial count (Davis et al., 1998). In order to investigate the applicability of this last method in extensive goat farming systems, the aim of this research was to assess the effects of feeding acidified milk replacer on the productive performances and metabolic status of Sarda goat kids.

2. Materials and methods

Thirty-two male kids of Sarda breed were selected for the trial in a traditional farm located in southwest Sardinia (39°00′N; 8°40′E, 400 m above sea level). They were born in the same week from single parturition and all the dams were SRLVs-free. Weight of kids was recorded at birth, and at weekly intervals throughout 6 weeks, until slaughter. During the whole experimental period, kids were examined by a veterinarian three times per week. Kids were individually identified by a plastic numbered neck-collar and housed in a pen of about 30 m^2 as a common group. They were randomly divided into two treatments, each made up of 16 animals. Kids of the first (NM, natural milk fed) were milk fed by their dams twice a day in the morning (6:00 AM) and in the afternoon (5:00 PM), according to local farming techniques, when goats came back to the farm from the pastures. Kids belonging to the second treatment (AR, acidified milk replacer) were artificially fed. They were separated from their dams immediately after birth and a total volume of about 300 mL of colostrum, divided in two administrations, was bottle-fed to kids on the same day of kidding. On the 2 days after kidding, colostrum was mixed with the acidified milk replacer (350 mL with a ratio of 1:1 at day 1; 350 mL with a ratio of 1:2 at day 2) and starting from the fourth day of age, AR kids were totally fed with the acidified milk replacer *ad libitum*. As reported above, AR kids were reared in the same indoor pen together with NM kids and twice a day (6:00 AM and 5:00 PM), in order to complete bottle-feeding, they were moved for a period of about half an hour, into individual boxes. The commercial milk powder (Starter Zero Acido, Cima s.r.l., Castiglione delle Stiviere, Italy) was dissolved in water at ambient temperature, at a concentration of 16.7% w/v during the first week, and 20% during the subsequent weeks. In order to achieve a comparison between composition of natural milk and acidified replacer, milk samples from the dams were collected 28 days after kidding and analysed for protein, fat, lactose and pH, using an infrared spectrophotometer (Milko-Scan 133B; Foss Electric, DK-3400 Hillerød, Denmark), and for moisture and ashes (AOAC, 1997). Composition of acidified milk replacer powder was provided by the producer. Energy of the two types of milk was computed from chemical composition using tabulated values proposed by the National Research Council (2007).

Before transportation of kids to slaughterhouse, a blood sample was taken from the jugular vein using tubes with lithium heparin as anticoagulant (Vacutest Kima, Azergrande, PD, Italy) to measure plasma levels of glucose, NEFA, triglycerides, total cholesterol, total proteins urea, creatinine, CK, GOT, GGT, calcium, phosphorus, magnesium and chlorides (spectrophotometer Ultrospec III, Pharmacia LKB, Uppsala, Sweden; reagents Sentinel CH, Milano, Italy) and potassium (Corning Flame Photometer 410, Corning Medical and Scientific, Corning, NY, USA). At a commercial slaughterhouse, kids were weighed after a 12-h fasting period with free access to water (pre-slaughter live weight, PSW) and later slaughtered according to the European Community Regulation 86/609. The hot carcass, blood, skin, head, feet, viscera and fat deposits were separately weighed. Gastro-intestinal content was later extracted and weighed to calculate empty body weight (EBW) by subtracting the digestive content from PSW. Carcasses were chilled for 24 h at 4 °C and classified according to the scheme proposed by the Council Regulation EEC n. 2137/92. Cold carcasses were measured and dissected according to the method by Vacca et al. (2008) and the following procedure. Carcass length, loin width and chest width were recorded and carcasses later divided into two symmetric half-carcasses to obtain internal length, chest depth, leg length and leg compactness (leg weight/leg length × 100) and carcass compactness (carcass weight/internal half carcass length × 100). The right half-carcasses were submitted to dissection into the following parts: neck, shoulder, breast, rib, loin, flank and leg. Muscle (M), separable fat (SF) and bone + tendons (BT) were later separated from all the anatomical parts. The pH values of *longissimus dorsi* and *semitendinosus* muscles, of hot and cold carcasses, were registered.

Data were processed by a General Linear Model procedure to investigate the diet effect (two levels, NM vs. AR); statistics was performed by Minitab statistical software version release 13.32 (Minitab Inc., State College, PA, USA) and results declared significant at $P < 0.05$.

3. Results and discussion

On the basis of the veterinarian's examination all kids were in healthy status until slaughtering, except one belonging to the AR group who died at day 11 because of acute diarrhoea. As regards feeding of AR group, during the first week kids were individually trained to the bottle. On the basis of the authors' personal experiences about bottle-feeding, the average time spent on training of Sarda kids was longer than that of lambs. This is probably due to the different ethological and feeding patterns of the two species, as sheep are follower and goats are hider species (Poindron et al., 2007). Training kids to bottle feeding might have been influenced by the particular methods performed in the extensive Sardinian goat farming, where kids are normally kept together in an indoor fence (named "*caprettile*" in Italian) and they are allowed to be in contact with their dams exclusively for milk-feeding, which corresponds to two short periods, each of about half an hour. Because AR kids were individually kept in boxes during bottle-feeding, they were not able to take advantage of social facilitation which is at its maximum level when activities and behaviour are conducted simultaneously within a herd (Miranda-de la Lama and Mattiello, 2010).

Table 1
Composition and pH of milk from dams and replacer powder.

Parameters	Feeds and feeding treatments	
	Dams' milk NM	Replacer powder# AR
Moisture (%)	85.5	3.3
Proteins (%)	4.2	20.5
Fat (%)	4.8	15.5
Lactose (%)	4.8	–
Ashes (%)	0.7	9.4
Energy MJ/kg	3.8	18.8
pH	6.7	5.2

NM, natural milk fed; AR, bottle fed with acidified replacer.
Values provided by the producer.

Table 1 reports average values of chemical composition, energy and pH of dams' milk and milk-replacer powder. Starting from the second week, the milk-replacer powder was mixed with water at a concentration of 20% to provide an energy value similar to the one estimated from data reported in the literature for Sarda goat milk (Pazzola et al., 2014). Indeed, after dilution milk replacer provided an estimated energy value of 3.77 MJ/kg and adequate concentrations of protein (4.1%), fat (3.0%) and carbohydrates (9.9%). As regards milk composition, this was in accordance to data reported for the Sarda goat breed in a similar stage of lactation (Pazzola et al., 2014) and provided an energy value corresponding to the one calculated for milk-replacer after dilution (3.80 MJ/kg).

Table 2 shows live weight and the average daily weight gain of kids from birth to slaughter. Kids belonging to the two feeding treatments had similar weight at birth (3.3 ± 0.65 kg for NM and 3.4 ± 0.50 kg for AR) and these results evidenced that successive records of weight were not biased by groups' differences. Significant differences were recorded from day 7 to day 21 with the highest mean values for NM and this was probably due to the initial difficulties in training to bottle-feeding of AR kids. Anyway, final weights at slaughtering time were similar between the treatments (9.2 ± 1.23 kg for NM and 8.7 ± 1.05 kg for AR) and they were on the whole higher than those registered for different autochthonous Spanish breeds slaughtered at 42–46 days (Horcada et al., 2012), even if those have shown higher birth weight than Sarda kids.

Table 2
Live-weight and average daily weight gain (LS means ± SD) of kids according to the feeding treatment and the days.

Days	Feeding treatments				*P*-value
	NM (n = 16)		AR (n = 15)		
LW (kg)					
At birth	3.3	±0.65	3.4	±0.50	ns
7	4.4^{b}	±0.59	3.6^{a}	±0.66	*
14	5.4^{B}	±0.62	4.3^{A}	±0.77	**
21	6.4^{b}	±0.82	5.4^{a}	±0.87	*
28	7.3	±0.98	6.5	±0.97	ns
35	8.4	±1.22	7.7	±0.99	ns
42	9.2	±1.23	8.7	±1.05	ns
ADG (g/die)					
birth–7	154^{B}	±16.22	33^{A}	± 8.30	**
8–14	144^{b}	±28.17	102^{a}	±14.02	*
15–21	135	±36.47	157	±37.71	ns
22–28	131	±45.07	154	±42.18	ns
29–35	154	±53.40	166	±32.79	ns
36–42	119	±62.47	150	±36.85	ns

LW, live-weight; ADG, average daily weight gain; NM, natural milk fed; AR, bottle fed with acidified replacer; means with different superscript with lower-case or capital letters in each row differ significantly in treatments comparison, respectively at $P < 0.05$ and $P < 0.01$; *, $P < 0.05$; **, $P < 0.01$; ns, non significant.

On the basis of live weight recorded throughout the experimental period, NM growth rate showed a steady pattern whereas, during the first two weeks, AR kids had lower gains in comparison with NM. Weight gains of AR were higher than those recorded by Yeom et al. (2003) in Dutch White kids, and similar to those recorded for artificially fed Saanen × Criollo kids (Tacchini et al., 2006). On the other hand, average weight gains calculated on the whole period of the trial were similar between the treatments (127 g/d for NM and 140 g/d for AR, data not shown); this result could be considered as positive because in the study by Argüello et al. (2007) artificially fed kids have significantly lower weight gains than the group naturally fed by their dams.

Table 3 shows slaughtering performance according to the two different feeding treatments. Percentage of internal deposit fat (mesenteric, kidney and pelvic) were higher at $P < 0.01$ in the NM group (3.6% *vs.* 2.7%), in accordance to the study by Argüello et al. (2007) regarding kids from the Canary islands. Regardless of the feeding treatment, percentages of internal fat deposits recorded in the present study were lower than those recorded in milk fed male kids of the breeds Florida (Peña et al., 2007) and Cabrito de Barroso (Santos et al., 2007). For both feeding treatments, dressing percentage, that is the proportion of carcass weight on empty body weight, were approximately 54%, and this value was similar to those recorded by Marichal et al. (2003) for Canary kids with a live weight at slaughter of 10 kg and by Horcada et al. (2012) in Blanca Andaluza and Pyrenean kids. Adding together the weights of the offal (head, heart, lungs and liver) and the carcass, parts which are traditionally sold together also in other Mediterranean countries (Santos et al., 2007), both groups of the present study reached a total yield of about 68%, which is similar to the value recorded for Girgentana kids slaughtered at lower age and live weight (Todaro et al., 2002).

Measurements and compactness indexes of carcasses are reported in Table 4. In both groups carcasses were classified as first quality and in the same class of weight (A), meat colour (pink) and adipose tissue (3rd class). Carcasses of NM kids were characterized by higher values of carcass length, loin widths, internal lengths and chest depths ($P < 0.05$); a similar result has been obtained by Argüello et al. (2007), who have reported longer carcass and leg in naturally milk fed kids than those artificially fed.

Table 5 shows half carcass weights, percentage of the different parts and pH of *longissimus dorsi* and *semitendinosus* muscles. The only significant differences were recorded for leg percentage which was higher at $P < 0.05$ for AR and the value of pH of *semitendinosus* muscle, higher at $P < 0.01$ in NM. The scarcity of differences regarding carcass measurements of the two treatments is in agreement with Argüello et al. (2007), who have not found any difference between natural and artificial fed kids. In both groups of

Table 3
Slaughtering performance and pH of hot carcasses of kids (LS means ± SD) according to the feeding treatment.

Parameters (*n*)	Feeding treatments				*P*-value
	NM (16)		AR (15)		
EBW (kg)	8.9	±1.60	8.5	±1.14	ns
Proportion (%) on EBW					
Hot Carcass	54.1	±1.31	53.9	±1.33	ns
Head	7.0	±0.81	7.5	±0.77	ns
Skin and limbs	12.9	±0.79	13.1	±0.71	ns
Internal deposit fat	3.6^{B}	±0.74	2.7^{A}	±0.25	**
Heart, lungs and liver	6.6	±0.43	6.4	±0.39	ns
Empty gastrointestinal tract	7.5	±0.54	7.9	±0.59	ns
Blood	4.8	±0.36	4.8	±0.29	ns
Urogenital organs and weight loss	3.5	±0.23	3.7	±0.74	ns
pH *longissimus dorsi* muscle	6.1	±0.33	6.3	±0.34	ns
pH *semitendinosus* muscle	6.4	±0.35	6.3	±0.54	ns

EBW, empty body weight; NM, natural milk-fed; AR, bottle fed with acidified replacer; means with different superscript with capital letters in each row differ significantly in treatments comparison at $P < 0.01$; **, $P < 0.01$; ns, non significant.

the present study, the sum of shoulder and leg percentages represented about 55% of the half carcass. This is a positive result because these are the parts of the carcass with the highest percentage of muscle and, as a consequence, the highest marketing value, and records were even higher than those reported by Marichal et al. (2003) and Peña et al. (2007) for other Mediterranean goat breeds slaughtered at similar weights.

Data regarding dissection of the anatomical parts are shown in Table 6. The highest percentage of separable fat (SF) was recorded for all the parts in kids belonging to the NM group, while the AR kids had higher muscle percentage (M) for shoulder, flank and leg. The amount of bone + tendons (BT) was not significantly influenced by the feeding treatment. As regards the total amount of tissue of the half carcass, the AR group had lower values of SF and a higher M percentage ($P < 0.01$). Also in the study by Argüello et al. (2007), artificial-feeding of Canary kids has a significant influence in order to produce leaner carcasses than those milk fed by their dams, and the carcasses of Sarda kids of the present study were furthermore leaner than those recorded for Canary breeds. In general, parts of AR kids had

Table 4
Carcass measurements and compactness indexes of kids (LS means ± SD) according to the feeding treatment.

Parameters (*n*)	Feeding treatments				*P*-value
	NM (16)		AR (15)		
Carcass length (cm)	53.7^{b}	±3.28	49.5^{a}	±2.94	*
Loin width (cm)	16.7^{b}	±1.86	15.6^{a}	±1.46	*
Chest width (cm)	12.6	±1.32	12.2	±1.11	ns
Half carcass internal length (cm)	43.9^{b}	±2.25	40.8^{a}	±2.44	*
Chest depth (cm)	11.8^{b}	±0.84	10.6^{a}	±0.93	*
Leg length (cm)	26.1	±1.56	24.6	±1.62	ns
Leg compactness	2.6	±0.35	2.5	± 0.27	ns
Carcass compactness	9.6	±1.53	10.2	± 1.07	ns

NM, natural milk fed; AR, bottle fed with acidified replacer; means with different superscript with lower-case letters in each row differ significantly in treatments comparison at $P < 0.05$; *, $P < 0.05$; ns, non significant.

Table 5
Proportion of parts and pH of cold carcasses of kids (LS means ± SD) according to the feeding treatment.

Parameters (*n*)	Feeding treatments				*P*-value
	NM (16)		AR (15)		
CHCW (kg)	2.4	±0.41	2.3	±0.29	ns
Proportion (%) on CHCW					
Neck	8.2	±1.59	8.2	±1.44	ns
Shoulder	23.4	±1.09	23.8	±0.90	ns
Breast	11.6	±0.96	10.9	±0.47	ns
Rib	14.2	±0.90	14.1	±1.75	ns
Loin	7.9	±0.52	7.4	±0.63	ns
Flank	3.3	±0.58	3.2	±0.39	ns
Leg	31.4^{a}	±0.88	32.4^{b}	±0.56	*
pH *longissimus dorsi* muscle	5.5	±0.12	5.4	±0.08	ns
pH *semitendinosus* muscle	5.7^{B}	±0.15	5.4^{A}	±0.06	**

CHCW, cold half carcass weight; NM, natural milk fed; AR, bottle fed with acidified replacer; means with different superscript with lower-case or capital letters in each row differ significantly in treatment comparison, respectively at $P < 0.05$ and $P < 0.01$; *, $P < 0.05$; **, $P < 0.01$; ns, non significant.

Table 6
Proportion of tissues (LS means ± SD) of kids according to the anatomical parts and the feeding treatment.

Parts (*n*)	Tissues	Feeding treatments				*P*-value
		NM (16)		AR (15)		
Neck	M	57.4	±5.01	61.9	±5.66	ns
	SF	12.2^{B}	±2.80	6.0^{A}	±1.64	**
	BT	30.4	±3.68	32.1	±5.72	ns
Shoulder	M	62.2^{A}	±2.07	66.6^{B}	±3.35	**
	SF	9.0^{B}	±1.89	5.7^{A}	±2.02	**
	BT	28.8	±1.50	27.7	±1.91	ns
Breast	M	50.6	±5.44	56.3	±7.73	ns
	SF	16.6^{B}	±4.12	7.3^{A}	±2.41	**
	BT	32.8	±3.66	36.4	±8.64	ns
Rib	M	58.9	±5.08	61.1	±5.27	ns
	SF	10.0^{b}	±6.92	4.3^{a}	±1.24	*
	BT	31.1	±4.57	34.6	±5.82	ns
Loin	M	56.4	±7.09	64.4	±4.03	ns
	SF	13.8^{B}	±3.00	9.1^{A}	±3.05	**
	BT	29.8	±8.02	29.5	±6.66	ns
Flank	M	70.8^{a}	±6.40	78.3^{b}	±8.04	*
	SF	29.2^{b}	±6.40	21.7^{a}	±8.04	*
Leg	M	64.7^{a}	±1.58	67.5^{b}	±2.91	*
	SF	6.0^{b}	±1.47	4.2^{a}	±8.87	*
	BT	29.3	±1.58	28.3	±2.13	ns
Half carcass	M	60.6^{A}	±1.71	64.3^{B}	±2.18	**
	SF	10.4^{B}	±1.15	6.0^{A}	±0.70	**
	BT	29.0	±1.81	29.7	±1.92	ns

M, muscle; SF, separable fat; BT, bone + tendons; NM, natural milk fed; AR, bottle fed with acidified replacer; means with different superscript with lower-case or capital letters in each row differ significantly in treatment comparison, respectively at $P<0.05$ and $P<0.01$; *, $P<0.05$; **, $P<0.01$; ns, non significant.

low percentage of SF with the sole flank which was higher than 10%, whereas in the NM group, four parts exceeded this value with breast representing the maximum value (16.6%). Comparison with other goat breeds evidenced that SF of the NM carcasses was similar to the one reported for Florida (Peña et al., 2007) and Serrana kids (Teixeira et al., 1995), and higher than those for "Cabrito de Barroso" kids (Santos et al., 2007).

Table 7 shows blood parameters registered at 42 days of age. Statistical analysis evidenced differences between the groups for total protein, creatinine and Mg ($P<0.05$). Even if statistical differences between the treatments were recorded, blood protein levels were similar to those reported for naturally fed Red Syrian kids by Celi et al. (2008) and also creatinine levels were similar to those found in kids of Sicilian breeds (Chiofalo et al., 2004; Zumbo

Table 7
Blood biochemical, enzymatic and mineral parameters of kids (LS means ± SD) according to the feeding treatment.

Parameters (*n*)	Feeding treatments				*P*-value
	NM (16)		AR (15)		
Glucose (mmol/L)	3.14	±0.19	3.20	±0.22	ns
NEFA (mmol/L)	0.38	±0.02	0.41	±0.01	ns
Total protein (g/dL)	6.32^{b}	±0.19	5.67^{a}	±0.24	*
Urea (mmol/L	4.92	±0.41	4.88	±0.31	ns
Triglycerides (mmol/L)	0.64	±0.07	0.68	±0.18	ns
Total cholesterol (mmol/L)	4.62	±0.60	4.80	±0.30	ns
CK (U/L)	174.46	±26.20	196.41	±33.32	ns
GOT (U/L)	87.01	±7.68	99.17	±7.90	ns
GGT (U/L)	54.98	±4.53	67.15	±9.21	ns
Creatinine (μmol/L)	54.62a	±3.59	68.68^{b}	±4.91	*
Ca (mmol/L)	2.51	±0.10	2.43	±0.14	ns
P (mmol/L)	3.24	±0.15	3.30	±0.17	ns
Mg (mmol/L)	1.16b	±0.02	1.04^{a}	±0.02	*
K (mmol/L)	3.21	±0.19	3.18	±0.32	ns
Clorures (mmol/L)	98.98	±2.65	96.88	±2.89	ns

NM, natural milk fed; AR, bottle fed with acidified replacer; means with different superscript with lower-case letters in each row differ significantly in treatment comparison at $P<0.05$; *, $P<0.05$; ns, non significant.

et al., 2011). As regards Mg values, to the best of our knowledge literature lacks of data for suckling kids at this age, whereas the other values of blood minerals are within the range reported for adult goats (Vazzana et al., 2013). Mbassa and Poulsen (1993) reported that urea blood levels are influenced by age with the highest values in young goats, but in the present study urea concentration was lower than that reported for primiparous lactating goats of Sarda breed reared with extensive methods (Pazzola et al., 2011b). Urea levels of Sarda kids, regardless of the feeding treatments, are similar to those recorded by Celi et al. (2008) in Red Syrian kids. Glucose and lipid blood values, which can indicate that the energetic supply of the replacer milk was adequate, are similar to the range reported for kids of different breeds (Chiofalo et al., 2004; Celi et al., 2008; Zumbo et al., 2011) at the same age of the kids of the present study.

4. Conclusions

Data of the present study showed that artificial feeding with an acidified milk replacer allowed to obtain, in kids of 42 days of age, live weights similar to those of kids naturally fed by their dams, as well as leaner carcasses. Feeding acidified milk replacer did not negatively affect the metabolic status of kids. This was an easy technique that could be implemented in extensive goat farming in order to produce suckling animals to slaughter or to help in eradication programs of SRLVs diseases.

Acknowledgement

This study was supported by FAR 2011 funds. (executive researcher G.M. Vacca).

References

Argüello, A., Castro, N., Capote, J., Solomon, M.B., 2007. The influences of artificial rearing and live weight at slaughter on kid carcass characteristics. J. Anim. Vet. Adv. 6, 20–25.

AOAC, Association of Official Analytical Chemists, 1997. Official Methods of Analysis, 16th ed. AOAC, Virginia, USA.

Balia, F., Pazzola, M., Dettori, M.L., Mura, M.C., Luridiana, S., Carcangiu, V., Piras, G., Vacca, G.M., 2013. Effect of CSN1S1 gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. J. Dairy Res. 80, 129–137.

Bertaglia, M., Joost, S., Roosen, J., Econogene Consortium, 2007. Identifying European marginal areas in the context of local sheep and goat breeds conservation: a geographic information system approach. Agr. Syst. 94, 657–670.

Bertolotti, L., Mazzei, M., Puggioni, G., Carrozza, M.L., Dei Giudici, S., Muz, D., Juganaru, M., Patta, C., Tolari, F., Rosati, S., 2011. Characterization of new small ruminant lentivirus subtype B3 suggests animal trade within the Mediterranean Basin. J. Gen. Virol. 92, 1923–1929.

Carcangiu, V., Vacca, G.M., Mura, M.C., Dettori, M.L., Pazzola, M., Luridiana, S., Bini, P.P., 2009. Relationship between MTNR1A melatonin receptor gene polymorphism and seasonal reproduction in different goat breeds. Anim. Reprod. Sci. 110, 71–78.

Celi, P., Di Trana, A., Claps, S., 2008. Effects of perinatal nutrition on lactational performance, metabolic and hormonal profiles of dairy goats and respective kids. Small Rumin. Res. 79, 129–136.

Chiofalo, V., Liotta, L., Chiofalo, B., 2004. Effects of the administration of Lactobacilli on body growth and on the metabolic profile in growing Maltese goat kids. Reprod. Nutr. Dev. 44, 449–457.

Daga, C., Paludo, M., Luridiana, S., Mura, M.C., Bodano, S., Pazzola, M., Dettori, M.L., Vacca, G.M., Carcangiu, V., 2013. Identification of novel SNPs in the Sarda breed goats POU1F1gene and their association with milk productive performance. Mol. Biol. Rep. 40, 2829–2835.

Davis, J.J., Sahlu, T., Puchala, R., Tesfai, K., 1998. Performance of Angora goat kids fed acidified milk replacer at two levels of intake. Small Rumin. Res. 28, 249–255.

Dettori, M.L., Rocchigiani, A.M., Luridiana, S., Mura, M.C., Carcangiu, V., Pazzola, M., Vacca, G.M., 2013. Growth Hormone gene variability and its effects on milk traits in primiparous Sarda goats. J. Dairy Res. 80, 255–262.

Dettori, M.L., Vacca, G.M., Carcangiu, V., Pazzola, M., Mura, M.C., Rocchigiani, A.M., 2009. A reliable method for characterization of the goat CSN1S1 E allele. Livest. Sci 125, 105–108.

Haenlein, G.F.W., 2004. Goat milk in human nutrition. Small Rumin. Res. 51, 155–163.

Horcada, A., Ripoll, G., Alcalde, M.J., Sañudo, C., Teixeira, A., Panea, B., 2012. Fatty acid profile of three adipose depots in seven Spanish breeds of suckling kids. Meat Sci. 92, 89–96.

Koknaroglu, H., Akunal, T., 2013. Animal welfare: an animal science approach. Meat Sci. 95, 821–827.

Konishi, M., Nagura, Y., Takei, N., Fujita, M., Hayashi, K., Tsukioka, M., Yamamoto, T., Kameyama, K., Sentsui, H., Murakami, K., 2011. Combined eradication strategy for CAE in a dairy goat farm in Japan. Small Rumin. Res. 99, 65–71.

Longobardi, F., Sacco, D., Casiello, G., Ventrella, A., Contessa, A., Sacco, A., 2012. Garganica kid goat meat: physico-chemical characterization and nutritional impacts. J. Food Compos. Anal. 28, 107–113.

Marichal, A., Castro, N., Capote, J., Zamorano, M.J., Argüello, A., 2003. Effects of live weight at slaughter (6, 10 and 25 kg) on kid carcass and meat quality. Livest. Prod. Sci. 83, 247–256.

Mbassa, G.K., Poulsen, J.S.D., 1993. Reference ranges for clinical chemical values in Landrace goats. Small Rumin. Res 10, 133–142.

Miranda-de la Lama, G.C., Mattiello, S., 2010. The importance of social behaviour for goat welfare in livestock farming. Small Rumin. Res. 90, 1–10.

National Research Council, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids. National Academy Press, Washington D.C., USA.

Pazzola, M., Balia, F., Carcangiu, V., Dettori, M.L., Piras, G., Vacca, G.M., 2012. Higher somatic cell counted by the electronic counter method do not influence renneting properties of goat milk. Small Rumin. Res. 102, 32–36.

Pazzola, M., Balia, F., Dettori, M.L., Mura, M.C., Carcangiu, V., Vacca, G.M., 2011a. Effects of different storage conditions, the farm and the stage of lactation on renneting parameters of goat milk investigated using the formagraph method. J. Dairy Res. 78, 343–348.

Pazzola, M., Dettori, M.L., Carcangiu, V., Luridiana, S., Mura, M.C., Vacca, G.M., 2011b. Relationship between milk urea, blood plasma urea and BCS in primiparous browsing goats with different milk yield level. Arch. Tierzucht 54, 546–556.

Pazzola, M., Dettori, M.L., Pira, E., Noce, A., Paschino, P., Vacca, G.M., 2014. Effect of polymorphisms at the casein gene cluster on milk renneting properties of the Sarda goat. Small Rumin. Res. 117, 124–130.

Peña, F., Bonvillani, A., Freire, B., Juárez, M., Perea, J., Gómez, G., 2009. Effects of genotype and slaughter weight on the meat quality of Criollo Cordobes and Anglonubian kids produced under extensive feeding conditions. Meat Sci. 83, 417–422.

Peña, F., Perea, J., García, A., Acero, R., 2007. Effects of weight at slaughter and sex on the carcass characteristics of Florida suckling kids. Meat Sci. 75, 543–550.

Peris, S., Caja, G., Such, X., Casals, R., Ferret, A., Torre, C., 1997. Influence of kid rearing systems on milk composition and yield of Murciano-Granadina dairy goats. J. Dairy Sci. 80, 3249–3255.

Pirisi, A., Lauret, A., Dubeuf, J.P., 2007. Basic and incentive payments for goat and sheep milk in relation to quality. Small Rumin. Res. 68, 167–178.

Poindron, P., Terrazas, A., de la Luz Navarro Montes de Oca, M., Serafín, N., Hernández, H., 2007. Sensory and physiological determinants of maternal behavior in the goat (Capra hircus). Horm. Behav. 52, 99–105.

Reina, R., Berriatua, E., Luja, L., Juste, R., Sanchez, A., de Andre, D., Amorena, B., 2009. Prevention strategies against small ruminant lentiviruses: an update. Vet. J. 182, 31–37.

Reina, R., Bertolotti, L., Dei Giudici, S., Puggioni, G., Ponti, N., Profiti, M., Patta, C., Rosati, S., 2010. Small ruminant lentivirus genotype E is widespread in Sarda goat. Vet. Microbiol. 144, 24–31.

Santos, V.A.C., Silva, A.O., Cardoso, J.V.F., Silvestre, A.J.D., Silva, S.R., Martins, C., Azevedo, J.M.T., 2007. Genotype and sex effects on carcass and meat quality of suckling kids protected by the PGI Cabrito de Barroso. Meat Sci. 75, 725–736.

Tacchini, F., Rebora, C., Van Den Bosch, S., Gascón, A., Pedrani, M., 2006. Formulation and testing of a whey-based kid goat's milk replacer. Small Rumin. Res. 63, 274–281.

Teixeira, A., Azevedo, J., Delfa, R., Morand-Fehr, P., Costa, C., 1995. Growth and development of Serrana kids from Montesinho Natural Park (NE of Portugal). Small Rumin. Res. 16, 263–269.

Todaro, M., Corrao, A., Barone, C.M.A., Schinelli, R., Occidente, M., Giaccone, P., 2002. The influence of age at slaughter and litter size on some quality traits of kid meat. Small Rumin. Res. 44, 75–80.

Vacca, G.M., Carcangiu, V., Dettori, M.L., Pazzola, M., Mura, M.C., Luridiana, S., Tilloca, G., 2008. Productive performance and meat quality of Mouflon × Sarda and Sarda × Sarda suckling lambs. Meat Sci. 80, 326–334.

Vacca, G.M., Daga, C., Pazzola, M., Carcangiu, V., Dettori, M.L., Cozzi, M.C., 2010a. D-loop sequence mitochondrial DNA variability of Sarda goat and other goat breeds and populations reared in the Mediterranean area. J. Anim. Breed. Genet. 127, 352–360.

Vacca, G.M., Ouled Ahmed Ben Alì, H., Carcangiu, V., Pazzola, M., Dettori, M.L., 2009a. Genetic structure of the casein gene cluster in the Tunisian native goat breed. Small Rumin. Res. 87, 33–38.

Vacca, G.M., Ouled Ahmed Ben Alì, H., Pazzola, M., Sanna, M., Dettori, M.L., Carcangiu, V., 2009b. An investigation on allele frequency at the CSN1S2 locus and its relationship with milk parameters in the Sarda goat. J. Anim. Feed Sci. 18, 628–637.

Vacca, G.M., Dettori, M.L., Carcangiu, V., Rocchigiani, A.M., Pazzola, M., 2010b. Relationships between milk characteristics and somatic cell score in milk from primiparous browsing goats. Anim. Sci. J. 81, 594–599.

Vazzana, I., Rizzo, M., Giambelluca, S., Zumbo, A., Piccione, G., Monteverde, V., 2013. The response of some blood constituents after administration of two different diets in goats. Comp. Clin. Pathol. 22, 691–695.

Yeom, K.-H., Van Trierum, G., Lee, K.-W., Beynen, A.C., 2003. Influence of feeding a milk replacer deficient in α-linolenic acid on fatty acid composition of various tissues in goat kids. Small Rumin. Res. 48, 141–148.

Zumbo, A., Scianò, S., Messina, V., Di Rosa, A., Assenza, A., Piccione, G., 2011. Screening metabolico nel capretto durante il periodo neonatale (Metabolical screening in the kid during neonatal period). Large Anim. Rev. 17, 63–67.

3.3 Paper III

Small Ruminant Research 117 (2014) 124–130

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Effect of polymorphisms at the casein gene cluster on milk renneting properties of the Sarda goat

M. Pazzola*, M.L. Dettori, E. Pira, A. Noce, P. Paschino, G.M. Vacca

Department of Veterinary Medicine, University of Sassari, via Vienna 2, 07100 Sassari, Italy

ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form 4 December 2013
Accepted 7 December 2013
Available online 15 December 2013

Keywords:
Goat
Milk
Casein genes
Renneting properties

ABSTRACT

This study investigated the influence of casein genes polymorphism on renneting properties of milk from Sarda goats. Milk yield and composition, and renneting properties (rennet coagulation time RCT, firming time k20 and curd firmness a30) were evaluated in 200 multiparous goats from three farms, at monthly intervals from March to July. Animals were genotyped at *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*. Statistical analysis was performed by a repeated measures General Linear Model. *CSN1S1* significantly affected milk traits with a lower fat content registered for goats carrying the F allele. The highest protein concentration was recorded for the *CSN1S1* AB goats. Milk from *CSN1S1* FF homozygote goats was characterized by a delayed k20 and the *CSN1S1* AB showed a higher a30. All the parameters were influenced by the *CSN2* locus, except milk yield. Polymorphism at *CSN1S2* influenced only daily milk yield and a30. The *CSN1S2* 0 null allele was detected for the first time in this breed. The influence of genotype effect was particularly marked for *CSN3*; RCT and k20 were delayed in *CSN3* BB goats and the highest level of a30, 47.88 mm registered for *CSN3* AA goats, could be considered remarkable when compared to other goat breeds or populations. In conclusion, this study improved knowledge on the effects of goat casein genes on milk renneting parameters, and could be useful in the future planning of breeding programs and specific dairy products linked to the Sarda breed (PDO/PGI).

1. Introduction

In the Mediterranean area, one of the possible tools to face up to the global livestock market has been indicated in the farming of sheep and goats by means of extensive methods and the EU-labeling of products as Protected Designation of Origin (PDOs) and geographical indication (PGIs); these are often associated to the concepts of typicality, respect for the environment and local breeds (Boyazoglu and Morand-Fehr, 2001). Indeed, many dairy products from small ruminant species with a PDO/PGI label are strictly linked to a specific breed (Martinez et al., 2011). Scientific research dealing with genetic and phenotypic characterization of local breeds is an important basis to define the uniqueness of typical products, as it has happened for La Serena, a PDO-cheese produced from Merinos sheep milk (Arqués et al., 2007).

One of the most important topics for the genetic characterization of dairy breeds is investigation of the casein genes. The goat casein genes *CNS1S1*, *CSN2*, *CSN1S2* and *CSN3* are clustered on chromosome 6 (Rijnkels, 2002) and have been the subject of several studies indicating that all of them have polymorphic variants (Moioli et al., 2007). Polymorphism of the α_{s1}-casein fraction, encoded by the *CSN1S1* gene, has been deeply considered in the literature (Mestawet et al., 2013a; Vassal et al., 1994) because of its

* Corresponding author at: Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, via Vienna 2, 07100 Sassari, Italy.
Tel.: +39 079 229509; fax: +39 079 229592.
E-mail addresses: pazzola@uniss.it (M. Pazzola), mldettori@uniss.it (M.L. Dettori), emanuelapira@hotmail.com (E. Pira), antonianoce83@tiscali.it (A. Noce), ppaschino@uniss.it (P. Paschino), gmvacca@uniss.it (G.M. Vacca).

0921-4488/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.smallrumres.2013.12.004

primary role in influencing composition and coagulation of milk and cheese-making characteristics.

In the island of Sardinia, a region of Italy, about 270 thousand goats are currently kept in 4.5 thousand farms (IZS, 2013), where the local breed, the Sarda, is generally reared under extensive and semi-extensive methods (Pazzola et al., 2011b; Vacca et al., 2010b). The Sarda is a Mediterranean dairy goat breed characterized by high morphological and genetic variability within the breed and high genetic diversity when compared to other goat breeds (Vacca et al., 2010a, 2011). The Sarda goat has been investigated for genetic variability of the *CSN1S1* (Balia et al., 2013) and *CSN1S2* (Vacca et al., 2009b) casein genes, revealing the occurrence of many genotype combinations and their effects on milk traits.

To the best of our knowledge, no study has yet considered the relationships between polymorphism at the complete casein gene cluster and coagulation properties in goats, in particular within the Sarda breed.

This study aimed to assess the influence of polymorphisms at *CNS1S1*, *CSN2*, *CSN1S2* and *CSN3* loci on renneting properties of milk from Sarda goats.

2. Materials and methods

2.1. Animals and samples

In three commercial farms located in Sardinia, Italy, a total number of 200 animals from Sarda breed were randomly selected among the multiparous goats which kidded in the first two weeks of February. Animals were reared according to the extensive method as described by Pazzola et al. (2011b). Individual milk samples were collected from each goat five times at monthly intervals, after kids' weaning, from March to July. During each sampling session, yield of the morning milking was registered for each goat and a milk sample was collected in a plastic disposable container. Milk samples were transported to the laboratory at 4 °C and analyzed no later than 2 h after collection. Furthermore, a blood sample from the jugular vein was taken from each goat using disposable needles and K_3EDTA vacuum tubes (BD Vacutainer, Plymouth, UK) in order to extract genomic DNA.

2.2. Genotyping

Genomic DNA was isolated from leucocytes using a commercial kit (Puregene DNA Isolation Kit Qiagen, Venlo, The Netherlands).

Animals were genotyped at *CSN1S1* according to the methods by Ramunno et al. (2000a) to detect the A, B, F and N alleles, Cosenza et al. (2003) for the 01 allele and Dettori et al. (2009) for the E allele.

Genotyping at *CSN2* was obtained according to Caroli et al. (2006) and the *CSN1S2* gene was analyzed for the A, B, C (Ramunno et al., 2000b), E (Veltri et al., 2000), F, D and 0 (Ramunno et al., 2001) alleles. Genotype at *CSN3* was investigated according to Chessa et al. (2003) and alleles were indicated according to the nomenclature proposed by Prinzenberg et al. (2005).

2.3. Analyses of milk and renneting properties

Milk composition was investigated using an infrared spectrophotometer (Milko-Scan™ 133B; Foss Electric, DK-3400 Hillerød, Denmark) and the International Dairy Federation (IDF) standard 141C:2000 for total protein, fat, lactose and pH; an automatic cell counter (Fossomatic™ 90, Foss Electric) was used according to the IDF 148A:1995 method for somatic cell count (SCC); an automatic counter (Bacto-Scan™, Foss Electric) was used according to the IDF 358:2000 method for total microbial count (TMC). Milk coagulation properties were analyzed by means of the Formagraph™ instrument (Foss Italia SPA, Padova, Italy) and the procedure already described by Pazzola et al. (2011a) to achieve rennet coagulation time (RCT) in minutes, curd firming time (k20) in minutes and curd firmness (a30) in millimeters.

2.4. Statistical analysis

Descriptive statistics were estimated from milk yield and composition data on 200 goats throughout lactation, in order to provide a general overview of milk characteristics (Table 1).

Renneting parameters data were analyzed after removing non-coagulating (NC) samples from dataset. NC are samples with a RCT higher than 30 min and as a consequence they cannot show recordable renneting properties; these samples were included in contingency tables, according to the different farm, month and casein genotype, and compared to coagulating samples by means of chi-square or Fisher's exact test as described by Devold et al. (2010) and Pazzola et al. (2012).

After removing NC samples, milk parameters were subjected to a General Linear Model (GLM) repeated measures procedure with the following model:

$$Y_{ijkl} = \mu + F_i + M_j + G_k + FM_{ij} + A_l(G_k) + e_{ijkl}$$

where Y_{ijkl} is the analyzed variable, μ is the general mean, F_i is the fixed effect of the farm ($i = 3$ levels), M_j is the fixed effect of the month as the stage of lactation ($j = 5$ levels from March to July), G_k is the fixed effect of genotype at one of the four different casein genes ($k = 4$ or 6 levels), FM_{ij} is the interaction effect between the farm and the month, $A_l(G_i)$ is the random effect of lth animal nested within the kth genotype and e_{ijkl} is the error effect.

Because of the low frequency of some genotypes and the resulting rank deficiency in contingency tables, each of the 4 different genes (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*) was analyzed at a time by a specific GLM procedure, and farm × stage of lactation was the only interaction effect considered. Genotypes of each casein gene, which are the levels of the genotype effect, are summarized in Table 2, together with the relative genotype frequencies. In order to avoid a bias of results linked to the low number of goats for each genotype level, only genotypes with a frequency higher than 4% were considered in the model. Multiple comparisons of the means were performed using the Bonferroni method and model effects were declared significant at $P < 0.05$.

Table 1
Descriptive statistics of milk yield and composition, pH, freezing point (FP), somatic cell count (SCC) and total microbial count (TMC); samples from 200 goats repeated for 5 months ($n = 1000$).

Parameters	Mean	SD	Min	Max	Skewness	Kurtosis
Milk yield (g/day)	1007	444	300	2800	0.301	0.821
Fat (g/100 ml)	4.82	0.77	3.04	6.50	−0.575	−0.024
Protein (g/100 ml)	4.07	0.47	3.02	5.19	−0.427	0.041
Lactose (g/100 ml)	4.58	0.25	4.00	5.19	−0.491	−0.047
FP (H°)	−0.567	0.008	−0.589	−0.550	−0.065	−0.385
pH	6.68	0.08	6.41	6.89	0.104	−0.211
Log SCC (cells/ml)	6.15	0.51	4.71	7.35	−0.346	−0.144
Log TMC (cells/ml)	4.42	0.98	3.30	7.23	−0.250	0.734

Data were processed by the statistic software Minitab™ release 13.32 (Minitab Inc. 2000, State College, PA).

3. Results

A preliminary Chi-square test indicated non-significant differences between the number of coagulating (C) and non-coagulating (NC) samples according to the different farm and stage of lactation (data not shown). Results regarding the comparison of C and NC milk samples according to each of the four casein genes are summarized in Table 3. The number of NC samples was influenced by *CSN1S1*, *CSN2* and *CSN1S2* genes.

Tables 4–7 report milk composition and renneting properties for *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* genes, respectively. The effects of farm, stage of lactation and interaction farm × stage of lactation significantly influenced milk yield and composition, regardless of the casein gene.

The *CSN1S1* locus significantly affected milk traits with a lower fat content registered for goats carrying the F allele in comparison with AB genotype. The highest protein concentration was recorded for *CSN1S1* AB goats and the lowest for *CSN1S1* AF. Milk renneting properties were influenced by *CSN1S1* genotype, as *CSN1S1* FF homozygote goats produced milk with a delayed firming time (k20) and *CSN1S1* AB showed a higher curd firmness (a30).

All milk traits and renneting properties, excluding milk yield, were influenced by the *CSN2* locus, with the highest significance level for protein content, which reached the highest value in milk from *CSN2* AA homozygotes and the lowest in *CSN2* C01.

Polymorphism at *CSN1S2* influenced only daily milk yield and a30, with a higher curd firmness for *CSN1S2* AC and CC animals.

CSN3 influenced renneting parameters with delayed RCT and k20 for goats carrying the *CSN3* BB genotype, which were the animals with the lowest milk pH; the highest curd firmness was registered for *CSN3* AA goats.

4. Discussion

Many studies investigated the relationships between casein polymorphism and milk traits in the caprine species, and the majority of those focus on the α_{s1}-casein locus (Martin et al., 2002; Mestawet et al., 2013b). To our knowledge, this is the first report which examined, at field level and within the same goat population, all the four casein genes and the effect of their polymorphisms on milk composition and coagulation properties.

Analysis of genotype frequencies at the casein gene cluster revealed the prevalence of *CSN1S1* AB and BB strong genotypes, which are associated with the production of 7 g/l of α_{s1}-casein, and the occurrence of the weak *CSN1S1* FF genotype, producing 0.9 g/l of milk (Remeuf, 1993), similar to those observed in the Sarda breed by Dettori et al. (2009) and Balia et al. (2013). Genotypes at the *CSN1S2* locus of the goat population analyzed were associated with a normal content of αs_2-casein in milk, as observed by Vacca et al. (2009b) for the same breed; in addition, the *CSN1S2* 0 null allele was detected for the first time in this breed. Genotype at the casein gene cluster described in the present study for the Sarda goat is typical of Mediterranean goat breeds (Sacchi et al., 2005; Vacca et al., 2009a).

Table 2
Genotype frequencies at the *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* loci in the analyzed Sarda goats, $n = 200$.

CSN1S1			CSN2			CSN1S2			CSN3		
Genotype	*n*	Frequency	Genotype	*n*	Frequency	Genotype	*n*	Frequency	Genotype	*n*	Frequency
AB	63	31.5	AC	87	43.5	CF	54	27	AB	83	41.5
BB	55	27.5	CC	73	36.5	AC	46	23	BB	78	39
BF	34	17	AA	26	13	AF	29	14.5	AA	15	7.5
AF	19	9.5	C01	9	4.5	FF	26	13	BD	14	7
AA	14	7	A01[a]	4	2	AA	19	9.5	BC[a]	5	2.5
FF	10	5	0101[a]	1	0.5	CC	17	8.5	AD[a]	4	2
AE[a]	2	1				A0[a]	4	2	CD[a]	1	0.5
EF[a]	2	1				C0[a]	2	1			
FN[a]	1	0.5				F0[a]	2	1			
						EF[a]	1	0.5			

[a] These genotypes were not considered for the statistical analysis of milk coagulation properties because the frequency is lower than 4%.

Table 3
Coagulating (RCT equal or lower than 30 min) and non-coagulating (RCT higher than 30 min) milk samples according to the genotype at the different casein genes.

	CSN1S1						CSN2				CSN1S2						CSN3			
	AB	BB	BF	AF	AA	FF	AC	CC	AA	C01	CF	AC	AF	FF	AA	CC	AB	BB	AA	BD
n	315	275	170	95	70	50	435	365	130	45	270	230	145	130	95	85	415	390	75	70
C	284	242	145	85	67	50	395	335	104	42	235	203	128	114	93	81	373	342	68	67
NC	31	33	25	10	3	0	40	30	26	3	35	27	17	16	2	4	42	48	7	3
Test	Fisher's exact						Fisher's exact				Fisher's exact						Fisher's exact			
P value	0.001						0.003				0.001						0.224			
Significance	***						**				***						ns			

C, coagulating samples; NC, non-coagulating samples, ns, non significant.
** $P < 0.01$.
*** $P < 0.001$

Table 4
Least square means and standard error of means (SEM) of rennet clotting time (RCT), rate of clot firming (k20) and clot firmness (a30) according to the genotype (G) at *CSN1S1*, the farm (F), stage of lactation (S) and interaction F × S.

	CSN1S1						SEM	Effect			
	AB	BB	BF	AF	AA	FF		G	F	S	F × S
n	284	242	145	85	67	50					
Milk yield# (g/day)	969	1010	995	1026	1085	965	18.2	ns	***	***	***
Fat (g/100 ml)	4.98^{B}	4.81^{AB}	4.69^{A}	4.66^{A}	4.71^{AB}	4.66^{A}	0.03	**	***	***	***
Protein (g/100 ml)	4.19^{D}	4.12^{C}	3.97^{B}	3.78^{A}	3.80^{AB}	3.97^{AB}	0.02	***	***	***	***
pH	6.67^{A}	6.67^{A}	6.69^{AB}	6.70^{AB}	6.69^{AB}	6.71^{B}	0.003	**	***	***	***
RCT (min)	12.90	13.54	13.14	12.60	13.09	14.06	0.13	ns	*	ns	***
k20 (min)	1.72^{A}	1.90^{A}	1.89^{A}	2.12^{AB}	1.92^{A}	2.36^{B}	0.04	**	ns	ns	**
a30 (mm)	48.61^{D}	45.08^{C}	43.93^{C}	41.07^{B}	43.15^{BC}	35.18^{A}	0.41	***	***	***	**

Yield of the morning milking.
A–D Means with different superscript with capital letters in each row differ significantly ($P < 0.01$) in genotype comparison.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$
ns, non significant.

The high values of protein and fat content in milk from *CSN1S1* AB goats were in agreement with those found in Cilentana goats by Zullo et al. (2005). The occurrence of either strong or defective alleles at the *CSN1S1* locus is important because defective alleles may impair the complex process of milk secretion and fat globule distribution (Cebo et al., 2012), thus affecting milk technological properties.

Genotype at *CSN1S1* did not influence RCT but only k20 and a30; the delayed firming time (k20) recorded for goats carrying *CSN1S1* F allele is in agreement with results observed by Zullo et al. (2005) in the Cilentana breed, even

Table 5
Least square means and standard error of means (SEM) of rennet clotting time (RCT), rate of clot firming (k20) and clot firmness (a30) according to the genotype (G) at *CSN2*, the farm (F), stage of lactation (S) and interaction F × S.

	CSN2				SEM	Effect			
	AC	CC	AA	C01		G	F	S	F × S
n	395	335	104	42					
Milk yield# (g/day)	1014	1012	921	1072	18.2	ns	***	***	***
Fat (g/100 ml)	4.82^{AB}	4.74^{A}	5.00^{B}	4.53^{A}	0.03	**	***	***	***
Protein (g/100 ml)	4.08^{C}	3.99^{B}	4.16^{D}	3.66^{A}	0.02	***	***	***	***
pH	6.67^{a}	6.69^{b}	6.68^{ab}	6.71^{b}	0.003	*	***	***	***
RCT (min)	12.98^{a}	12.95^{a}	14.24^{b}	13.50^{ab}	0.13	*	*	ns	***
k20 (min)	1.82^{A}	1.90^{A}	2.09^{AB}	2.43^{B}	0.04	**	***	ns	**
a30 (mm)	46.03^{b}	43.67^{a}	44.67^{ab}	42.59^{a}	0.38	*	***	***	***

Yield of the morning milking.
A–D Means with different superscript with capital letters in each row differ significantly ($P < 0.01$) in genotype comparison.
a–b Means with different superscript with lower-case letters in each row differ significantly ($P < 0.05$) in genotype comparison.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
ns, non significant.

Table 6
Least square means and standard error of means (SEM) of rennet clotting time (RCT), rate of clot firming (k20) and clot firmness (a30) according to the genotype (G) at *CSN1S2*, the farm (F), stage of lactation (S) and interaction F × S.

	CSN1S2						SEM	Effect			
	CF	AC	AF	FF	AA	CC		G	F	S	F × S
n	235	203	128	114	93	81					
Milk yield[#] (g/day)	1058^{B}	1029^{B}	1006^{AB}	913^{AB}	983^{AB}	898^{A}	18.3	**	***	***	***
Fat (g/100 ml)	4.83	4.75	4.77	4.86	4.81	5.02	0.03	ns	***	***	***
Protein (g/100 ml)	3.98	4.06	4.02	4.05	4.04	4.16	0.02	ns	***	***	***
pH	6.69	6.68	6.69	6.67	6.69	6.67	0.003	ns	***	***	***
RCT (min)	13.11	13.17	12.80	13.46	13.65	12.89	0.13	ns	**	ns	***
k20 (min)	1.91	1.84	1.95	1.83	2.24	1.77	0.04	ns	***	ns	*
a30 (mm)	43.58^{A}	46.34^{B}	43.32^{A}	43.99^{AB}	43.72^{AB}	48.07^{B}	0.39	**	***	***	**

[#] Yield of the morning milking.
[A–B] Means with different superscript with capital letters in each row differ significantly ($P<0.01$) in genotype comparison.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.
ns, non significant.

if milk protein content and curd firmness from Sarda were noticeably higher than those from Cilentana goats.

The absence of a significant influence of the *CSN1S1* genotype on RCT has been also evidenced by Clark and Sherbon (2000) in eight different caprine pure-breeds and crossbreds. In contrast, variation of rennet coagulation time has been observed when α_{s1}-casein variants were defined based on "high" and "low" type electrophoretic mobility (Ambrosoli et al., 1988) or when milk samples with high or low α_{s1}-casein synthesis rate have been compared (Pirisi et al., 1994).

On the other hand, *CSN1S1* polymorphism influenced the occurrence of non-coagulating samples; this effect has been already reported in goats by Devold et al. (2010), even if in that study genotype distribution, with a marked prevalence of null alleles, was quite different than in the Sarda goat.

Values of curd firmness registered for the Sarda goat in the present study, regardless of the high significant effect ($P<0.001$) of *CSN1S1* genotype, ranged between 42.42 and 47.88 mm and were similar to those previously recorded for the same breed (Pazzola et al., 2011a, 2012). These values may be considered very high when compared to other goat breeds or populations. Indeed, in a recent paper by Mestawet et al. (2013b), novel mutations at the *CSN1S1* gene, in autochthonous goat breeds from Ethiopia, influence milk protein concentration and significantly improve curd firmness, to reach an a30 mean value of 45.23 mm, which is described as exceptionally high.

The failure to measure the interaction effects among the casein genes, and statement that the whole casein cluster and its haplotype should be considered rather than a single gene at a time (Sacchi et al., 2005), could lead to speculate that this certainly diminished the possible information from this study. But, in one of the few paper which investigated interaction effects among casein genes (Caravaca et al., 2009) it has been conversely evidenced that interaction between genes *CSN1S1* and *CSN3* was not significantly influencing milk traits.

Not only the genetic effect of polymorphism at *CSN1S1*, but also the non-genetic effects considered in the statistical model, the farm and stage of lactation, showed a significant influence on milk traits, as observed by Zullo et al. (2005).

The influence of *CSN2*, *CSN1S2* and *CSN3* casein genes on milk composition were in accordance with results recorded in goat breeds both from Europe (Caravaca et al., 2009; Chiatti et al., 2007; Valenti et al., 2012) and Africa

Table 7
Least square means and standard error of means (SEM) of rennet clotting time (RCT), rate of clot firming (k20) and clot firmness (a30) according to the genotype (G) at *CSN3*, the farm (F), stage of lactation (S) and interaction F × S.

	CSN3				SEM	Effect			
	AB	BB	AA	BD		G	F	S	F × S
n	373	342	68	67					
Milk yield[#] (g/day)	997	999	1071	1001	18.5	ns	***	***	***
Fat (g/100 ml)	4.86^{AB}	4.68^{A}	5.02^{B}	4.85^{B}	0.03	***	***	***	***
Protein (g/100 ml)	4.07	3.99	4.08	3.95	0.02	ns	***	***	***
pH	6.68^{A}	6.69^{B}	6.67^{A}	6.64^{A}	0.003	***	***	***	***
RCT (min)	12.85^{B}	13.81^{C}	12.74^{B}	11.69^{A}	0.13	***	ns	ns	***
k20 (min)	1.82^{A}	2.13^{B}	1.68^{A}	1.61^{A}	0.04	***	***	ns	**
a30 (mm)	45.74^{B}	42.42^{A}	47.88^{C}	45.94^{AB}	0.41	***	***	***	**

[#] Yield of the morning milking.
[A–C] Means with different superscript with capital letters in each row differ significantly ($P<0.01$) in genotype comparison.
** $P<0.01$.
*** $P<0.001$.
ns, non significant.

(Scheepers et al., 2010), but their possible effect on milk renneting properties has not been investigated yet.

The influence of genotype effect recorded in this study was particularly marked just for *CSN3*, even if the amount of non-coagulating samples was not affected by this genotype. As deeply explored in dairy cows (Bonfatti et al., 2010), the κ-casein has a fundamental role in influencing size and stabilization of casein micelles, and consequently cheese-making processes.

We observed that milk from *CSN3* BB goats, when compared to *CSN3* AA, had a higher RCT value. This parameter indicates a difference in the speed of clot formation and is especially affected by the pH value of milk, which plays an important role in milk coagulation properties and has a relative high value of heritability in cattle (Bittante et al., 2012). The milk pH can be influenced by the isoelectric point (p*I*) of proteins. We measured the p*I* value of the *CSN3* A and B variants, based on the amino acid sequences, by using the Scansite software (Obenauer et al., 2003) and the outcome was the same for the two variants. The observed differences may be also due to variation in the number of phosphorylation or glycosylation sites, as stated by Jensen et al. (2012) for bovine κ-casein. In order to support this hypothesis, we analyzed the deduced amino acid sequences of the *CSN3* A and B alleles by means of NetPhos 2.0 (Blom et al., 1999). This analysis revealed that the p.Thr129 amino acid is a potential phosphorylation site in the *CSN3* A variant, which was lost in the *CSN3* B variant. The p.Thr129 amino acid is positioned near the p.Val131Ile variation site, which represents the only difference between the two alleles. Analysis of the potential glycosylation sites by using the software NetOGlyc 4.0 (Steentoft et al., 2013) revealed that the p.Ser166 amino acid may be a potential glycosylation site in the *CSN3* B allele, which was lost in the *CSN3* A allele.

The strongest a30 was recorded for *CSN3* AA, even though in absence of significant differences for protein content. This last occurrence is not in agreement with previous papers on the same topic (Caravaca et al., 2009; Chiatti et al., 2007) in which protein content was influenced by genotype at *CSN3*, but those studies have considered only differences at nucleotide position 471, which allows to distinguish *CSN3* A from all the other alleles.

Besides the differences in milk renneting properties, it was also evidenced a high variability at the *CSN3* locus in Sarda goats, as well as the occurrence of the *CSN3*C allele in two rare genotypes, BC and CD, removed from the statistical model because of frequency lower than 4%, which has been evidenced with very low frequencies in some Italian autochthonous (Caroli et al., 2006; Sacchi et al., 2005) and hair goat populations (Prinzenberg et al., 2005).

5. Conclusions

Results illustrated in this study could be useful in characterization of specific dairy products linked to the Sarda breed (PDO/PGI), which are certainly a suitable tool to safeguard local breeds and biodiversity. It was evidenced that milk from the Sarda goat had optimal renneting properties which were influenced by the different genotypes at casein genes, especially at *CSN3*. Furthermore, knowledge on the effects of casein genes on milk renneting parameters in the goat species and in the Sarda breed was improved. On the whole some genotypes, as *CSN1S1* AB, *CSN1S2 CC*, *CSN3 AA* and *BD*, could be preferred in future planning of breeding programs and selection schemes for enhancing coagulation traits.

Acknowledgements

Research supported by the RAS, Regional Government of Sardinia (P5a-Animal Biodiversity funds).

References

Ambrosoli, R., Di Stasio, L., Mazzocco, P., 1988. Content of α_{s1}-casein and coagulation properties in goat milk. J. Dairy Sci. 71, 24–28.

Arqués, J.L., Garde, S., Fernández-García, E., Gaya, P., Nuñez, M., 2007. Volatile compounds, odor, and aroma of La Serena Cheese high-pressure treated at two different stages of ripening. J. Dairy Sci. 90, 3627–3639.

Balia, F., Pazzola, M., Dettori, M.L., Mura, M.C., Luridiana, S., Carcangiu, V., Piras, G., Vacca, G.M., 2013. Effect of CSN1S1 gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. J. Dairy Res. 80, 129–137.

Bittante, G., Penasa, M., Cecchinato, A., 2012. Genetics and modeling of milk coagulation properties. J. Dairy Sci. 95, 6843–6870.

Blom, N., Gammeltoft, S., Brunak, S., 1999. Sequence- and structure-based prediction of eukaryotic protein phosphorylation sites. J. Mol. Biol. 294, 1351–1362.

Bonfatti, V., Di Martino, G., Cecchinato, A., Degano, L., Carnier, P., 2010. Effects of β–κ-casein (CSN2-CSN3) haplotypes, β-lactoglobulin (BLG) genotypes, and detailed protein composition on coagulation properties of individual milk of Simmental cows. J. Dairy Sci. 93, 3809–3817.

Boyazoglu, J., Morand-Fehr, P., 2001. Mediterranean dairy sheep and goat products and their quality. A critical review. Small Rumin. Res. 40, 1–11.

Caravaca, F., Carrizosa, J., Urrutia, B., Baena, F., Jordana, J., Amills, M., Badaoui, B., Sanchez, A., Angiolillo, A., Serradilla, J.M., 2009. Effect of α_{S1}-casein (CSN1S1) and κ-casein (CSN3) genotypes on milk composition in Murciano-Granadina goats. J. Dairy Sci. 92, 2960–2964.

Caroli, A., Chiatti, F., Chessa, S., Rignanese, D., Bolla, P., Pagnacco, G., 2006. Focusing on the goat casein complex. J. Dairy Sci. 89, 3178–3187.

Cebo, C., Lopez, C., Henry, C., Beauvallet, C., Ménard, O., Bevilacqua, C., Bouvier, F., Caillat, H., Martin, P., 2012. Goat α_{s1}-casein genotype affects milk fat globule physicochemical properties and the composition of the milk fat globule membrane. J. Dairy Sci. 95, 6215–6229.

Chessa, S., Budelli, E., Gutscher, K., Caroli, A., Erhardt, G., 2003. Short communication: simultaneous identification of five κ-casein (CSN3) alleles in domestic goat by polymerase chain reaction-single strand conformation polymorphism. J. Dairy Sci. 86, 3726–3729.

Chiatti, F., Chessa, S., Bolla, P., Cigalino, G., Caroli, A., Pagnacco, G., 2007. Effect of κ-casein polymorphism on milk composition in the Orobica goat. J. Dairy Sci. 90, 1962–1966.

Clark, S., Sherbon, J.W., 2000. Alphas1-casein, milk composition and coagulation properties of goat milk. Small Rumin. Res. 38, 123–134.

Cosenza, G., Illario, R., Rando, A., Di Gregorio, P., Masina, P., Ramunno, L., 2003. Molecular characterization of the goat CSN1S101 allele. J. Dairy Res. 70, 237–240.

Dettori, M.L., Vacca, G.M., Carcangiu, V., Pazzola, M., Mura, M.C., Rocchigiani, A.M., 2009. A reliable method for characterization of the goat CSN1S1 E allele. Livest. Sci. 125, 105–108.

Devold, T.G., Nordbø, R., Langsrud, T., Svenning, C., Brovold, M.J., Sørensen, E.S., Christensen, B., Adnøy, T., Vegarud, G.E., 2010. Extreme frequencies of the α_{s1}-casein 'null' variant in milk from Norwegian dairy goats-implications for milk composition, micellar size and renneting properties. Dairy Sci. Technol. 91, 39–51.

IZS, 2013. Italian National Data Bank for the animal husbandry register at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", (Abruzzo and Molise Experimental Zooprophilactic Institute), founded by the Italian Ministry of Health. http://www.izs.it/it/anagrafe.zootecnica/anagrafe.zootecnica.intro.html (accessed 01.07.13).

Jensen, H.B., Holland, J.W., Poulsen, N.A., Larsen, L.B., 2012. Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties. J. Dairy Sci. 95, 2891–2903.

Martin, P., Szymanowska, M., Zwierzchowski, L., Leroux, C., 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. Reprod. Nutr. Dev. 42, 433–459.

Martinez, S., Franco, I., Carballo, J., 2011. Spanish goat and sheep milk cheeses. Small Rumin. Res. 101, 41–54.

Mestawet, T.A., Girma, A., Ådnøy, T., Dagnachew, B., Sundsaasen, K.K., Lien, S., Kent, M.P., Devold, T.G., Narvhus, J.A., Vegarud, G.E., 2013a. New genetic polymorphism at the α_{s1}-casein gene region in Ethiopian indigenous goat breeds. Small Rumin. Res. 113, 329–334.

Mestawet, T.A., Girma, A., Ådnøy, T., Devold, T.G., Vegarud, G.E., 2013b. Newly identified mutations at the CSN1S1 gene in Ethiopian goats affect casein content and coagulation properties of their milk. J. Dairy Sci. 96, 4857–4869.

Moioli, B., D'Andrea, M., Pilla, F., 2007. Candidate genes affecting sheep and goat milk quality. Small Rumin. Res. 68, 179–192.

Obenauer, J.C., Cantley, L.C., Yaffe, M.B., 2003. Scansite 2.0: proteome-wide prediction of cell signaling interactions using short sequence motifs. Nucleic Acids Res. 31, 3635–3641.

Pazzola, M., Balia, F., Carcangiu, V., Dettori, M.L., Piras, G., Vacca, G.M., 2012. Higher somatic cell counted by the electronic counter method do not influence renneting properties of goat milk. Small Rumin. Res. 102, 32–36.

Pazzola, M., Balia, F., Dettori, M.L., Mura, M.C., Carcangiu, V., Vacca, G.M., 2011a. Effect of different storage conditions, the farm and the stage of lactation on renneting parameters of goat milk investigated using the Formagraph method. J. Dairy Res. 78, 343–348.

Pazzola, M., Dettori, M.L., Carcangiu, V., Luridiana, S., Mura, M.C., Vacca, G.M., 2011b. Relationship between milk urea, blood plasma urea and BCS in primiparous browsing goats with different milk yield level. Archiv Tierzucht 54 (5), 546–556.

Pirisi, A., Colin, O., Laurent, F., Scher, J., Parmentier, M., 1994. Comparison of milk composition, cheesemaking properties and textural characteristics of the cheese from two groups of goats with a high or low rate of α_{S1}-casein synthesis. Int. Dairy J. 4, 329–345.

Prinzenberg, E.M., Gutscher, K., Chessa, S., Caroli, A., Erhardt, G., 2005. Caprine kappa-casein (CSN3) polymorphism: new developments in molecular knowledge. J. Dairy Sci. 88, 1490–1498.

Ramunno, L., Cosenza, G., Pappalardo, M., Longobardi, E., Gallo, D., Pastore, N., Di Gregorio, P., Rando, A., 2001. Characterization of two new alleles at the goat CSN1S2 locus. Anim. Genet. 32, 264–268.

Ramunno, L., Cosenza, G., Pappalardo, M., Pastore, N., Gallo, D., Di Gregorio, P., Masina, P., 2000a. Identification of the goat CSN1S1F allele by means of PCR-RFLP method. Anim. Genet. 31, 342–343.

Ramunno, L., Pappalardo, M., Cosenza, G., Pastore, N., Gallo, D., Grimaldi, G., Rubino, R., Calandrelli, M., Rando, A., 2000b. Genetic characterization at α_{S1}, β and α_{S2} loci of a goat population reared in the Sorrentine Peninsula. In: Proceedings of the XIV Congress SIPAOC (Italian Society of Pathology and Breeding of Sheep and Goat), Vietri sul mare, Salerno, Italy, pp. 321–324.

Remeuf, F., 1993. Influence du polymorphisme génétique de la caséine αs_1caprine sur les caractéristiques physico-chimiques et technologiques du lait. Lait 73, 549–557.

Rijnkels, M., 2002. Multispecie comparison of the casein gene loci and evolution of casein gene family. J. Mammary Gland Biol. 7, 327–345.

Sacchi, P., Chessa, S., Budelli, E., Bolla, P., Ceriotti, G., Soglia, D., Rasero, R., Cauvin, E., Caroli, A., 2005. Casein haplotype structure in five Italian goat breeds. J. Dairy Sci. 88, 1561–1568.

Scheepers, R.C., van Marle-Köster, E., Visser, C., 2010. Genetic variation in the kappa-casein gene of South African goats. Small Rumin. Res. 93, 53–56.

Steentoft, C., Vakhrushev, S.Y., Joshi, H.J., Kong, Y., Vester-Christensen, M.B., Schjoldager, K.T., Lavrsen, K., Dabelsteen, S., Pedersen, N.B., Marcos-Silva, L., Gupta, R., Bennett, E.P., Mandel, U., Brunak, S., Wandall, H.H., Levery, S.B., Clausen, H., 2013. Precision mapping of the human O-GalNAc glycoproteome through Simple Cell technology. EMBO J. 32, 1478–1488.

Vacca, G.M., Daga, C., Pazzola, M., Carcangiu, V., Dettori, M.L., Cozzi, M.C., 2010a. D-loop sequence mitochondrial DNA variability of Sarda goat and other goat breeds and populations reared in the Mediterranean area. J. Anim. Breed. Genet. 127, 352–360.

Vacca, G.M., Dettori, M.L., Carcangiu, V., Rocchigiani, A.M., Pazzola, M., 2010b. Relationships between milk characteristics and somatic cell score in milk from primiparous browsing goats. Anim. Sci. J. 81, 594–599.

Vacca, G.M., Ouled Ahmed Ben Alì, H., Carcangiu, V., Pazzola, M., Dettori, M.L., 2009a. Genetic structure of the casein gene cluster in the Tunisian native goat breed. Small Rumin. Res. 87, 33–38.

Vacca, G.M., Ouled Ahmed Ben Alì, H., Pazzola, M., Sanna, M., Dettori, M.L., Carcangiu, V., 2009b. An investigation on allele frequency at the CSN1S2 locus and its relationship with milk parameters in the Sarda goat. J. Anim. Feed Sci. 18, 628–637.

Vacca, G.M., Pazzola, M., Pisano, C., Carcangiu, V., Diaz, M.L., Nieddu, M., Robledo, R., Mezzanotte, R., Dettori, M.L., 2011. Chromosomal localisation and genetic variation of the SLC11A1 gene in *Capra hircus*. Vet. J. 190, 60–65.

Valenti, B., Pagano, R.I., Avondo, M., 2012. Effect of diet at different energy levels on milk casein composition of Girgentana goats differing in CSN1S1 genotype. Small Rumin. Res. 105, 135–139.

Vassal, L., Delacroix-Buchet, A., Bouillon, J., 1994. Influence des variants AA, EE et FF de la caséine $\alpha s1$ caprine sur le rendement fromager et les caractéristiques sensorielles de fromages traditionnels: premières observations. Lait 74, 89–103.

Veltri, C., Lagonigro, R., Pietrolà, E., D'Andrea, M., Pilla, F., Chianese, L., 2000. Molecular characterisation of the goat α_{s2}-casein E allele and its detection in goat breeds of Italy. In: Proceedings of the 7th International Conference on Goats, Tours, France, p. 727.

Zullo, A., Barone, C.M.A., Chianese, L., Colatruglio, P., Occidente, M., Matassino, D., 2005. Protein polymorphisms and coagulation properties of Cilentana goat milk. Small Rumin. Res. 58, 223–230.

3.4 Paper IV

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi: 10.1111/age.12188

Goat casein genotypes are associated with milk production traits in the Sarda breed

G. M. Vacca*†, M. L. Dettori*, G. Piras*, F. Manca*, P. Paschino* and M. Pazzola*

*Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, via Vienna 2, Sassari 07100, Italy. †Centro di Competenza Biodiversità Animale, viale Adua 2C, Sassari 07100, Italy.

Summary

The aim of the current work was to analyze, in the Sarda breed goat, genetic polymorphism within the casein genes and to assess their influence on milk traits. Genetic variants at the *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* gene loci were investigated using PCR-based methods, cloning and sequencing. Strong alleles prevailed at the *CSN1S1* gene locus and defective alleles also were revealed. Null alleles were evidenced at each calcium-sensitive gene locus. At the *CSN3* gene locus, we observed a prevalence of the *CSN3 A* and *B* alleles; the occurrence of rare alleles such as *CSN3 B''*, *C*, *C'*, *D*, *E* and *M*; and the *CSN3 S* allele (GenBank KF644565) described here for the first time in *Capra hircus*. Statistical analysis showed that all genes, except *CSN3*, significantly influenced milk traits. The *CSN1S1 BB* and *AB* genotypes were associated with the highest percentages of protein (4.41 and 4.40 respectively) and fat (5.26 and 5.34 respectively) ($P < 0.001$). A relevant finding was that *CSN2* and *CSN1S2* genotypes affected milk protein content and yield. The polymorphism of the *CSN2* gene affected milk protein percentage with the highest values recorded in the *CSN2 AA* goats (4.35, at $P < 0.001$). The *CSN1S2 AC* goats provided the highest fat (51.02 g/day) and protein (41.42 g/day) ($P < 0.01$) production. This information can be incorporated into selection schemes for the Sarda breed goat.

Keywords casein cluster, genotypes, milk traits, sarda goat

Introduction

In the Mediterranean Basin, autochthonous goats with high hardiness prevail and, among these breeds, the Sarda (Sardinia, Italy) with over 200 000 heads is certainly one of the most important ones (Vacca *et al.* 2010a). The Sarda goat is reared under a traditional management system, and feeding is based on the extensive use of natural pasture (Usai *et al.* 2006). This breed has not undergone high selective pressure to improve milk production or reproductive seasonality (Carcangiu *et al.* 2009).

Goat milk can display variable composition, which determines its use as drinking milk or for cheese making. This potentiality depends mainly on the milk protein component and, more specifically, on the casein fraction, the genetic variants of which can affect milk technological properties (Grosclaude *et al.* 1994; Barillet 2007). Four casein genes have been mapped to chromosome 6 in goat and are organized as a cluster: *CSN1S1–CSN2–CSNS2–CSN3* (Rijnkels 2002). The *CSN1S1*, *CSN2* and *CSN1S2* genes encode the calcium-sensitive caseins and are evolutionarily related, whereas *CSN3* is a physically linked gene having the functional role of stabilizing the casein micelle (Rijnkels *et al.* 2003). The αs_1-casein fraction is encoded by the *CSN1S1* gene, which has been the one most studied and shows at least 18 alleles classified as strong (*A*, *B1*, *B2*, *B3*, *B4*, *B'*, *C*, *H*, *L*, *M*), intermediate (*E*, *I*), weak (*D*, *F*, *G*) and null (*01*, *02*, *N*), based on gene expression levels (Grosclaude & Martin 1997; Martin *et al.* 2002). The β-casein fraction is encoded by the *CSN2* gene, which includes at least eight alleles: *A*, A_1, *B*, *C*, *D*, *E*, *0* and *01* (Marletta *et al.* 2007). The αs_2-casein is encoded by the *CSN1S2* gene, which includes seven alleles characterizing different expression levels: strong (*CSN1S2 A*, *B*, *C*, *E*, *F*), intermediate (*CSN1S2 D*) and null (*CSN1S2 0*) (Ramunno *et al.* 2001a). The k-casein fraction is encoded by the *CSN3* gene, which as at least 21 known alleles: *A*, *B*, *B'*, *B''*, *C*, *C'*, *D*, *E*, *F*, *G*, *H*, *I*, *J*, *K*, *L*, *M*, *N*, *O*, *P*, *Q* and *R* (Prinzenberg *et al.* 2005; Gupta *et al.* 2009).

The composition and technological properties of Sarda goat milk have been reported (Pazzola *et al.* 2011, 2012), but its association with casein genotypes has been

Address for correspondence

G. M. Vacca, Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, via Vienna, 2 - 07100 Sassari, Italia.
E-mail: gmvacca@uniss.it

Accepted for publication 07 May 2014

investigated only for *CSN1S1* and *CSN1S2* (Vacca *et al.* 2009b; Balia *et al.* 2013). Although *CSN1S1* causal mutations have been analyzed in depth in goats, the other three genes and their effects on milk traits have been studied only to a very limited extent. The purpose of this research was to analyze genetic polymorphism within the casein genes *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* in Sarda goats and to assess the influence of the casein genotype on milk traits.

Material and methods

Sampling of Sarda goats and milk analysis

This research was carried out on 33 farms of Sardinia (Italy) which have traditional management systems. Milking was carried out once per day by hand. On each farm, 24–25 lactating goats and three to four adult males were randomly chosen, for a total of 814 females and 121 males, from which blood samples were taken. Milk production was recorded and individual milk samples were collected from each female goat. Milk protein, fat and lactose contents were determined by infrared spectrophotometry according to the International Dairy Federation (IDF) 2000 standard 141C: 2000 (Milko-Scan 133B; Foss Electric). Milk energy, which represents the estimated calories provided by total solid components, was calculated according to the National Research Council (2001).

Genotyping of casein genes

Genomic DNA was extracted using the Puregene DNA isolation kit (Gentra Systems, Inc.). The *CSN1S1 A, B, F* and *N* alleles were analyzed simultaneously using PCR-RFLP (polymerase chain reaction, restriction fragment length polymorphism) as described by Ramunno *et al.* (2000a). The *CSN1S1 E* allele was analyzed by AS-PCR (allele-specific PCR) according to Dettori *et al.* (2009). The *CSN1S1 01* allele was investigated using AS-PCR (Cosenza *et al.* 2003). The *CSN2 A, C, E* and *01* alleles were genotyped simultaneously by SSCP (single-strand conformation polymorphism) according to Caroli *et al.* (2006). The *CSN1S2 A, B* and *C* alleles were determined by multiplex AS-PCR (Ramunno *et al.* 2000b). The *CSN1S2 F* and *D* alleles were investigated by PCR (Ramunno *et al.* 2001a), and the *CSN1S2 E* and *0* alleles were detected by PCR-RFLP (Ramunno *et al.* 2001b). The *CSN3* gene was analyzed by PCR-SSCP (Chessa *et al.* 2003) to determine the *CSN3 A, B, B'', C, C', D, E, F, G, H, I, J, K, L, M, N, O, P, Q* and *R* alleles, named according to the nomenclature proposed by Prinzenberg *et al.* (2005). All genotyping methods are described in Appendix S1. The nucleotide changes defining each variant were named according to the recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/) (Tables S1 to S4). One to three PCR amplicons showing the same SSCP pattern were analyzed by Sanger sequencing in both directions. Sequencing was performed with an ABI 3730xl DNA Analyzer (Applied Biosystems) after purification with the Charge-Switch PCR Clean-Up Kit (Invitrogen).

Allele frequencies and Hardy–Weinberg equilibrium were analyzed using GENEPOP software (Raymond & Rousset 1995). Casein haplotype frequencies were estimated using PHASE software (Stephens *et al.* 2001). Pairwise linkage disequilibrium (LD) measures (D' and r^2) were determined and plotted using the HAPLOVIEW software package (Barrett *et al.* 2005), utilizing the Gabriel criteria to define the LD blocks (Gabriel *et al.* 2002).

Cloning of a new allele at the *CSN3* gene

The *CSN3* PCR amplification products of three goats, showing an unknown genotype after sequencing, were cloned into the pCR4-TOPO vector and then transformed into Mach1-T1 chemically competent cells (Invitrogen Life Technologies). Recombinant plasmids were purified with the Qiagen Plasmid Midi Kit (Qiagen GmbH) and then sequenced in both directions using M13F and M13R universal primers.

Statistical analysis

To identify associations between casein genotypes and milk traits, data were analyzed with a general linear model as follows:

$$Y_{ijkl} = \mu + F_i + S_j + G_k + e_{ijkl},$$

where Y_{ijkl} is the analyzed trait, μ is the overall mean, F_i is the fixed effect of the farm (33 levels), S_j is the fixed effect of the stage of lactation (three levels: 1–80, 81–160 and >160 days in milk), G_k is the fixed effect of the casein genotype and e_{ijkl} is the error. Genotypes with a frequency lower than 2.5 percent were excluded from statistical analyses. Because of rank deficiency, association effects were not included in the model and each of the four different loci was analyzed with a GLM (general linear model) procedure because the high number of alleles did not allow a combined genotype analysis. The number of G_k levels was 7, 4, 6 and 4 for *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* respectively. The Bonferroni method was used to correct for multiple testing of the means and significance was set at $P < 0.05$.

Results

Genotype frequencies at the casein genes

Descriptive statistics estimated from milk traits on 814 goats is reported in Table S5. Molecular analysis of the four casein loci (Table 1) revealed that, at *CSN1S1*, the most frequent

Table 1 Allele frequencies at casein gene cluster loci in Sarda breed goats.

CSN1S1				*CSN2*				*CSN1S2*				*CSN3*			
Allele	Females $n = 814$	Males $n = 121$	Total $n = 935$	Allele	Females $n = 814$	Males $n = 121$	Total $n = 935$	Allele	Females $n = 814$	Males $n = 121$	Total $n = 935$	Allele	Females $n = 814$	Males $n = 121$	Total $n = 935$
A	0.197	0.235	0.202	*A*	0.379	0.343	0.375	*A*	0.309	0.343	0.313	*A*	0.282	0.235	0.2765
B	0.523	0.500	0.520	*C*	0.597	0.599	0.597	*B*	0.007	–	0.006	*B*	0.679	0.665	0.6770
E	0.029	0.033	0.030	*01*	0.024	0.058	0.028	*C*	0.272	0.260	0.271	*B''*	0.001	–	0.0005
F	0.244	0.227	0.242					*E*	0.020	0.008	0.018	*C*	0.014	0.046	0.0182
N	0.006	0.004	0.006					*F*	0.383	0.380	0.383	*C'*	0.001	0.004	0.0016
01	0.001	–	0.001					*0*	0.009	0.008	0.009	*D*	0.021	0.046	0.0246
												E	–	0.004	0.0005
												M	0.001	–	0.0005
												S	0.001	–	0.0005
P value			0.023				0.304				0.083				0.019
Hardy–Weinberg test															

allele in the total population was *B*, followed by *F* and *A*. The *E*, *N* and *01* alleles were rare, and the latter was not found in bucks. Sequencing of PCR amplicons showing differing SSCP patterns allowed to assign genotypes at the *CSN2* locus. The most frequent allele was *CSN2 C* in both sexes, whereas the *CSN2 01* allele had a frequency higher than 5 percent only in males. No *CSN2 E* allele was detected. At the *CSN1S2* locus, the most frequent alleles were $F > A > C$ in both sexes. The rare *CSN1S2 E*, *0* and *B* alleles were also identified, with frequencies lower than 2 percent and the *CSN1S2 B* allele being found only in females. At the *CSN3* locus, *B* and *A* were the most frequent alleles. The remaining may be considered as rare alleles, as they showed a frequency lower than 3 percent (*C* and *D*) or lower than 1 percent (*B''*, *C'*, *E*, *M* and *S*). At the *CSN1S1* and *CSN3* loci, the population did not meet Hardy–Weinberg expectations, probably due to a heterozygote excess.

Discovery of a new *CSN3* allele

Cloning and sequencing of three unknown SSCP patterns revealed that two of them corresponded to the *CSN3 C'E* and *CSN3 BM* genotypes, and one corresponded to the *CSN3 AS* genotype, *CSN3 S* being a new allele variant (Fig. S1). We considered *CSN3 S* a new allele, according to the criteria proposed by Prinzenberg *et al.* (2005), and the *S* letter was given following the alleles identified thus far (Gupta *et al.* 2009). The *CSN3 S* sequence was submitted to the GenBank database and given accession number KF644565.

Haplotype distribution

Haplotypes at the casein gene cluster locus of Sarda goats with frequencies higher than 1 percent are shown in Table 2. In females, seven of 21 haplotypes had a frequency higher than 5 percent, whereas 26 haplotypes were identified in males. Seven of these showed frequencies higher than 5 percent. A total of 15 of 26 polymorphic sites investigated were deemed valid for analysis with HAPLOVIEW. The resulting set of polymorphisms covered the entire casein cluster, spanning about 216 kb from nucleotide 23 at *CSN1S1* exon 9 to nucleotide 591 at *CSN3* exon 4. We observed a *D'* value of 0.79 between *CSN1S1* and *CSN2*, a *D'* value of 1 within the *CSN2* gene and several regions of high LD within the *CSN3* gene (Fig. S2).

Association analysis between casein genotypes and milk traits

Statistical analysis showed that farm is a fixed factor that has a significant effect on all the traits studied, whereas stage of lactation did not affect fat percentages and energy (data not shown). Genotype at the *CSN1S1* locus significantly influenced all milk parameters: the *CSN1S1 BB* and *AB* genotypes

Table 2 Haplotype frequencies at the casein gene cluster loci in Sarda breed goats.

Haplotype[1]				Females		Males	
CSN1S1	*CSN2*	*CSN1S2*	*CSN3*	Frequency[2]	SE[3]	Frequency[2]	SE[3]
A	C	A	A	0.013	0.003	0.032	0.006
A	C	A	B	**0.050**	0.003	0.036	0.009
A	C	A	D			0.012	0.004
A	C	C	A	0.020	0.003	0.016	0.004
A	C	C	B	0.024	0.003	0.019	0.006
A	C	C	D			0.020	0.003
A	C	F	A			0.012	0.008
A	C	F	B	0.032	0.003	0.022	0.007
A	01	F	B	0.016	0.000	**0.054**	0.004
B	A	A	A	0.020	0.003	0.025	0.011
B	A	A	B	**0.061**	0.004	**0.091**	0.013
B	A	C	A	**0.091**	0.004	**0.055**	0.009
B	A	C	B	**0.055**	0.004	**0.053**	0.011
B	A	C	C			0.014	0.004
B	A	F	A	0.023	0.003		
B	A	F	B	0.038	0.004	0.022	0.012
B	C	A	A	0.011	0.002	0.022	0.007
B	C	A	B	**0.068**	0.003	**0.067**	0.011
B	C	C	A	0.010	0.002	0.023	0.007
B	C	C	B			0.014	0.009
B	C	F	A	0.010	0.002		
B	C	F	B	**0.079**	0.004	**0.055**	0.010
E	A	A	B			0.015	0.003
E	A	F	B			0.011	0.003
F	C	A	B	0.039	0.004	0.011	0.007
F	C	C	B	0.011	0.002	0.021	0.007
F	C	F	A	0.027	0.002	0.014	0.008
F	C	F	B	**0.134**	0.004	**0.143**	0.010

[1]Only haplotypes with frequency >0.01 are shown.
[2]Haplotypes showing frequencies >0.05 are in bold.
[3]SE standard deviations (square root of the variance of the posterior distribution) for the frequencies.

showed the highest protein and fat percentages and energy level but a lower daily milk yield than did the *CSN1S1 AA, BF* and *EF* genotypes ($P < 0.001$). The *CSN1S1 EF* genotype provided the highest daily production of protein (Table 3). The *CSN2* gene significantly affected the milk protein percentage ($P < 0.001$) and protein daily production ($P < 0.01$) with the *CSN2 C01* genotype showing the lowest values. The highest protein percentages were recorded for the *CSN2 AA* genotype. The *CSN1S2 CF* goats provided the highest daily milk yield ($P < 0.05$), and as well, goats with the *CSN1S2 AC* genotype had the highest ($P < 0.01$) fat and protein daily production. Goats carrying the *CSN1S2 FF* genotype showed the lowest protein percentage ($P < 0.05$). Genotypes at the *CSN3* locus did not significantly influence any of the milk parameters under consideration.

Discussion

Goat *CSN2* and *CSN1S2* genotypes are associated with milk protein content and yield

At the *CSN2* locus, the Sarda goat was characterized by the prevalence of the *CSN2 C* and the occurrence of the *CSN2 01* alleles, as previously evidenced in caprine breeds from southern Italy (Chessa *et al.* 2005). We observed huge variability at *CSN1S2*, where, except for *CSN1S2 D*, all known alleles segregated. The *CSN1S2* genetic pool of the Sarda goat was similar to that observed in other Italian breeds (Marletta *et al.* 2005; Sacchi *et al.* 2005). In this study, the *CSN1S2 0* allele was identified in the Sarda goat for the first time, being undetected in a previous investigation (Vacca *et al.* 2009b). Some authors have suggested that goat milk lacking the αs_2-casein fraction is more similar to human milk than is αs_1-casein-free milk (Ramunno *et al.* 2001b). Polymorphic sites within the *CSN1S2* gene showed low LD, which is consistent with the fact that the *CSN1S2* gene, in bovids, is phylogenetically more recent when compared with the other two calcium-sensitive *CSN1S1* and *CSN2* casein genes (Rijnkels 2002).

Because of the linkage of the casein genes in a cluster, a statistical model including the combined genotypes for all four loci would have been more correct but, due to the high number of genetic variants found and of their multiple combinations, it was infeasible. The most important finding in this study is that the *CSN1S2* genotype affected milk yield as well as milk fat and protein daily production and protein

Table 3 Association analysis between casein genotypes and milk traits.

	Milk yield		Fat		Protein		Lactose	Energy
	n	g/day	%	g/d	%	g/day	%	MJ/kg
CSN1S1		4.71***	5.40***	2.43*	24.17***	4.15***	1.69 ns	10.66***
AA	31	1010.2^{B}	5.04^{B}	51.44^{b}	4.05^{B}	40.42^{B}	5.00	3.75^{B}
AB	167	924.0^{A}	5.34^{C}	50.75^{b}	4.40^{C}	40.53^{B}	4.91	3.91^{C}
AF	80	857.9^{A}	5.11^{B}	43.83^{a}	4.09^{B}	34.67^{A}	4.97	3.78^{B}
BB	234	942.8^{A}	5.26^{C}	48.93^{b}	4.41^{C}	41.14^{B}	4.90	3.88^{C}
BF	199	1020.8^{B}	5.00^{B}	50.39^{b}	4.10^{B}	41.45^{B}	4.93	3.72^{B}
EF	22	1191.3^{C}	4.55^{A}	51.98^{b}	3.73^{A}	43.59^{C}	4.90	3.45^{A}
FF	47	937.0^{A}	4.78^{A}	44.42^{a}	3.82^{A}	35.87^{A}	4.91	3.56^{A}
SEM		1.561	0.004	0.081	0.002	0.064	0.001	0.002
CSN2		2.16ns	0.31ns	1.38ns	6.42***	4.35**	0.92ns	0.62ns
AA	112	975.6	5.20	50.26	4.35^{C}	41.62^{B}	4.92	3.84
AC	378	976.4	5.11	49.74	4.25^{BC}	40.99^{B}	4.91	3.79
CC	286	952.2	5.11	48.06	4.19^{B}	39.40^{B}	4.91	3.78
C01	22	783.3	5.12	43.12	3.91^{A}	31.22^{A}	5.01	3.78
SEM		1.441	0.004	0.075	0.002	0.055	0.001	0.002
CSN1S2		2.57*	1.17 ns	3.15**	2.95*	3.31**	2.22 ns	1.58 ns
AA	91	931.5^{a}	5.19	48.78^{AB}	4.25^{b}	39.89^{AB}	4.92	3.82
AC	134	924.4^{a}	5.25	51.02^{B}	4.27^{b}	41.42^{B}	4.90	3.84
AF	167	924.7^{a}	5.11	46.02^{A}	4.23^{b}	38.89^{A}	4.92	3.78
CC	466	903.2^{a}	5.18	48.41^{AB}	4.24^{b}	37.89^{A}	4.97	3.83
CF	203	1021.5^{b}	5.06	51.99^{B}	4.26^{b}	42.64^{B}	4.95	3.78
FF	115	923.1^{a}	5.01	46.33^{A}	4.07^{a}	37.75^{A}	4.87	3.71
SEM		1.272	0.003	0.066	0.002	0.048	0.001	0.001
CSN3		1.18ns	0.01ns	1.76ns	1.22ns	1.53ns	0.66ns	0.03ns
AA	72	931.9	5.14	48.09	4.22	39.13	4.88	3.79
AB	306	976.7	5.15	50.17	4.25	41.00	4.92	3.80
BB	377	955.6	5.15	48.48	4.18	39.68	4.93	3.80
BD	22	1073.7	5.15	56.03	4.16	44.43	4.94	3.80
SEM		1.502	0.004	0.077	0.002	0.057	0.001	0.002

SEM, standard error of the mean; A–C, means with different superscript with capital letters ($P < 0.01$) or lower case letter ($P < 0.05$) in each row differ significantly in genotype comparison.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

percentage, because no effects had been found for the *CSN1S2* locus in a previous investigation conducted on the same breed (Vacca *et al.* 2009a). The *CSN1S2* genotypes associated with the highest values of milk traits were both heterozygous (*AC* and *CF*), whereas the *AA*, *CC* and *FF* homozygous genotypes showed no differences. Thus, the observed differences are probably not explainable with the only nucleotide changes characterizing these alleles. The association pattern evidenced at the *CSN1S2* locus partly reflects that observed at the *CSN1S1* locus in Sarda goats. Nevertheless, at the *CSN1S2* locus, we observed differences between non-defective alleles that deserve further studies to be explained, although many comprehensive studies have been carried out on αs1-casein explaining the molecular basis of the genotype effect at the genomic, mRNA and protein levels as well as its effects on the cytology of the goat mammary epithelial cell (Martin *et al.* 2002).

The higher protein percentage of genotype *CSN2 AA* compared with *CC* may be due to different unknown causes. Nevertheless, if one were to observe the casein gene cluster haplotypes derived from the analysis with PHASE software, one can see that, in females, the *CSN2 A* variant was always linked with *CSN1S1 B*, whereas the *CSN2 C* variant was linked to the *CSN1S1 A*, *B* or *F* alleles. Hence, the higher protein content associated with the *CSN2 AA* genotype may result from the association of *CSN2 A* with *CSN1S1 B*. In fact, although mRNA transcription shows the same efficiency for the four casein genes (about 25% each), the *CSN1S1* and *CSN2* mRNAs are translated about four times more efficiently than are *CSN1S2* and *CSN3* mRNAs in cow, sheep and goats (Bevilacqua *et al.* 2006). The lowest values of protein percentage and protein daily production associated with the *CSN2 C01* genotype can be explained by the fact that the *CSN2 01* allele is characterized by the nucleotide change c.544C>T, causing the premature introduction of a stop codon and, thus, a protein truncated at amino acid position 182 (p.Gln182X). This mutation involves a 10-fold decay in mRNA expression (Ramunno *et al.* 1995).

Variability at the *CSN1S1* gene influences the milk quality and yield of Sarda goats

Strong alleles at the *CSN1S1* gene prevailed over the others; in particular, *CSN1S1 B* was the most widespread one in the

Sarda breed. Conversely, the *A* allele is the most frequent one in other Mediterranean breeds such as Arbi, a native of Tunisia, or Maltese (Sacchi *et al.* 2005; Vacca *et al.* 2009a). We hypothesize that the presence of *CSN1S1 A* in the Sarda goat may be due to the genetic influence of the Maltese breed, which for many years has been used to increase Sarda goat milk production (Vacca *et al.* 2010b). The occurrence of the null *CSN1S1 0*1 and *N* alleles, although in low percentage, enriches the genetic potential of the Sarda goat. LD analysis showed that the most frequent haplotype was *FCFB* in both groups, similar to other Italian goat breeds (Caroli *et al.* 2006). LD association was observed between the *CSN1S1* and *CSN2* loci, a region of high LD at the *CSN3* locus and a recombination hotspot between *CSN2* and *CSN1S2*, as previously observed by Hayes *et al.* (2006) in a Norwegian goat population.

If we refer to association analysis of the *CSN1S1 AA* and *FF* homozygous genotypes, our findings are in line with the literature, as we evidenced that *CSN1S1 AA* goats have higher protein and fat content than do the *CSN1S1 FF* goats. The impact of these two αs1-casein genotypes on milk traits was initially evidenced in Alpine breeds, where *CSN1S1 AA* goats produced milk with higher casein, protein and fat content and with smaller casein micelles than did *CSN1S1 EE* and *FF* goats (Grosclaude *et al.* 1994), and the *CSN1S1 FF* genotype was associated with smaller milk fat globules (Martin *et al.* 2002; Neveu *et al.* 2002). The *CSN1S1 F* allele has been shown to be defective due to incorrect splicing of the primary transcript, leading to the production of at least nine different classes of mRNA. The most transcribed of these defective mRNAs is characterized by the skipping of exons 9, 10 and 11, and this produces a form of αs1-casein lacking a 37-aa peptide, which comprises a major multiple phosphorylation site (Leroux *et al.* 1992, 2003). The reduced synthesis level of the *CSN1S1 E* allele, which was detected only in heterozygosis in the present study, is due to the insertion of a 458-bp LINE element within exon 19, which destabilizes the primary transcript, reducing its translatability (Pérez *et al.* 1994). Research on the cytology of the lactating mammary epithelium has shown that the lower fat content in milk from *CSN1S1 FF* goats is due to the accumulation of proteins not perfectly processed in the mammary epithelial RER (rough endoplasmic reticulum), especially αs1-casein FF, which causes a retention of the fat globules (Neveu *et al.* 2002).

But the main finding of this research was that the *CSN1S1 BB* genotype, occurring at high frequencies in the study population, was associated with higher fat and protein contents than were the *CSN1S1 AA* and *FF* genotypes. The *CSN1S1 BB* genotype has been reported to be associated, in the Sarda goat, with higher percentages of total caseins and αs1-casein, as well as with long chain fatty acids and essential amino acids than is *CSN1S1 AA* (Balia *et al.* 2013). High amounts of αs1-casein expressed by the *CSN1S1 BB* genotype have also been reported in Spanish goats (Caravaca *et al.* 2009). The molecular events underlying the higher milk protein content of the *CSN1S1 BB* genotype compared with *AA* should be investigated. In fact, both alleles are 'high type', not defective, and should express similar protein levels in milk. One may hypothesize that the effect of each casein allele on milk traits could be influenced by neighboring genes, as the four casein genes are closely linked within 250 kb on chromosome 6, where the casein cluster is enclosed. In this case, the *CSN1S1 B* allele could be the genetic marker of an unknown causal mutation, or its effect may be modulated by interaction with other genes. The *CSN1S1 A* allele differs from the *CSN1S1 B* allele (including *B1*, *B2*, *B3*, *B4* and *C*) on the basis of several non-synonymous SNPs (Brignon *et al.* 1989). These coding SNPs may be responsible for the differences in milk protein levels. Recent studies have shown that the protein-coding regions (codons) may be recognized at the genomic level and bound by transcription factors in a large number of human genes, according to a 'binding code' which may influence gene expression and codon choice, regardless of the structure or function of the protein (Stergachis *et al.* 2013). In contrast with the *CSN1S1 A*, *N* and *01*, the *B* allele has not been fully sequenced yet. Such information might reveal variability other than non-synonymous SNPs, which could potentially explain the molecular basis of the observed differences. For example, synonymous changes may introduce optimal codons in a position to cause a 'codon bias', which may improve translation rates and accuracy in some variants, leading to translational optimization (Xia 1996). In addition, differences between *CSN1S1 BB* and *AA* genotypes regarding milk protein and fat content may be due to variations in promoter regions or in miRNA target sites (Bartel 2004).

The *CSN1S1 EF* heterozygote genotype was characterized by the highest daily protein production. Although defective alleles cause a lower synthesis of αs1-casein in milk than do high-type alleles, the highest daily protein production of the *CSN1S1 EF* goats could be explained by the higher milk yield of these goats, which is under the influence of environmental, hormonal and genetic factors.

We also evidenced an effect of the *CSN1S1* genotype on milk yield, which had not been highlighted in Alpine breeds (Mahé *et al.* 1994; Barbieri *et al.* 1995) or in the Sarda breed (Balia *et al.* 2013).

The *CSN3* genotype is not associated with dairy traits in Sarda goats

The Sarda goat was characterized by the occurrence of: (i) the *CSN3 C*, *C'*, *D* and *M* alleles, reported to occur together only in the Roccaverano (Sacchi *et al.* 2005) and Girgentana (Gigli *et al.* 2008) breeds; (ii) the *CSN3 B''*, reported only once in a European breed (Jann *et al.* 2004); (iii) the *CSN3 E* allele, evidenced so far only in the Montefalcone breed (Angiolillo *et al.* 2002); (iv) the newly identified *CSN3*

S allele. The *CSN3 S* allele (accession no. KF644565) was similar to the *CSN3 C* allele (accession no. AY350425) except for the nucleotide change c.530T>C, causing the putative amino acid change p.Val156Ala. As c.530T characterizes the *CSN3 C* and *CSN3 C'* variants, the *CSN3 S* allele might be phylogenetically considered as an intermediate allele between the *CSN3 A* and *CSN3 C* variants, or it might be produced by a back mutation of the *CSN3 C* allele. Probably the first hypothesis is more credible, because the frequency of the *CSN3* C allele is <2 percent in the population study, which makes it unlikely to detect a back mutation.

Data analysis revealed no association between the *CSN3* genotype and milk yield and composition. This result differs from those reported by Caravaca *et al.* (2009) and Chiatti *et al.* (2007) in the Murciano-Granadina and Orobica breeds respectively (*AB* and *BB* genotypes were associated with higher protein and casein contents than was *AA*). In addition, a strong influence of the promoter region of the *CSN3* gene on fat and protein content was detected in Norwegian goats, although the causative mutation has not been identified yet (Hayes *et al.* 2006).

Conclusions

Analysis of the casein gene cluster in the Sarda breed goat evidenced that the strong alleles prevailed at the calcium-sensitive casein *CSN1S1*, *CSN2* and *CSN1S2* genes. The intermediate *CSN1S1 F* allele accounted of about 25 percent of allele frequencies, and the *CSN1S1 01* and *N*, *CSN2 01* and *CSN1S2 0* null alleles also occurred. Rare alleles such as *CSN3 C'*, *B''*, *E*, *M* and the newly identified *CSN3 S* made the Sarda goat different from the other known breeds. The main finding was that non-defective genotypes at the *CSN1S2* and *CSN2* loci were associated with the phenotypic variation of dairy traits. It is noteworthy that *CSN1S1* and *CSN1S2* genotypes affected milk yield. In conclusion, our data showed that the Sarda goat has a high genetic variability and this information can be incorporated in selection schemes in order to exploit to the best its genetic potential.

Acknowledgement

Research supported by the RAS, Regional Government of Sardinia (P5a-Animal Biodiversity funds).

References

Angiolillo A., Yahyaoui M.H., Sanchez A., Pilla F. & Folch J.M. (2002) Characterization of a new genetic variant in the caprine κ-casein gene. *Journal of Dairy Science* 85, 2679–80.

Balia F., Pazzola M., Dettori M.L., Mura M.C., Luridiana S., Carcangiu V., Piras G. & Vacca G.M. (2013) Effect of *CSN1S1* gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. *Journal of Dairy Research* 80, 129–37.

Barbieri M., Manfredi E., Elsen J.M., Ricordeau G., Bouillon J., Grosclaude F., Mahé M. & Bibé B. (1995) Influence du locus de la caséine αs1 sur les performances laitières et les paramètres génétiques des chèvres de race Alpine. *Genetics, Selection, Evolution* 27, 437–50.

Barillet F. (2007) Genetic improvement for dairy production in sheep and goats. *Small Ruminant Research* 70, 60–75.

Barrett J.C., Fry B., Maller J. & Daly M.J. (2005) HAPLOVIEW: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21, 263–5.

Bartel D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–97.

Bevilacqua C., Helbling J.C., Miranda G. & Martin P. (2006) Translational efficiency of casein transcripts in the mammary tissue of lactating ruminants. *Reproduction, Nutrition, Development* 46, 567–78.

Brignon G., Mahe M.F., Grosclaude F. & Ribadeau-Dumas B. (1989) Sequence of caprine αs1-casein and characterization of those of its genetic variants which are synthesized at a high level, αs1-CnA, B and C. *Protein Sequences & Data Analysis* 2, 181–8.

Caravaca F., Carrizosa J., Urrutia B., Baena F., Jordana J., Amills M., Badaoui B., Sanchez A., Angiolillo A. & Serradilla J.M. (2009) Short communication: effect of αS1-casein (*CSN1S1*) and κ-casein (*CSN3*) genotypes on milk composition in Murciano-Granadina goats. *Journal of Dairy Science* 92, 2960–4.

Carcangiu V., Vacca G.M., Mura M.C., Dettori M.L., Pazzola M., Luridiana S. & Bini P.P. (2009) Relationship between MTRN1A *melatonin receptor* gene polymorphism and seasonal reproduction in different goat breeds. *Animal Reproduction Science* 110, 71–8.

Caroli A., Chiatti F., Chessa S., Rignanese D., Bolla P. & Pagnacco G. (2006) Focusing on the goat casein complex. *Journal of Dairy Science* 89, 3178–87.

Chessa S., Budelli E., Gutscher K., Caroli A. & Erhardt G. (2003) Short communication: simultaneous identification of five κ-casein (*CSN3*) alleles in domestic goat by polymerase chain reaction-single strand conformation polymorphism. *Journal of Dairy Science* 86, 3726–9.

Chessa S., Budelli E., Chiatti F., Cito A.M., Bolla P. & Caroli A. (2005) Predominance of β-casein (*CSN2*) *C* allele in goat breeds reared in Italy. *Journal of Dairy Science* 88, 1878–81.

Chiatti F., Chessa S., Bolla P., Cigalino G., Caroli A. & Pagnacco G. (2007) Effect of k-casein polymorphism on milk composition in the Orobica goat. *Journal of Dairy Science* 90, 1962–6.

Cosenza G., Illario R., Rando A., di Gregorio P., Masina P. & Ramunno L. (2003) Molecular characterization of the goat *CSN1S101* allele. *Journal of Dairy Research* 70, 237–40.

Dettori M.L., Vacca G.M., Carcangiu V., Pazzola M., Mura M.C. & Rocchigiani A.M. (2009) A reliable method for characterization of the goat *CSN1S1* E allele. *Livestock Science* 125, 105–8.

Gabriel S.B., Schaffnerv S.F., Nguyen H. *et al.* (2002) The structure of haplotype blocks in the human genome. *Science* 296, 2225–9.

Gigli I., Maizon D.O., Riggio V., Sardina M.T. & Portolano B. (2008) Short communication: casein haplotype variability in Sicilian dairy goat breeds. *Journal of Dairy Science* 91, 3687–92.

Grosclaude F. & Martin P. (1997) Casein polymorphisms in the goat. In *Proceedings of International Dairy Federation, Milk Protein Polymorphism Seminar II.* pp. 41–253. IDF, Bruxelles, Belgium.

Grosclaude F., Ricordeau G., Martin P., Remeuf F., Vassal L. & Bouillon J. (1994) Du gène au fromage: le polymorphisme de la caséine αs1 caprine, ses effets, son évolution. *INRA Production Animals* **7**, 3–19.

Gupta S.C., Kumar D., Pandey A., Malik G. & Gupta N. (2009) New k-casein alleles in Jakhrana goat affecting milk processing properties. *Food Biotechnology* **23**, 83–96.

Hayes B., Hagesaether N., Adnøy T., Pellerud G., Berg P.R. & Lien S. (2006) Effects on production traits of haplotypes among casein genes in Norwegian goats and evidence for a site of preferential recombination. *Genetics* **174**, 455–64.

International Dairy Federation. (2000) Determination of milk fat, protein and lactose content. Guide for the operation of mid-infrared instruments. IDF Standard No. 141C, Brussels, Belgium.

Jann O.C., Prinzenberg E.M., Luikart G., Caroli A. & Erhardt G. (2004) High polymorphism in the κ-casein (*CSN3*) gene from wild and domestic caprine species revealed by DNA sequencing. *Journal of Dairy Research* **71**, 188–95.

Leroux C., Mazure N. & Martine P. (1992) Mutations away from splice site recognition sequences might cis-modulate alternative splicing of goat aSl-casein transcripts. *The Journal of Biological Chemistry* **267**, 6147–57.

Leroux C., Le Provost F., Petit E., Bernard L., Chilliard Y. & Martin P. (2003) Real-time RT-PCR and cDNA macroarray to study the impact of the genetic polymorphism at the αS1-casein locus on the expression of genes in the goat mammary gland during lactation. *Reproduction, Nutrition, Development* **43**, 459–69.

Mahé M.F., Manfredi E., Ricordeau G., Piacère A. & Grosclaude F. (1994) Effet des variants de la caséine as1 caprine sur les performances laitières: analyse intradescendance de boucs de race Alpine. *Genetics, Selection, Evolution* **26**, 151–7.

Marletta D., Bordonaro S., Guastella A.M., Criscione A. & D'Urso G. (2005) Genetic polymorphism of the calcium sensitive caseins in Sicilian Girgentana and Argentata dell'Etna goat breeds. *Small Ruminant Research* **57**, 133–9.

Marletta D., Criscione A., Bordonaro S., Guastella A.M. & D'Urso G. (2007) Casein polymorphism in goat's milk. *Lait* **87**, 491–504.

Martin P., Szymanowska M., Zwierzchowski L. & Leroux C. (2002) The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reproduction Nutrition and Development*, **42**, 433–59.

National Research Council. (2001) *Nutrient Requirements of Dairy Cattle: Seventh Revised Edition*. National Academic Press, Washington DC, USA.

Neveu C., Riaublanc A., Miranda G., Chich J.F. & Martin P. (2002) Is the apocrine milk secretion process observed in the goat species rooted in the perturbation of the intracellular transport mechanism induced by defective alleles at the alpha(s1)-Cn locus? *Reproduction Nutrition and Development* **42**, 163–72.

Pazzola M., Balia F., Dettori M.L., Mura M.C., Carcangiu V. & Vacca G.M. (2011) Effect of different storage conditions, the farm and the stage of lactation on renneting parameters of goat milk investigated using the Formagraph method. *Journal of Dairy Research* **78**, 343–8.

Pazzola M., Balia F., Carcangiu V., Dettori M.L., Piras G. & Vacca G.M. (2012) Higher somatic cell counted by the electronic counter method do not influence renneting properties of goat milk. *Small Ruminant Research* **102**, 32–6.

Pérez M.J., Leroux C., Bonastre A.S. & Martin P. (1994) Occurrence of a LINE sequence in the 3′ UTR of the goat αs1-casein E-encoding allele associated with reduced protein synthesis level. *Gene* **147**, 179–87.

Prinzenberg E.M., Gutscher K., Chessa S., Caroli A. & Erhardt G. (2005) Caprine κ-casein (*csn3*) polymorphism: new developments in molecular knowledge. *Journal of Dairy Science* **88**, 1490–8.

Ramunno L., Mariani P., Pappalardo M., Rando A., Capuano M., Di Gregorio P. & Cosenza G. (1995) Un gene ad effetto maggiore sul contenuto di caseina β nel latte di capra, XI Convegno ASPA, Grado, Italy, pp. 185–6.

Ramunno L., Cosenza G., Pappalardo M., Pastore N., Gallo D., Di Gregorio P. & Masina P. (2000a) Identification of the goat *CSN1S1* F allele by means of PCR-RFLP method. *Animal Genetics* **31**, 333–46.

Ramunno L., Pappalardo M., Cosenza G., Pastore N., Gallo D., Grimaldi G., Rubino R., Calandrelli M. & Rando A. (2000b) Genetic characterization at αS1, β and αS2 loci of a goat population reared in the Sorrentine Peninsula. In: *Proceedings of the XIV Congress SIPAOC (Italian Society of Pathology and Breeding of Sheep and Goat)*, Vietri sul mare, Salerno (Italy), 321–4.

Ramunno L., Cosenza G., Pappalardo M., Longobardi E., Gallo D., Pastore N., Di Gregorio P. & Rando A. (2001a) Characterization of two new alleles at the goat *CSN1S2* locus. *Animal Genetics* **32**, 264–8.

Ramunno L., Longobardi E., Pappalardo M., Di Gregorio P., Cosenza G., Mariani P., Pastore N. & Masina P. (2001b) An allele associated with a non-detectable amount of αs2 casein in goat milk. *Animal Genetics* **32**, 19–26.

Raymond M. & Rousset F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–9.

Rijnkels M. (2002) Multispecies comparison of the casein gene loci and evolution of casein gene family. *Journal of Mammary Gland Biology and Neoplasia* **7**, 327–45.

Rijnkels M., Elnitski L., Miller W. & Rosen J.M. (2003) Multispecies comparative analysis of a mammalian-specific genomic domain encoding secretory proteins. *Genomics* **82**, 417–32.

Sacchi P., Chessa S., Budelli E., Bolla P., Ceriotti G., Soglia D., Rasero R., Cauvin E. & Caroli A. (2005) Casein haplotype structure in five Italian goat breeds. *Journal of Dairy Science* **88**, 1561–8.

Stephens M., Smith N.J. & Donnelly P. (2001) A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics* **68**, 978–89.

Stergachis A.B., Haugen E., Shafer A. *et al.* (2013) Exonic transcription factor binding directs codon choice and affects protein evolution. *Science* **342**, 1367–72.

Usai M.G., Casu S., Molle G., Decandia M., Ligios S. & Carta A. (2006) Using cluster analysis to characterize the goat farming system in Sardinia. *Livestock Science* **104**, 63–76.

Vacca G.M., Ouled Ahmed Ben Alì H., Carcangiu V., Pazzola M. & Dettori M.L. (2009a) Genetic structure of the casein gene cluster in the Tunisian native goat breed. *Small Ruminant Research* **87**, 33–8.

Vacca G.M., Ouled Ahmed Ben Alì H., Pazzola M., Sanna M., Dettori M.L. & Carcangiu V. (2009b) An investigation on allele frequency at the *CSN1S2* locus and its relationship with milk parameters in the Sarda goat. *Journal of Animal and Feed Sciences* **18**, 628–37.

Vacca G.M., Daga C., Pazzola M., Carcangiu V., Dettori M.L. & Cozzi M.C. (2010a) D-loop sequence mitochondrial DNA variability of Sarda goat and other goat breeds and populations reared in the Mediterranean area. *Journal of Animal Breeding and Genetics* **127**, 352–60.

Vacca G.M., Dettori M.L., Carcangiu V., Rocchigiani A.M. & Pazzola M. (2010b) Relationships between milk characteristics and somatic cell score in milk from primiparous browsing goats. *Animal Science Journal* **81**, 594–9.

Xia X. (1996) Maximizing transcription efficiency causes codon usage bias. *Genetics* **144**, 1309–20.

Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1. SSCP patterns of *CSN3* gene.
Figure S2. Linkage disequilibrium structure of the Sarda goat casein cluster.
Table S1. Descriptive statistics of milk yield and composition (samples from 814 goats).
Table S2. *CSN1S1* gene variants.
Table S3. *CSN2* gene variants.
Table S4. *CSN1S2* gene variants.
Table S5. *CSN3* gene variants (modified from Gupta *et al.* 2009 with nomenclature as proposed by Prinzenberg *et al.* 2005).
Appendix S1. Supplementary Genotyping Methods.

4. Conclusions

In a dairy farming system that is still conducted in traditional ways, the possibilities for increasing the income are related to few factors: the improvement of management, by the introduction for example of nutrition schemes or by the use of automatic milking machines, or the proper valorisation of products. This should be the case of Sarda goats.

Our results, in fact, shows that the Sarda goat breed has a genetic uniqueness that must be well-kept in the optic of biodiversity preservation, and the increased knowledge about genetic traits and products characteristics should consent to farmers to reach the right income with the introduction of PDO products and the increase of prices and commercial value.

Goat kids provide a meat with good quality, and milk from Sarda goats presents excellent characteristics for cheese making and, with genetic chosen lines, high performances animals could be selected for reproduction. Also the rare but not absent presence of null alleles can be exploited for selection schemes, in the case a production of milk for direct drinking consumption is the aim of the farmers.

Furthermore, quality paid productions, a well experienced system already present in the dairy cattle industry, should and might be applied, as it consents the improvement of the products quality and an increase of income for farmers. This should consent the maintenance of Sarda Goat farming with extensive methods, with benefits for the environment too,

and the constant control of the bushlands in areas where is impossible to rear any other species, and is also hard for men to arrive.

Sarda goat valorisation could and should help to rediscover the mountain areas of the region and create employment, while maintaining the economic and social importance that for centuries it has had.

This PhD work, its results and the future work that is requested to better understand and define Sarda goat and its products has had and will have the only aim to give this breed the right place I think it deserve in the goat farming panorama.

5. References

Agriculture, http://www.agriculture.com/livestock/speciality-livestock/pygmy-goat-profile_293-ar13324 last visited on September, 20, 2015.

Asso.Na.Pa., 2015. *Associazione Nazionale della Pastorizia, Online: http://www.assonapa.it/* last visited on September, 20, 2015.

Attaie, R., Richter, R.L. 2000. *Size distribution of fat globules in goat milk.* Journal of Dairy Science 83: 940-944.

Awad, S., Luthi-Peng, Q. Q., Puhan, Z. 1998. *Proteolytic activities of chymosin and porcine pepsin on buffalo, cow, and goat whole and beta-casein.* Journal of Agricultural and Food Chemistry, 46: 4997-5007.

Balia, F., Pazzola, M., Dettori, M.L., Mura, M.C., Luridiana, S., Carcangiu, V.,Piras, G., Vacca, G.M., 2013. *Effect of CSN1S1 gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats.* J. Dairy Res. 80, 129–137.

Barillet F. 2007. *Genetic improvement for dairy production in sheep and goats.* Small Ruminant Research 70, 60–75.

Bittante, G., Andrighetto, I., Ramanzin, M., 2005. *Tecniche di produzione animale.* Ed Liviana.

Boi R., 2010. *L'allevamento caprino in Sardegna.* XX Congresso Nazionale S.I.P.A.O.C. Siracusa, 26/29 Settembre.

Brandano, P., Piras, B., 1978. *La capra Sarda: Nota III. Le caratteristiche dell'allevamento.* Studi Sassaresi, Ann. Fac. Agr. Univ. Sassari, XXVI.

Casu S., Cappai P. and Branca A., 1981. *L'attività sessuale della capra Sarda nei diversi periodi dell'anno.* IV Congresso S.I.P.A.O.C. Alghero 22-24 Ottobre 1981.

Cristofori, F. 1994. *Il problema del latte nei paesi in via di sviluppo.* Atti Convegno: "Un alimento antico in viaggio verso il futuro". Torino 3-4 Novembre 1994, 121-130.

Debski, B., Picciano, M. F., Milner, I. A. 1987. *Selenium content and distribution of human, cow and goat milk.* Journal of Nutrition, 117: 1091-1097.

Delatouche R., 1968. *Storia agraria del Medioevo*. Ed. Il saggiatore.

Dettori, M.L., Pazzola, M., Paschino, P., Pira, M.G., Vacca, G.M., 2015a. *Variability of the caprine whey protein genes and their association with milk yield, composition and renneting properties in the Sarda breed. 1. The LALBA gene.* Journal of Dairy Research, in press.

Dettori, M.L., Pazzola, M., Pira, E., Puggioni, O., Vacca, G.M., 2015b. *Variability of the caprine whey protein genes and their association with milk yield, composition and renneting properties in the Sarda breed: 2. The BLG gene*. Journal of Dairy Research, in press.

Dubeuf J.P., Sayadi S. 2014. *Multi-functionality issues for small ruminants: What changes are needed in territorial public policies*

and training? Report of two round tables on territorial issues and training for the development of goat farming. Small Ruminant Research 121 (2014) 136–145

Dubeuf J-P., Boyazoglu J., 2009. *An international panorama of goat selection and breeds.* Livestock Science, 120, 225-231.

Durante M., 1958. *Osco hirpo, lupo o capro?* Ed. La Parola del Passato. 412-417.

FAOSTAT, 2012. *http://www.faostat.fao.org.* FAO, Rome, Italy, last visited on September, 20, 2015.

Gall, C., 1996. *Goat Breeds of the World.* Technical Centre for Agricultural and Rural Cooperation, Wageningen, The Netherlands, 186 pp.

Grosclaude F. and Martin P. 1997. *Casein polymorphisms in the goat.* In Proceedings of International Dairy Federation, Milk Protein Polymorphism Seminar II. pp. 41–253. IDF, Bruxelles, Belgium.

Grosclaude F., Ricordeau G., Martin P., Remeuf F., Vassal L. and Bouillon J. 1994. *Du gène au fromage: le polymorphisme de la casèine αs1 caprine, ses effets, son èvolution.* INRA Production Animals 7, 3–19.

Gupta S.C., Kumar D., Pandey A., Malik G. and Gupta N. 2009. *New k-casein alleles in Jakhrana goat affecting milk processing properties.* Food Biotechnology 23, 83–96.

ICAR *www.icar.org*, last visited on September, 20, 2015

Laore, 2013. *Comparto ovi-caprino e zootecnia regionale, dati strutturali*. Regione Autonoma della Sardegna.

Lucey, J. A., 2004. *Rennet Coagulation of Milk.* Encyclopedia of dairy sciences 2004, 286-293.

Macciotta, N. P. P., Cappio-Borlino, A., Steri, R., Pulina, G., Brandano, P., 2002. *Somatic variability of Sarda goat breed analysed by multivariate methods*. Livestock Production Science, 75, 51-58.

MacKinnon M., 1999. *Animal Production and Consumption in Roman Italy: The Zooarchaeological and Textual Evidence.* Ph.D. diss. University of Alberta.

Marletta D., Criscione A., Bordonaro S., Guastella A.M. and D'Urso G. 2007. *Casein polymorphism in goat's milk.* Lait 87, 491–504.

Marshal K., 2014 *Optimizing the use of breed types in developing country livestock production systems: a neglected research area.* Journal of Animal Breeding and Genetics, 131, 329-340.

Martin P., SzymanowskaM., Zwierzchowski L. and Leroux C. 2002. *The impact of genetic polymorphisms on the protein composition of ruminant milks*. Reproduction Nutrition and Development, 42, 433–59.

Mehaia, M. A and Al-Kenehal, M. A. 1992. *Taurine and other free amino acids in milk of camel, goat, cow and man.* Milchwissenschsft, 47: 351-353.

Park, Y. W. 2007. *Rheological characteristics of goat and sheep milk.* Small Ruminant Research, 68: 73-87.

Pazzola, M., Carcangiu, V., Fanari, U., Pisano, B., Bini, P. P., Vacca G. M., 2002. *L'allevamento caprino in Sardegna: Realtà e prospettive.* Atti X. Congr. Int. Fe.Me.S.P.Rum., CD-ROM.

Peris, S., Caja, G., Such, X., Casals, R., Ferret, A., Torre, C., 1997. *Influence of kid rearing systems on milk composition and yield of Murciano-Granadina dairy goats.* J. Dairy Sci. 80, 3249–3255.

Pirisi, A., Lauret, A., Dubeuf, J.P., 2007. *Basic and incentive payments for goat and sheep milk in relation to quality.* Small Ruminant Research 68, 167–178.

Prinzenberg E.M., Gutscher K., Chessa S., Caroli A. and Erhardt G. 2005. *Caprine k-casein (CSN3) polymorphism: new developments in molecular knowledge.* Journal of Dairy Science 88, 1490–8.

Ramunno L., Cosenza G., Pappalardo M., Longobardi E., Gallo D., Pastore N., Di Gregorio P. and Rando A. 2001. *Characterization of two new alleles at the goat CSN1S2 locus*. Animal Genetics 32, 264–268.

Reina, R., Berriatua, E., Luja, L., Juste, R., Sanchez, A., de Andre, D. and Amorena, B., 2009. *Prevention strategies against small ruminant lentiviruses: an update.* Vet. J. 182, 31–37.

Rijnkels M. 2002. *Multispecies comparison of the casein gene loci and evolution of casein gene family.* Journal of Mammary Gland Biology and Neoplasia 7, 327–45.

Rijnkels M., Elnitski L., Miller W. and Rosen J.M. 2003. *Multispecies comparative analysis of a mammalian-specific genomic domain encoding secretory proteins*. Genomics 82, 417–32.

Rubino, R., 1990. *L'allevamento della capra. Guida pratica alla progettazione, realizzazione e gestione di un allevamento caprino.* Ed. Ars Grafica-villa D'Agri.

Santillo Frizell B., 2004. *Man and Animal in Antiquity.* Proceedings of the conference at the Swedish Institute in Rome, 2004. 54-60.

Sayadi, S., Calatrava,J. 2001. *Agroturismo y desarrollo rural: situación actual, potencial y estrategias en zonas de monta˜na del sureste espa˜ nol.* Cuadernos Turismo7,131–157.

Scherf, B.D., 2000. *World Watch List of Domestic Animal Diversity.* 3rd ed. Food and Agriculture Organization of the United Nations, Rome, Italy.

Simon, D.L. 1990. *The global animal genetic data bank.* FAO Animal Production and Health Paper 80, pp. 153–166.

Trujillo, A. J., Guamis, B., Carretero, C. 1997. *Hydrolysis of caprine k-casein by plasmin*. Journal of Dairy Science, 80: 2258–2263.

Vacca G. M., Dettori M. L., Piras G., Manca F., Paschino P. and Pazzola M. 2014. *Goat casein genotypes are associated with milk production*

traits in the Sarda breed. Animal Genetics 45, Issue 5, pages 723–731.

Vacca, G.M., Dettori, M.L., Piras, G., Manca, F., Paschino, P., Pazzola, M. 2014. *Goat casein genotypes are associated with milk production traits in the Sarda breed.* Animal Genetics 45, Issue 5, 2014, pp 723-731

Vacca, G.M., Carcangiu, V., Mura, M.C., Dettori, M.L. and Bini, P. P. 2005. *Effect of parity and number of kids in yield and milk composition in Sarda goat*. Proc. 56th EAAP, 274, Uppsala, 5-8 June 2005.

Vacca, G.M., Carcangiu, V., Porqueddu, M., Dettori, M.L. and Bini, P.P. 2006. *Milk yield and composition in Sarda goats and their relations across lactations.* In: Animal products from the Mediterranean area, EAAP publication n. 119, pp 255–260. Wageningen Academic Publishers, The Netherlands.

Zeder, M. A. and Hesse, B. 2000. *The initial domestication of goats (Capra hircus) in the Zagros mountains 10,000 years ago.* Science, 287(5461): 2254-2257.

Acknowledgements

I'd like to thank all the farmers whom consented us to work in their farms, my Tutor Professor Giuseppe Massimo Vacca, the Special Zootechnics guys and gals, Professor Tormod Ådnøy and all the people at NMBU.

A special thank to my family for their unconditioned support and love.

Pietro