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The Garfagnina goat: A zootechnical overview of a local dairy population

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ABSTRACT

Domestic livestock with a limited distribution are increasingly recognized in the action plans of the European Union as a reason for protecting rural land. The preservation and enhancement of the native germplasm and traits selected through the ages in different areas of farming is the first step in increasing typical products at a time when high quality products are increasingly in demand. This is the first time that a zootechnical overview has been performed on the Italian native goat population named "Garfagnina," which is registered on the Tuscan regional repertory of genetic resources at risk of extinction. The aim of the study was to give added value to this population by focusing on particular traits that could be used for promoting typical products. Data on the size of the local goats, zoometric measures, breeding system, milk quality, and genetic polymorphisms were collected to get insight into the current state of the population of this type of goat. The native goat population is reared in Tuscany in central Italy, mostly for its milk. The local goat farms considered in our study are located in the hills and mountains of the northwestern Tuscan Apennine area. For every farm we measured at least 10% of the reproductive females (273), randomly chosen, and all reproductive males (47) for a total of 320 subjects. Regarding the management of the animals and the feeding system, semi-extensive farming is practiced in all the flocks. From a morphological point of view the animals are relatively homogeneous, especially in terms of zoometric data, whereas they show a wider variability regarding coat. Milk gross and fatty acid composition were similar to that reported in the literature for bulk goat milk. Moreover, the average of somatic cell count and standard plate count found in Garfagnina goat milk indicated good hygienic farm management and correct milking practices, although milking is mainly manual. The average number of globules per milliliter found in Garfagnina goat milk was almost double compared with the literature, whereas the average diameter was lower. Milk coagulation properties were scarce, thus indicating poor cheesemaking aptitude of Garfagnina milk. Selecting haplotypes carrying alleles associated with a higher expression of the specific casein could help improve milk cheesemaking aptitude. Moreover, the rather high frequency of the faint *CSN1S1*F* allele and the occurrence of *CSN2*0* might suggest that Garfagnina goat milk could be used, after an appropriate selection, for direct consumption of milk at low casein content for intolerant human subjects.

Key words: endangered goat population, milk quality, genetic polymorphism, farm management

INTRODUCTION

Domestic livestock with a limited distribution are increasingly recognized in the action plans of the European Union as a reason for protecting rural land. Although much negative pressure is in favor of introducing more profitable domestic animals, local breeds show a better adaptability to marginal environments and a greater exploitation of poor pastures.

The preservation and enhancement of the native germplasm and traits selected through the ages in different areas of farming is the first step in increasing typical products at a time when high quality products are increasingly in demand. Consumers are searching for traditional products in the belief that they are from animals reared in natural conditions, which results in products of the best quality in terms of both organoleptic and health properties.

The combination of biodiversity and quality of products is therefore a current issue. This might also be linked to the selection of genes with important effects on the different nutritional needs of the human population (children, the elderly, athletes, those who are sick, and so on); that is, genes associated with reduced energy level or with a lower content of specific proteins in the case of food intolerances (Haenlein, 2004).

The Registro Anagrafico of native populations with a limited distribution is an important tool for the pres-

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ervation of biodiversity in Italy. According to the European Commission (2004), a local breed is considered to be endangered if fewer than 10,000 breeding females exist.

Over the last 20 yr, the number of goats around the world has increased by about 58% (Morand-Fehr et al., 2004) because of their excellent adaptability to marginal areas combined with their high production per unit of live weight and the high nutritional properties of goat's products. Interest in goat's milk has increased not only for cheese production but also as an alternative source of liquid milk.

A few authors have suggested the influence of polymorphisms in goat's milk in terms of allergenic effects of milk, but these aspects need further investigation because for goat breeding programs this knowledge could be both challenging and rewarding (Haenlein, 2004). In all cases, a direct relationship has been found in cattle between the variability at these loci and the human tolerance to lactose (Beja-Pereira et al., 2003), as well as a significant effect of the milk protein genotype on fatty acid composition and fat globule size (Martini et. al., 2007a,b). Thus, the existence of a high number of genetic protein polymorphisms in goat's milk (Chessa et al., 2003) suggests their potential use for human nutrition. This is because a direct relationship has been found in cattle between the variability at these loci and the human tolerance to lactose (Beja-Pereira et al., 2003), as well as a significant effect of the milk protein genotype on fatty acid composition and fat globule size (Martini et al., 2007a,b).

This is the first time that a zootechnical overview has been performed on the Italian native goat population named "Garfagnina," which is registered on the Tuscan regional repertory of genetic resources at risk of extinction. The origins of this population are still not certain, even if they seem to derive from crossings between native goats from Alpine Arc and from the Tuscan-Emilian Apennines; local breeders report that the population was reared for generations for its milk and meat production. The aim of the study was to give added value to this population by promoting its typical products such as fresh and ripened cheeses (Caprino delle Apuane) and meat kid (Controneria kid). Data on the size of the local goats, zoometric measures, breeding system, milk quality, and genetic polymorphisms were collected to get insight into the current state of the population of this type of goat.

MATERIALS AND METHODS

The native goat population is reared in Tuscany in central Italy, mainly for its milk. The local goat farms considered in our study are located in the hills and mountains of the northwestern Tuscan Apennine area.

To characterize how the farms are organized we collected information on the farm typology, specifically, details on breeding system, geographical location, and demographic parameters. Data on the social, economic, and management structure of the farms are necessary to make decisions on the conservation of the population and marketing strategies.

An accurate description of the animals is also necessary to support the farmers' decisions about the sustainability of a breed. Thus, we collected zoometric data on a sample randomly chosen from the overall population. To obtain a representative number of animals, on the basis of a stratification (WinEpiscope, 2001), on every farm we measured at least 10% of the reproductive females (273), randomly chosen, and all reproductive males (47) for a total of 320 subjects.

Zoometric Data

To evaluate the morphology of the animals we took the following measurements, using a Lydtin stick or a flexible meter, on every subject kept in posture: withers height (from the ground to the top of the withers); chest height (from the withers to the ventral face of the breastbone, immediately behind the shoulder blade); trunk length (from the tip of the shoulder to the tip of the buttock); rump width; chest girth (immediately behind the shoulder blade); chest depth (from the cranial face of the first rib to the tail face of the last rib); and foreshank girth.

Sampling and Analysis of Milk

Bulk milk samples were collected twice from the morning milking of each flock, when goats were in mid lactation. Each sample was collected in duplicate for a total of 124 samples. Samples were taken to the laboratory in tanks at 4°C; no preservatives were added. Bulk milk samples were analyzed for the following parameters: DM, protein, fat, lactose, and urea by infrared analysis (MilkoScan, Italian Foss Electric, Padova, Italy); freezing point, pH, casein, ash, phosphorus and calcium (AOAC, 1990), SCC (Fossomatic, Italian Foss Electric), and SPC (plate count agar; 30°C, 72 h). Solids-not-fat was calculated from the difference between DM and fat content.

Lactodynamographic Analysis

The analysis of milk rheological parameters (Zannoni and Annibaldi, 1981) was performed without pH

standardization by Formagraph (Italian Foss Electric), recording the following parameters (ASPA, 1995): clotting time, which represents the time (min) from the addition of rennet to the beginning of coagulation; curd firming time, which represents the time (min) needed until the curd is firm enough to be cut and is usually measured when the Formagraph diagram reaches the width of 20 mm; and curd firmness, which is the diagram width (mm) measured 30 min after the addition of the rennet.

Morphometric Analysis of Milk Fat Globules

The morphometric analysis of fat globules (number of fat globules per milliliter of milk and diameter) was performed according to Scolozzi et al. (2003). This is a simple method and enabled us to directly measure the diameter of every single, visible, native milk fat globule in fresh milk with an image analyzer system.

Milk Fatty Acid Composition

Milk samples were stored at −20°C before carrying out the fatty acid analysis. Milk fat extraction was performed according to Röse-Gottlieb's method (AOAC, 1995) modified by Secchiari et al. (2003). Fatty acid methyl esters were obtained after transesterification with sodium methoxide (Christie, 1982). The composition of the fatty acids was determined by gas chromatography using a PerkinElmer Auto System apparatus (Norwalk, CT) equipped with a flame ionization detector and a capillary column (30 m × 0.25 mm, film thickness 0.25 μm; FactorFour Varian, Middelburg, the Netherlands). The helium carrier gas flow rate was 1 mL/min. The oven temperature program was as follows: level 1, 120°C held for 1 min; level 2, 120 to 180°C at 5°C/min then held for 18 min; level 3, 180 to 200°C at 2°C/min then held for 1 min; level 4, 200 to 230°C at 2°C/min then held for 19 min. The injector and detector temperatures were set at 270 and 300°C, respectively. All the results concerning the milk fatty acid composition are expressed as a percentage in total fatty acid methyl esters.

Genotyping Analyses of Casein Variability

Blood samples were collected from a total of 72 animals randomly chosen from the overall sample. A commercial kit, Kapa Blood Direct 2× Ready Mix (Kapa Biosystems, Boston, MA), was successfully used for the PCR reactions, starting directly from the blood samples and skipping the DNA extraction step. The samples were typed by 8 different methods, described in detail in Rignanese (2009).

Statistical Analysis

Means and standard deviations of the physical and chemical, hygienic, and rheological parameters of milk were calculated. The frequency distribution of the total counted and measured milk fat globules was evaluated according to their size: diameters of the fat globules were divided into 10 classes of 1-μm width (from 0 μm to >9 μm). For each milk sample, the percentages of fat globules within each class size were calculated. All 10 classes were represented in all the milk samples evaluated. Therefore, each milk sample was characterized by a different percentage of fat globules for each diameter size class. The 10 classes were subsequently grouped into 3 categories of fat globule size: small globules with a diameter of <2 μm, medium globules with a diameter from 2 to 5 μm, and large globules with a diameter of >5 μm.

The casein and haplotype distributions were analyzed by the EH program (Xie and Ott, 1993). Haplotype frequencies were calculated in the independence hypothesis (independence frequencies) and estimated by the EH program, taking the association between the casein genes into account (association frequencies). The descriptive statistical analyses were performed using JMP software (version 5.0 for PC; SAS Institute, 2002).

RESULTS AND DISCUSSION

Breed Size and Distribution

The Garfagnina goat population was estimated to be approximately 2,500 head distributed over 31 farms with an altitude ranging from 230 to 900 m above sea level in the provinces of Lucca (18 farms), Massa Carrara (12 farms), and Pistoia (1 farm). Seventy-five percent of the goats are situated in the province of Lucca, in the Garfagnana area (longitude: 10°20′36″E; latitude: 44°8′19″N). This area is situated in the middle and upper valleys of the river Serchio, surrounded by the Apuane Alps and Tuscan-Emilian Apennines. Approximately 23% of the total number of goats is found in the Apuane Alps in the province of Massa Carrara (longitude: 11°26′30″E; latitude: 42°41′00″N). The remaining 2% of the population is located in the province of Pistoia. More than 50% of the total population is bred in 7 farms with several goats greater than 100 head. The largest farms are located at higher altitudes.

Farm Typology and Management

Regarding the management of the animals and the feeding system, which is known to influence body measurements and animals' development (Riva et al., 2004),

semi-extensive farming is practiced in all the flocks: goats graze during the morning, often using woodland pastures and state-owned lands, and are housed during the night (nocturnal shelters are necessary because of a high predation rate by wolves). Pond et al. (1995) report that walking activity and exposure to the sun could promote calcium absorption and the growth of bones. Shelters, mangers, and drinking troughs are not normally present in the pastures. Feed supplements are given mainly in the winter, involving the use of rough pastures and small amounts of concentrate. In 29% of the farms, the animals are transferred to the mountain area at the beginning of the spring and are returned in late autumn.

Only 1 farm has a special room for mechanical milking, whereas in the other farms animals are milked manually in the stable. Flock management is of a family farm type, and goat breeding represents one of the main sources of income for the majority of breeders in this area, through the sale of kids, milk, and milk products.

The first delivery usually happens around 1 yr of age; normally, goats deliver once a year in the January to February period and some authors (Shrestha et al., 1984; Al-Tarayrah and Tabbaa, 1999) report in sheep that the month of birth can affect some morphological traits. The rate of twin births is approximately 40%, quite low if compared with some Italian goat breeds such as Maltese and Girgentana (Lucifero, 1981) but similar to the Garganica goat (Rubino, 1993).

The length of the productive career of females is high (7–10 yr) and some breeders prefer to let animals die a natural death. Males are inserted into the flock from a minimum age of 3 yr until 7 yr. The rate of replacement is quite low, around 15%. Kids remain with their mother until slaughtering, at an average age of 60 d and a weight of between 13 and 15 kg.

Zoometric Data

Morphological characters provide useful information to detect the genetic relationship of breeds in different domestic species (Zaitoun et al., 2005) and can be a useful criterion for selection (Mabrouk et al., 2008). Some authors have used morphological measurement for breed characterization and to describe change in size and shape (Riva et al., 2004).

Means and standard deviations of the morphological parameters referring to adult females and males are reported in Table 1. From a morphological point of view, the animals are relatively homogeneous, especially in terms of zoometric data, whereas they show a wider variability regarding coat. The long hair type coat is of several colors, with a prevalence of black, gray, and brown of different tones; white spots are frequently visible on limbs, abdomen, and snout. This is attributed to the limited selection carried out within the population; in fact, selection was found to reduce the variability and at the same time help to establish a breed standard (Panella et al., 1998).

Table 1. Zoometric data of adult female and male Garfagnina goats

	Female		Male	
Item, cm	Mean	SD	Mean	SD
Withers height	75.60	3.851	83.50	4.579
Chest height	31.99	2.480	36.72	4.675
Chest depth	40.77	3.604	47.17	3.434
Trunk length	79.74	5.433	88.67	7.806
Rump width	17.94	1.478	18.83	2.065
Chest girth	93.05	6.065	101.00	9.048
Fore shank girth	10.00	0.951	11.71	1.404

The Garfagnina goat is long legged; this is preferred in grazing and dairy animals because the mammary apparatus is more distant from the ground and therefore less subject to trauma. The shape and structure of the head is considered a racial character and suggest the productive aptitude. In both sexes the head is light. The profile is rectilinear or lightly convex in males, the neck is always long and thin in females and muscular in males, and the ears are long and horizontally inclined; the study of head and the neck morphology reveal a typical conformation of a dairy animal according to that reported by Lucifero (1981). Ninety-six percent of females and all males have beards, and wattles are present in 61.5% of females and 38% of males (Figure 1).

The horns, long and turned backward, are present in 70% of the females and only in 52.38% of the males as a result of the selection carried out by breeders who prefer animals without horns, especially regarding males. However this type of selection could not be favorable because some anomalies of the reproductive apparatus are related to the absence of horns (Lucifero, 1981).

Udder morphology is one of the factors determining milkability and is a useful tool for the screening of animals that are more adapted to machine milking. In fact, as reported by Caja et al. (2000) for dairy sheep, a well-shaped udder for machine milking should have great volume, globose shape, and clearly defined teats and should be of medium size and implanted near to vertical. In Garfagnina goats, the mammary apparatus is of a medium size in 46.4% of the animals, abundant in 36%, and insufficient in 16%; in some adult animals (1.6%), the udder is not developed.

The udder is symmetrical and well supported in 80% of the animals, and the teats are placed low in 93.6% of the subjects. The udder is pyriform in 76% of the goats and globose in 22.4%. The teats are cylindrical

Table 2. Physical, chemical, health, and rheological characteristics of milk

Item	Mean	SD
DM, %	12.43	1.279
Fat, %	3.97	0.987
Protein, %	3.32	0.371
Casein, %	2.80	0.303
Lactose, %	4.38	0.140
SNF, %	8.46	0.366
Casein/fat	0.74	0.171
Protein/fat	0.87	0.163
Ash, %	0.78	0.045
Ca, %	0.18	0.024
P, %	0.12	0.016
Ca/P	1.58	0.213
Freezing point, °C	−0.537	0.008
SPC, $\times 10^3$ cfu/mL	183.55	307.98
SCC, $\times 10^3$ cells/mL	772.42	607.054
Urea, mg/dL	33.43	2.370
pH	6.53	0.049
Clotting time, min	9.95	2.150
Curd firming time, min	—	—
Curd firmness, mm	11.45	3.247

in 52.4% of the goats and conical in the other 47.6% of the animals. Supernumerary teats occur in only 1.4% of the goats. This highlights the attention of breeders for selecting udder morphology to improve the adaptability to machine milking.

Milk Quality Properties

The daily average milk yield is about 2 L/head. The mean milk gross composition (Table 2) is similar to that reported by Guo et al. (2004) for bulk goat's milk. However, our results were higher in fat and protein percentages compared with the values reported for dairy goat breeds such as British Saanen, Alpine, and Saanen but lower compared with the values reported for Garganica, Nubian, and Mediterranean local breeds (Boyazoglu and Morand Fehr, 2001; Albenzio et al., 2006).

The average casein content was greater than values reported by Park et al. (2007) and identified in the Alpine (Soryal et al., 2005) and British Saanen breeds (Albenzio et al., 2006) but lower than in Nubian breeds (Soryal et al.,2005). Although the limit for SCC in goat's milk has not yet been definitely established, the average SCC found in Garfagnina goat milk was approximately half of the threshold of 1,500,000 cells/mL advised in Europe for fresh milk by some authors (Delgado-Pertiñez et al., 2003). In all cases, a high variability was found among the farms.

The average SPC value was lower than the limits imposed by the European Union (500,000 bacteria/mL of fresh milk). This indicates good hygienic farm manage-

Figure 1. A flock of Garfagnina goats. Color version available in online PDF.

Table 3. Morphometric characteristics of milk fat globules

Item[1]	Mean	SD
Globules, $\times 10^9$/mL	3.09	1.079
Mean diameter, μm	2.20	0.203
Small globules, %	54.01	10.198
Medium globules, %	42.57	9.667
Large globules, %	3.42	1.883

[1]Small globules = <2 μm; medium globules = 2–5 μm; large globules = >5 μm.

ment and correct milking practices, although milking is mainly manual. A first possible source of milk microbial contamination is its handling from leaving the udder to refrigeration (Delgado-Pertiñez et al., 2003).

The average content of milk urea is in agreement with the value reported for goats reared in Sicily (Scatassa et al., 2002). The freezing point is important to verify the addition of water in milk, but for goat's milk there is no tolerance limit. Nevertheless, in our study the freezing point value was lower than that reported by Park et al. (2007). The pH value was within the range reported by Park et al. (2007) for goat's milk.

The lactodynamographic analysis evaluates the rennet clotting aptitude of milk, thus enabling the cheesemaking properties to be predicted. In this study, milk coagulation properties were scarce: the rennet clotting time was shorter than in Santillo et al. (2009) and the rate of firming was not detectable. Curd firmness was lower compared with data on Saanen (Fantuz et al., 2001), Girgentana (Todaro et al., 2005), and Garganica goats (Santillo et al., 2009). This suggests that Garfagnina goat milk could be used for direct consumption, given the potential hypoallergenic characteristics of some goat's milk (Rignanese, 2009). The rather high frequency of the faint *CSN1S1*F* allele and the occurrence of *CSN2*0*, discussed in the next section, could be related to this potential use of Garfagnina milk.

The variability of fat globule sizes is linked to several factors such as species and breeds (Mehaia, 1995; Martini et al., 2006a). Some studies highlight that the variability in milk fat globule size affects milk digestibility (Attaie and Richter, 2000), fatty acid content (Martini et al., 2006b), and the capability to retain water in cheese (Wiking et al., 2004; Martini et al., 2008).

The average number of globules per milliliter found in Garfagnina goat milk (Table 3) was almost double compared with the literature, whereas the average diameter was lower (Mehaia, 1995; Attaie and Richter, 2000; Martini et al., 2009). In this respect a negative correlation between the size and number of milk fat globules was found (Martini et al., 2008). Approximately 78% of milk fat globules of the Garfagnina goat were within the range of 1 to 3 μm, confirming data on Camosciata delle Alpi goats (Martini et al., 2009).

The fatty acid composition (Table 4) was within the range reported by Park et al. (2007), confirming that C10:0, C14:0, C16:0, C18:0, and C18:1 account for more than 75% of total fatty acids and that goat conjugated linoleic acid is approximately 60% of the corresponding value in cow's and ewe's milks. Caproic, caprylic, and capric fatty acids have been used for the treatment of malabsorption syndromes and intestinal disorders because of their metabolic activity, which provides energy (Jandal, 1996). In our study these 3 fatty acids represented approximately 14% of the total fatty acid methyl esters, which is in agreement with Boza and Sanz Sampelayo (1997).

Table 4. Fatty acid composition of milk

Fatty acid,[1] % of total fatty acids	Mean	SD
C4	1.73	0.219
C6	2.09	0.266
C8	2.60	0.374
C10	9.28	1.203
C11	0.06	0.028
C12	3.77	0.921
C13	0.07	0.023
C14	9.85	1.119
C14:1	0.26	0.050
C15	1.08	0.183
C15:1	0.17	0.018
C16	28.09	3.462
C16:1	0.69	0.085
C17	0.67	0.166
C17:1	0.22	0.057
C18	11.95	2.674
C18:1 *trans*-9	1.97	0.818
C18:1 *cis*-9	20.37	2.483
C18:2 *trans*	0.44	0.146
C18:2 *cis*	1.84	0.426
C18:3 n-6	0.03	0.008
C18:3 n-3	0.05	0.023
C20	0.32	0.083
CLA *cis*-9,*trans*-11	0.60	0.175
C20:1	1.24	0.300
C21	0.07	0.018
C20:3	0.01	0.004
C22	0.14	0.059
C22:1	0.11	0.031
C23	0.06	0.024
C22:2	0.05	0.030
C24	0.09	0.052
C24:1	0.02	0.006
C22:6	0.05	0.017
SCFA	3.06	1.847
MCFA	25.03	4.448
LCFA	71.92	4.686
SFA	39.38	2.543
MUFA	44.93	2.425
PUFA	15.70	0.475

[1]CLA = conjugated linoleic acid; SCFA: short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 5. Casein allele and haplotype frequencies in the Garfagnina goat[1]

Casein[2]	Allele	Frequency	*CSN1S1*	*CSN2*	*CSN1S2*	*CSN3*	IF	AF
			B	*A*	*A*	*B*	0.0763	0.3117
CSN1S1	*A*	0.1724	*F*	*C1*	*A*	*B*	0.0588	0.1294
	B	0.4397	*F*	*C1*	*B*	*B*	0.0123	0.1008
	F	0.3879	*F*	*C1*	*F*	*A*	0.0063	0.0657
			F	*C1*	*F*	*B*	0.0256	0.0463
			A	*C*	*E*	*B*	0.0012	0.0431
CSN2	*A*	0.4052	*A*	*C*	*C*	*B*	0.0022	0.0429
	C	0.2069	*B*	*A*	*A*	*A*	0.0189	0.0297
	C1	0.3534	*B*	*A*	*F*	*B*	0.0332	0.0296
	0	0.0345	*A*	*C*	*F*	*A*	0.0016	0.0289
			F	*C*	*F*	*A*	0.0037	0.0263
			A	*0*	*A*	*B*	0.0025	0.0259
CSN1S2	*A*	0.5345	*B*	*C*	*C*	*B*	0.0057	0.0170
	B	0.1121	*F*	*C*	*F*	*B*	0.0150	0.0168
	C	0.0776	*A*	*C*	*A*	*B*	0.0153	0.0117
	E	0.0431	*A*	*C*	*B*	*A*	0.0008	0.0112
	F	0.2328	*B*	*A*	*F*	*A*	0.0082	0.0106
			B	*C*	*A*	*B*	0.0390	0.0088
			A	*A*	*A*	*A*	0.0074	0.0086
CSN3	*A*	0.1983	*B*	*0*	*A*	*A*	0.0016	0.0086
	B	0.8017	*B*	*C1*	*F*	*A*	0.0072	0.0086

[1]IF = haplotype frequencies under the independence hypothesis; AF = haplotype frequencies estimated by EH program (Xie and Ott, 1993) taking association into account. Haplotypes are reported in descending AF value. Only haplotypes with AF higher than 0.008 are shown.
[2]*CSN1S1* = α_{S1}-CN; *CSN2* = β-CN; *CSN1S2* = α_{S2}-CN; *CSN3* = κ-CN.

Milk Protein Polymorphisms

A total of 14 alleles were found in the breed (Table 5). The most polymorphic gene was α_{S2}-CN (***CSN1S2***; 5 alleles), followed by β-CN (***CSN2***; 4 alleles), α_{S1}-CN (***CSN1S1***; 3 alleles), and κ-CN (***CSN3***; 2 alleles). In *CSN1S1*, 2 alleles associated with a high content of the specific casein were found (*A* and *B*), as well as the "faint" *F* allele at a rather high frequency (>0.38). In addition to 3 alleles coding for a normal β-CN content, a null *CSN2* allele was found showing a rather high frequency in one flock (>0.08). At *CSN3*, only 2 alleles were detected (*A* and *B*) that do not result in an AA exchange affecting the isoelectric point of casein. Of the 120 haplotypes expected from the possible combinations of casein genes, only 17 and 4 showed association frequencies higher than 0.01 and 0.05, respectively (Table 6). The predominant haplotype was considered the ancestral one, *CSN1S1*B-CSN2*A-CSN1S2*A-CSN3*B* (Caroli et al., 2006), followed by 3 haplotypes (*F-C1-A-B*, *F-C1-B-B,* and *F-C1-F-A*) carrying the faint *CSN1S1*F* allele associated with *CSN2*C1* while differing in the last 2 genes of the casein cluster. These 4 haplotypes represent more than 60% of the casein cluster variability in the Garfagnina.

It is well known that the casein haplotype structure is highly different among breeds. The casein variation of the Garfagnina goat seems more limited compared with other Italian or foreign breeds typed at the casein haplotype level (Caroli et al., 2006, 2007). The low variability is especially evident for *CSN3* and *CSN1S1*, whereas the genes occupying an intermediate position within the casein cluster, namely *CSN2* and *CSN1S2*, were more polymorphic.

The ancestral haplotype *B-A-A-B* was usually found at the highest frequencies in breeds that are rarely or never selected for milk production, similarly to the African breeds considered by Caroli et al. (2007) and Küpper et al. (2010). The relationship between the variability in goat casein haplotypes and the productive purposes of the breed still needs further investigation. The high frequency of strong *CSN1S1* alleles in the Garfagnina indicates that selection for haplotypes carrying these alleles should be an easy breeding objective to improve milk composition and cheesemaking aptitude. In addition, a differential casein haplotype selection could be implemented within flocks depending on their productive aim, namely fresh or ripened cheese, which could be improved by selecting goats carrying faint (i.e., *F-C1-A-B*, *F-C1-B-B*, *F-C1-F-A*) or strong (i.e., *B-A-A-B*, *A-C-E-B*, *A-C-C-B*) haplotypes, respectively. On the other hand, the occurrence of haplotypes carrying null and faint alleles at *CSN2* and *CSN1S1*, respectively, could be exploited for the production of milk with specific nutritional properties, also in the light of the poor milk rennet clotting properties of the population.

CONCLUSIONS

This study is an initial cognitive step toward the protection and promotion of the Garfagnina popula-

Table 6. Genotyping analyses and identifiable alleles or groups of alleles

Casein gene[1]	DNA method[2]	Identifiable alleles or groups of alleles
CSN1S1	PCR-SSCP	*F*, *N*, *A*/*0*$_1$, *B*/*E*, *B*′
	AS-PCR	*E*, non-*E*
	AS-PCR	*0*$_1$, non-*0*$_1$
CSN2	mPCR-SSCP	*A*, *C*, *C1*, *E*, *0*′, *0*
CSN1S2	PCR-SSCP	*A*, *B*, *C*, *E*
	PCR-RFLP	*D*, *0*
	PCR-RFLP	*F*
CSN3	PCR-SSCP	*A*, *B*/*B*′, *B*″, *C*, *C*′, *E*, *D*/*I*/*K*/*L*, *G*, *H*, *J*, *M*

[1]*CSN1S1* = α_{S1}-CN; *CSN2* = β-CN; *CSN1S2* = α_{S2}-CN; *CSN3* = κ-CN.

[2]Methods: PCR-SSCP = PCR-single strand conformation polymorphism; AS-PCR = allele-specific PCR; mPCR-SSCP = multiplex PCR-single strand conformation polymorphism.

tion within its breeding area in that it provides an overview of zootechnical data, including milk quality and nutritional properties. As a result of our study, we believe that selection should maintain morphological characteristics, thus encouraging farmers to breed pure populations. The alternative use of milk for direct human consumption should be further investigated in light of the data presented on milk quality and genetic polymorphisms. An agreement between national and local institutions is essential for safeguarding native gene pools and at the same time obtaining practical solutions aimed at promoting innovation in terms of the nutritional properties of typical food products. We recommend that ecopromoting policies and agrozootechnical activities should focus on the quality of typical products in order to promote the involvement of young people and create new employment opportunities.

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