The Grey Goat of Lanzo Valleys (Fiurinà): Breed characteristics, genetic diversity, and quantitative-qualitative milk traits

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ABSTRACT

The aim of this study was to provide an overview on breed's characteristics of a small dairy goat population recently identified in the Piedmont region (NW Italy): the Grey Goat of Lanzo Valleys. This goat, locally named *Fiurinà*, is composed of about 150 heads. Increasing knowledge on threatened breeds is an effective tool to develop

conservation programs aiming at preserving loss of genetic resources, economically valorizing animal-derived food products, maintaining traditions and cultural values, as well as appropriately managing native habitats. Data and samples were collected in 15 representative farms to obtain information on geographical distribution, breeding systems, morphometric measures, and genetic diversity. Milk traits, including fatty acids profile and caseins polymorphism, were also investigated. The prevalent breeding system is extensive or semi-extensive, mostly with vertical transhumance from lowland to alpine pastures during the grazing season. The breed is characterized by a peculiar color of the fleece (mixture of white, grey and black course outer hairs with brown under-down) from which the local name "*Fiurinà*" (=speckled) derives. The breed is of medium size, with a quite high frequency of well-developed and turned backward horns. More than 80% of does have pear-shaped udder, typical of goats, with cylindrical teats directed downward. The microsatellite analysis pointed out significant distances between *Fiurinà* and other goat breeds reared in the same area. Consequently, *Fiurinà* has to be considered as a unique breed. Although highly variable, quite appreciable milk yields were observed. Considering milk gross composition and fatty acids profile, *Fiurinà* goat showed comparable results to other local breeds reared in alpine environment and mainly fed with fresh and conserved forages. A remarkable amount of α-linolenic acid (0.82 g 100 g^{-1} fat) was detected. The breed is also characterized by an interesting and wide variability in the casein cluster, with some haplotypes (i.e., *A-C-F-C'*, *E-A-C-B*, *F-C_1-F-C')* detected only in *Fiurinà*, confirming the genetic uniqueness of the breed. The balanced frequency of medium-strong and weak-null *CSN1S1* alleles could be exploited for different breeding strategies.

Key Words: local goat breed, breeding system, genetic diversity, milk quality.

INTRODUCTION

The positive trend in the number of goats around the world (+58%) occurred from 1980 to 2000 has recently been confirmed in the last 10 years when goat stocks increased by about 23%. These data are even more interesting if compared to the modest increase in cattle and sheep stocks (+9% and +3%, respectively). The growing success in goat farming is noticed worldwide, in developing countries as well as in industrialized areas (FAOSTAT, 2010).

Goats play an important role in rural economy of developing countries, especially in areas with unfavorable environmental and climatic conditions, thanks to their capacity for adaptation to very different environments and because they are important users of marginal and rural lands (Kalantzopoulos et al., 2004). In developed countries, goats are presently considered as 'ecological' animals and seem to adequately respond to increasing consumers' demand concerning product quality, animal welfare, and environment respect (Morand-Fehr et al., 2004). In these contexts, goat dairy products often gain niche market reaching higher prices than other dairy products. Furthermore, goat milk has some distinctive traits, such as high digestibility and low allergenicity, which bring it to be widely considered the alternative to bovine milk, especially for people with allergies and other gastrointestinal disorders (El-Agamy, 2007; Park et al., 2007).

The worldwide increase in goat stocks has also been possible with the diffusion of some specialized breeds (e.g., Saanen). However, the widespread use of a reduced number of high producing breeds led to a dramatic reduction of autochthonous ones, placing most of them in an endangered status. In Europe, for example, although there is only about 4% of the world's goat population, there is the largest share of goat genetic resources, approximately 33% (Galal, 2005). However, the 35% of European

local breeds are endangered (Bertaglia et al., 2007).

Effective management of farm animal resources requires comprehensive knowledge of the breeds' characteristics, including data on population size and structure, geographical distribution, production environment, and within- and between-breed genetic diversity (Groeneveld et al., 2010). Such an exhaustive overview on breeds' characteristics, as well as on products' quality, represents the starting point for the development of a preservation program (Canali, 2006; Lauvie et al., 2011; Verrier et al., 2005). An effective safeguard of a local breed prevents extinction and erosion of genetic resources and allows the economic valorization of derived products, the maintenance of traditions and cultural values as well as the appropriate management of native habitats (Dubeuf, 2011; Rosa García et al., 2012).

In Italy, goats are mainly reared in the Centre-South, where the age-long dairying tradition counts several cheeses obtained from sheep and goat milk (Pirisi et al., 2011). Nevertheless, goats are also reared in the Italian alpine regions where dairy products are recently increasing their relevance on the market. An Italian native goat dairy population, officially named Grey Goat of Lanzo Valleys (*Capra Grigia delle Valli di Lanzo*) and locally known as "*Fiurinà*" has been recently identified in the Piedmont region (Cornale et al., 2012, 2010).

To prove comprehensive information, the goat population has been the subject of a multidisciplinary project concerning with population size, geographical distribution, breeding systems, morphometric measures, and genetic diversity (microsatellite and mitochondrial DNA analyses). Furthermore, since the main purpose of the *Fiurinà* breed is milk production, quanti-qualitative traits of milk were investigated, including fatty acid profile, for its well known human health implications, and caseins polymorphisms, for their relation with milk composition and

101 technological properties.

102

103

104 **MATERIALS AND METHODS**

105 The *Fiurinà* goat population is reared in the Piedmont region (NW Italy). All
106 the investigated farms breeding the *Fiurinà* goats are located in uplands and highlands
107 of the northwestern territories of the Torino province.

108 The 3-year research project on the *Fiurinà* breed lasted from the beginning of
109 2009 to the end of 2011, and was developed on two main research lines. The first
110 topic included the collection of data related to population size, geographical
111 distribution, farm management, morphometric characteristics, as well as the analysis
112 of genetic diversity to evaluate if the *Fiurinà* goat could be considered a new breed.
113 These data were collected between 2009 and 2010 by examining 15 farms, breeding
114 approximately the 50% of the *Fiurinà* population. The second topic focused on milk
115 production and quality, including fatty acids and caseins polymorphism analyses.
116 These surveys were carried out between 2010 and 2011, by collecting milk samples in
117 11 representative farms.

118

119 ***Morphometric Measurements***

120 Body measurements are of primary importance for breed phenotypical
121 description and they represent a starting point in the definition of breed standards
122 (Zaitoun et al., 2005; Dossa et al., 2007). In the 15 investigated farms, all reproductive
123 females (n = 77), corresponding approximately to 50% of the registered population,
124 were measured.

125 The goats were weighed using a spring balance. Height at withers, rump height,

rump width, trunk length, and chest girth were also measured by using a flexible tape or a Lydtin stick. With the animal standing upright, the height at withers and the rump height were measured as the distance from the floor to the shoulders and to the highest point of the rump, respectively. The rump width was measured as the distance between the pin bones. The trunk length was the distance between the crown and the sacrococcygeal joint. The chest girth was the circumference of the thoracic cavity taken just behind the forelimbs.

A five-point scale was carried out to assess traits of udder morphology based on the scoring system proposed by de la Fuente et al. (1996) for ewes and modified for dairy goats as stated below. The udder depth was evaluated by considering the position of the udder floor with respect to hock (score 3: at the same hock's level; score <3: above the hock; score >3: below the hock). The fore udder attachment evaluates the strength of the attachment of the lateral ligaments to the body wall. The score can range from extremely loose (score 1) to snug and strong (score 5) attachment. The rear udder attachment was evaluated through the insertion to the abdominal wall by assessing the udder height. The cistern shape was assessed through the degree of separation between the left and right halves (score 1, pronounced separation; score 5, no separation). Finally, halves' symmetry was recorded.

Concerning teats' traits, their shape, inclination, and orientation were assessed. The teats' shape can range from short and small (score 1) to squat and cone-shaped (score 5). The physiological inclination of the teats is almost vertical (score 3), but it can be directed cranially (score 1) or caudally (score 5). The teats' orientation can be divergent (score 1), parallel (score 3) or convergent (score 5). Furthermore, symmetry of teats and presence/absence of supernumerary teats were also evaluated. All the morphometric assessments were performed by the three same operators.

151

152 ***Genetic Diversity***

153 In order to characterize the genetic diversity of the *Fiurinà* breed, DNA was
154 extracted from hair root samples using proteinase k digestion.

155 ***Microsatellite analysis.*** A group of *Fiurinà* (n = 26) reared in 15 different farms
156 was compared with two officially recognized autochthonous Piedmontese breeds,
157 Sempione (n = 22) and Vallesana (n = 36), reared in other 26 farms located in the
158 same region. The individuals were selected in order to avoid close relatives (the
159 number of samples collected per farm varied between 1 and 6). The evaluation of the
160 genetic structure was investigated using 12 microsatellites (INRA005, MAF65,
161 INRA063, MCM527, ETH10, SRCRSP5, INRA023, OarFCB20, TGLA53, SRC247,
162 CSRD247, ILST87), most of them recommended by ISAG/FAO (FAO, 2004).
163 Genotype determinations were performed on an ABI Prism 3100 DNA Sequencer,
164 equipped with Genscan and Genotyper software.

165 ***Mitochondrial DNA analysis***. A subsample of 10 animals belonging to the
166 *Fiurinà* breed was also analyzed for the mitochondrial DNA (mtDNA). The goats
167 were selected by respecting phenotypic standards, maximizing geographical spread of
168 the farms, and avoiding relationships among the sampled flocks. The primer pairs
169 CAP-F (5'-CGTGTATGCAAGTACATAC-3') and CAP-R (5'-
170 CTGATTAGTCATTAGTCCATC-3') and the amplification conditions were used to
171 determine the sequence of the mtDNA control region, as described by Luikart et al.
172 (2001). Amplified products were used for sequencing with the CAP-F or CAP-R
173 primer. Sequence reactions were performed for both DNA strands by means of an
174 ABI Prism 3100 DNA Sequencer, according to the manufacturer's instructions. All
175 sequences were deposited in GenBank (Accession Numbers JQ655153-JQ655162).

The sequences of mtDNA d-loop region from 10 sequences of the *Fiurinà* goats belonging to this study were aligned with the mtDNA complete sequence of *Capra hircus* (GenBank NC_005044) using ClustalX, version 2.0.11 (Thompson et al., 1997). The same software was used to compare the *Fiurinà* goats' sequences with the mtDNA of the 22 reference individuals of the 6 domestic goat haplogroups reported by Naderi et al. (2007). The alignments were imported in MEGA, version 4.0 (Kumar et al., 2004) and a neighbour-joining haplotype tree was constructed using Kimura 2-parameter distance model with 1,000 bootstrap replications.

Milk Yield, Gross Composition and Fatty Acids Analysis

The individual daily milk yield was measured by using recording jars. In the selected 11 farms, individual milk samples were collected from 52 goats during lactation. Two aliquots of each individual milk sample were collected during the morning milking, immediately stored at 4°C in a portable refrigerator, and transported to the laboratory. One aliquot (50 mL) was then immediately analyzed for fat, protein, lactose, casein, urea, solids-non-fat (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark), and somatic cell count (SCC) (Fossomatic 5000, Foss Electric, Hillerød, Denmark). The other aliquot (150 mL) was frozen at –20°C and successively analyzed for the FA composition as previously reported by Renna et al. (2012). Milk fat extraction was obtained by centrifugation at 7,300 rpm for 30 min at –4°C. The resulting molten butter was filtered through a hydrophobic filter (Whatman 1, Whatman International Ltd, Maidstone, England). The pure milk fat was then dissolved in 5 mL of internal standard solution (nonanoic acid in heptane) and fatty acid methyl esters (FAME) were obtained by trans-esterification of glycerides by using a solution of potassium hydroxide in methanol (IOfS, 2002). FAME were

analyzed by high-resolution gas chromatography (Shimadzu GC17A, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan) with flame ionization detector according to Collomb and Bühler (2000). FAME were separated on a CP-Sil 88 capillary column (100 m × 0.25 mm ID, 0.20 μm film thickness; Varian Inc., Lake Forest, CA, USA). The column temperature was held at 45°C for 5 min, then raised 20°C min^{-1} up to 195°C and maintained for 65 min. The temperatures of the injector and detector were maintained at 250°C and 280°C, respectively. The injection volume was 0.1 μL. Nitrogen constant linear flow rate was set at 40 mL min^{-1}. Peaks were identified by comparing their retention times with pure FAME standards (Matreya Inc., Pleasant Gap, PA, USA and Restek Corporation, Bellefonte, PA, USA). Quantification was assessed by using nonanoic acid as internal standard. The results are expressed as both absolute values (g $100g^{-1}$ fat) and percentages of each FAME per total FAME detected.

Genotyping Analysis of Caseins Variability

Milk was also used as starting material for DNA extraction. Individual milk samples were collected from 52 does randomly chosen in the selected 11 farms. The GFX Genomic Blood DNA Purification kit (Amersham Biosciences, Piscataway, NJ, USA) was used for DNA extraction directly from milk. Extraction was performed starting with 300 μL of milk to recover a 130 μL final volume of genomic DNA solution. All samples were analyzed using a NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) to assess DNA concentration and purity. To analyze most of the 50 alleles described (Küpper et al., 2010) at the casein gene cluster (19, 8, 7 and 16 variants for *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*, respectively), the samples were typed by 8 different methods, first

screening the major alleles by means of the PCR - Single Strand Conformation Polymorphism (SSCP) methods and then applying the PCR - Allele Specific (AS) and the PCR-RFLP methods available to discriminate alleles grouping together within the PCR-SSCP, as described in Table 1. All the PCR protocols were applied using at least 25 ng of DNA.

Statistical Analysis

The software Arlequin ver. 3.11 (Excoffier et al., 2005) was used to calculate: i) the number of alleles per locus, the observed and expected heterozygosity; ii) to test for Hardy-Weinberg Equilibrium using exact test and sequential Bonferroni correction; iii) to compute Wright's F_{IS}, F_{ST} and F_{IT} fixation indexes (Weir and Cockerham, 1984; Wright, 1965) and to evaluate the significance of genetic differentiation between populations with an Analysis of Molecular Variance (AMOVA, Excoffier et al., 1992). Reynolds's genetic distance (Reynolds et al., 1983) was obtained using GENEDIST, included in the PHYLIP computer package, version 3.65 (Felsenstein, 1989). FSTAT software (Goudet, 2001) was used to calculate the allelic richness (AR) standardized for variation in sample size.

Means and standard deviations (or median and interquartile range, depending on the considered variables) of morphometric data as well as of chemical and hygiene parameters of milk were calculated with the MEANS procedure of SAS (SAS Institute, 2008).

The casein alleles and haplotypes distributions were analyzed by the ALLELE and HAPLOTYPE procedures of SAS (SAS Institute, 2008). The ALLELE procedure uses the notation and concepts described by Weir (1996). Haplotype frequencies were calculated under the null hypothesis of no linkage disequilibrium and under the

alternative hypothesis of associations between casein genes.

Haplotype frequencies were also analyzed by the PRINCOMP procedure of SAS (SAS Institute, 2008) to underline the differences among the *Fiurinà* goats and other Italian goat breeds (Caroli et al., 2006; Martini et al., 2010).

RESULTS AND DISCUSSION

Breed Geographical Distribution and Size

The *Fiurinà* goats are mainly reared for milk production in the territories of Lanzo Valleys (longitude: 7°48'03''E; latitude: 45°27'56''N) from which the official name Grey Goat of Lanzo Valleys (*Capra Grigia delle Valli di Lanzo*) derives. This area, gathering three alpine valleys (*Val Grande di Lanzo*, *Val d'Ala*, and *Valle di Viù*), is located in the northwestern area of the Torino province. Farmers rearing the *Fiurinà* subjects have been also reported in adjacent areas (*Val Susa* and *Canavese*) of the same region. All investigated farms are located between 400 and 1,400 m a.s.l. About 150 heads out of 1,250 goats in the territories of Lanzo Valleys are identified as belonging to the *Fiurinà* breed, distributed in 56 farms, which correspond to the 82% of goat farms in the area. The average herd size is small, ranging from 13 to 42 goats. Only in few farms the flock is mainly composed of *Fiurinà* subjects, otherwise few *Fiurinà* heads are reared with other cosmopolitan (e.g., Camosciata delle Alpi) or local (e.g., Valdostana) goat breeds, or even crossbreds. The presence of few subjects per farm justifies the high percentage of goat farms in the area rearing the *Fiurinà* breed.

Breeding Systems

276 As commonly happens in the alpine territories, all investigated farms are of
277 small and medium size and the most common types of business are family and single-
278 worker types. Historically, farms chiefly base their livestock activity on bovine milk
279 production. Goat milk and meat often represent complementary activities, especially
280 because of the high ability of small ruminants to exploit marginal mountain areas,
281 which are otherwise not utilized.

282 Extensive or semi-extensive farming (highly dependent on grazing) is generally
283 practiced in all farms. In winter months (from November to March-April) the goats
284 are stabled indoors and mainly fed with local hay. The supplementation, with cereals
285 (corn and wheat bran) and, occasionally, with other feedstuffs (e.g., chestnuts), is
286 limited and depends on both availability of hay and nutrient requirements of the goats.
287 As soon as fresh grass is available, the flocks are moved outdoors and the goats graze
288 on pastures located near the farms. In just one of the investigated farms, the goats are
289 always housed and fresh cut grass is offered in through. Vertical transhumance (from
290 lowland to alpine pastures) is diffused in 87% of the investigated farms. The alpine
291 grazing season averages from April-May to October-November, in accordance with
292 weather conditions and fresh forages availability. After alpine grazing, the goats graze
293 on pastures near the farms until the winter arrival. Consequently, the feeding system
294 is mostly based on local fresh and conserved forages. Although in free grazing
295 conditions goats usually utilize trees and shrubs in woodland due to their feeding
296 behavior (Dwyer, 2009), a regional law generally forbids goats to graze in forests.

297 As stated above, the main purpose of the *Fiurinà* goat is milk production. Hand-
298 milking is practiced in 100% of the investigated farms. The absence of machine-
299 milking is probably related to the small flock size that does not justify such an
300 investment. After kids weaning, goat milk, often mixed with bovine milk, is processed

at farm level into fresh and matured cheeses and directly sold to consumers.

The reproductive career of does is quite long, reaching sometimes 15 years. Artificial insemination is not used and natural mating occurs with the introduction of a buck in the flock of all the investigated farms. Average age of does at first kidding is 13 - 14 months. Mating season aims at converging kidding in autumn-winter months to ensure the availability of kids in Christmas and, particularly, in Easter times. Kids are maintained with the mother until slaughtering weight (10 - 12 kg). Concerning reproductive performance, fertility rate (calculated as the percentage of does that kid per does exposed to buck), fecundity rate (calculated as the percentage of kids born alive per does exposed to buck), and prolificacy (calculated as the percentage of kids per does kidding) were 92%, 136%, and 147%, respectively. The *Fiurinà* breed showed a slightly higher (+2%) fertility rate and lower fecundity rate and prolificacy (-8% and -13%, respectively) if compared to the Saanen goat reared in the same region (Bigi and Zanon, 2008). Higher results (95%, 152%, and 160%) were reported for the Camosciata delle Alpi reared in the same area (Deitos, 2001).

Morphometric Characteristics

The *Fiurinà* breed is of medium size, alertly graceful. The hair is medium to short. This breed is characterized by a peculiar color of the fleece (mixture of white, grey and black course outer hairs with brown under-down) from which the local name "*Fiurinà*" (=speckled) derives (Figure 1). Legs are long and well developed, allowing the breed to easily move in unfavorable territories such as steep pastures. The head is straight and light, with quite long upright ears. The neck is long and thin in females, much short and muscular in males. Beards and wattles are occasionally present in both bucks and does. All characteristics refer to the typical shape of the dairy goat

breeds (Lucifero, 1981). The breed showed a quite high frequency of well-developed and turned backward horns in males (85.71%), while only 60% of females have horns, usually shorter than in males.

Average morphometric traits measured on adult females of the *Fiurinà* breed are reported in Table 2. By comparing the results with morphometric measurements of other cosmopolitan (Saanen and Camosciata delle Alpi) and local (Sempione and Vallesana) breeds commonly reared in the same area, the *Fiurinà* does showed a slightly reduced size. Such characteristic makes grazing easier in mountain marginal areas and *Fiurinà* appeared to be well adapted to environmental and climatic conditions of alpine valleys.

Udder morphology is normally assessed in dairy ewes (Caja et al. 2000, Casu et al., 2006) and in dairy goats (Capote et al., 2006; Peris et al., 1999; Salama et al., 2004). These authors agreed with the importance of udder morphology assessment in relation to milkability, especially when related to machine milking. Although hand milking is diffused in all investigated farms, the evaluation of udder traits appears to be relevant because of some relationships pointed out between morphological and productive traits (i.e., milk yield) (Casu et al., 2006). Therefore, such information should be taken into account in a future genetic selection program. Concerning udder morphology, more than 80% of the considered subjects had pear-shaped udder, typical of goats, with cylindrical teats directed downward (85.19%). A weak median suspensory ligament, resulting in the udder floor below the hock, rarely occurred in the *Fiurinà* goats (<2%). The udder was usually well supported, with the floor at the same level of the hock (16.67%) or slightly above (>80%). This is important for the udder functionality because, as reported by Altinçekiç and Koyuncu (2011) for dairy ewes, grazing animals with deep udder are more exposed to accidental injuries.

Concerning fore udder attachment, none of the goats showed a snug and strong attachment. Lateral ligaments were intermediate in strength and tightness in 35% of the goats, while the remaining ones showed a score <3. The height of the rear udder attachment is an indication of the goat's potential capacity for milk production. Almost 90% of the goats obtained a score ≥3. Only 11% of the goats had a low udder height. Usually the cistern was well shaped and only a small percentage of subjects showed a pronounced separation of the halves (<7%) or a separation almost absent (<9%). The symmetry in two halves was detected in almost 80% of the goats.

The ease of milking is particularly reflected in teats' evaluation. A good teat should be cylindrical, with a constant diameter and should have a medium length (Le Du and Benmederbel, 1984). In the *Fiurinà* population less than 6% of goats had very narrow teats. The majority (68.52%) showed good cylindrical shape, while tight or funnel shaped teats occurred in about 1/4 of the goats. Teats' inclination was almost vertical in more than 85% of goats. The remaining heads showed teats cranially inclined. Caudal inclination was not detected. Similarly, none of the goats showed teats pointed inward. Teats were almost parallel (28%) or slightly pointed outward (72%). More than 85% of goats had symmetric teats and supernumerary ones were present only in one doe.

Genetic Diversity

Microsatellites. A total of 103 alleles were detected across the 12 microsatellites loci analyzed, which were polymorphic in the three considered breeds (*Fiurinà*, Sempione, and Vallesana). The number of alleles ranged between 4 (ET10) to 17 (SRC247). Observed and expected heterozygosities, AR and F_{IS} for each goat breed are presented in Table 3. The highest value of AR was found in Sempione (6.41) and

376 the lowest in Vallesana (5.80) while *Fiurinà* showed an intermediate value (6.19).
377 The *Fiurinà* goat showed the lowest level of observed (0.59) and expected (0.63)
378 heterozygosities. F_{IS} value within populations was statistically significant only for
379 Vallesana breed due to a deficiency of heterozygosity. Out of the total of 36 Hardy-
380 Weinberg equilibrium tests, only two (both in Vallesana breed) gave significant
381 deviations at the 1% level.

382 *F* statistics per microsatellite locus are shown in Table 4. As expected, because
383 of the close geographical origin of the three goat populations, levels of apparent breed
384 differentiation were quite low. The average F_{ST}, which was significantly different
385 from zero ($P<0.001$), indicated that about 3% of the total genetic variation was
386 explained by differences among breeds, with the remaining 97% corresponding to
387 differences among individuals. Genetic differentiation among breeds was significant
388 ($P<0.001$) only for two loci (SRC247 and ILST87). A significant excess of
389 homozygotes across all breeds ($P<0.001$) was found for MCM527, CSRD247, and
390 ILST87 loci. On average, breeds had a 7.6% ($P<0.001$) deficit of heterozygotes,
391 whereas the total population had a 10.3% ($P<0.001$) deficit of heterozygotes.

392 Reynolds's genetic distance values ranged from 0.031 between Sempione and
393 Vallesana to 0.062 between *Fiurinà* and Sempione; F_{ST} values expressed similar
394 relationships between the same pairwise breed combinations (Table 5). The distance
395 between *Fiurinà* and the other two breeds is therefore greater than the distance
396 between Sempione and Vallesana. Pairwise F_{ST} values between *Fiurinà* and the other
397 two breeds reached significant levels ($P<0.001$), indicating that *Fiurinà* can be
398 considered as a separate breed. F_{ST} value between Sempione and Vallesana was
399 instead significant only at $P<0.05$. Some other genetic studies about Italian goat
400 breeds have been carried out using microsatellite markers (Iamartino et al., 2005;

401 Negrini et al., 2012), but no information about Piedmontese goat breeds have been
402 reported till now.

403 ***Mitochondrial DNA***. The mtDNA fragments of the d-loop region in the 10
404 *Fiurinà* samples were highly polymorphic, with 51 variable sites over the 481 bp of
405 the alignment (Figure 2). A total of 9 haplotypes were identified.

406 As shown in Figure 3, using the available goat mtDNA haplogroup
407 classification system, 8 *Fiurinà* goats could be classified into haplogroup A, whereas
408 the remaining 2 animals were assigned to haplogroup C. Six mitochondrial
409 haplogroups A, B, C, D, G, and F have been identified by many authors (Naderi et al.,
410 2007; Royo et al., 2009; Sardina et al. 2006). The most ancient population expansion
411 is probably represented by the haplogroup A, which is observed worldwide with high
412 frequencies (Luikart et al., 2001; Royo et al., 2009) ranging from 89% in Asia to 98%
413 in Europe (Pereira et al., 2005). Haplogroups B and D were found in Asia while
414 haplogroup C was found in Asia and Europe with low frequencies. Haplogroups B
415 and C can be the consequence of a second domestication in Asia, with a more recent
416 expansion (Luikart et al., 2001). A haplogroup G has been found in the Middle East
417 and North Africa (Naderi et al., 2007) while the haplogroup F was limited to Sicily
418 (Sardina et al., 2006).

419 Haplogroup A is the most observed haplogroup among the 20 Italian goat
420 breeds that have been studied till now (Luikart et al., 2001; Naderi et al., 2007; Vacca
421 et al., 2010). The Girgentana breed represents an exception, because it showed the
422 haplogroup F (Sardina et al., 2006). Apart from *Fiurinà* goat, sequences belonging to
423 the haplogroup C have been recently found in the Sarda breed (Piras et al., 2012),
424 even if with very low frequencies (0.008). This finding gives a further element of

interest on the *Fiurinà* breed and its genetic distinctiveness compared to the other breeds reared in the same region.

Milk Yield and Gross Composition

The average length of lactation was about 200 days. A high variability in milk yield was observed: the recorded levels varied from less than 1.0 to approximately 3.5 L head^{-1} day^{-1}. Although the performance was variable, such result has to be considered quite appreciable, particularly for a local breed usually fed with fresh or conserved forages with only a limited and occasional use of concentrates.

The main milk constituents are reported in Table 6. They were in the range reported by Park et al. (2007) for goat milk, with the exception of the average protein content that showed a slightly lower value (29.9 g kg^{-1} compared to 34.0 g kg^{-1}). The obtained results agreed also with those reported by Raynal-Ljutovac et al. (2008) for goat milk, including the protein content (reported lower limit: 26.1 g kg^{-1}).

Considering milk gross composition of other goat breeds (Bigi and Zanon, 2008) reared in the same area, *Fiurinà* reported comparable protein but higher milk fat levels *(Fiurinà*: 35.8 g kg^{-1}; Vallesana: 30.3 g kg^{-1}; Sempione: 31.9 g kg^{-1}). It is worth mentioning that, besides breed, milk main constituents depend on several factors, such as feeding, lactation stage, season, etc.

While in the United States the legal limit established for milk SCC is 1,000 × 10^3 cells mL^{-1}, currently there is no legal limit for goat milk in the European Union (Paape et al., 2007). The observed value for the *Fiurinà* milk (median: 490 × 10^3 cells mL^{-1}) was largely included in the threshold limit of 1,500,000 cells mL^{-1} proposed by Delgado-Pertiñez et al. (2003) and even in the more restrictive grading scheme

449 suggested by Leitner et al. (2008): grade A (the best) with SCC ≤ 840 × 10^3 cells
450 mL^{-1}.
451
452 ***Milk Fatty Acids Profile***
453 The individual FA and the groups of FA in *Fiurinà* milk are presented in Tables
454 7 and 8, respectively. Results showed that five FA (C10:0, C14:0, C16:0, C18:0, and
455 C18:1 *c*9) accounted for 72% of total FA. Caproic (C6:0), caprylic (C8:0), and capric
456 (C10:0) acids are among the most characteristic FA in goat milk and derived dairy
457 products, being more abundant than in cow milk fat. They are named after the species
458 name (*Capra hircus*) and are generally associated with the characteristic flavor of
459 goat cheeses (Mele et al., 2008). Mainly caprylic and capric acids have become
460 established medical treatments for a wide range of clinical disorders, being
461 consequently considered of particular importance in human nutrition (Haenlein, 2004).
462 In milk fat from the *Fiurinà* goat caproic, caprylic, and capric acids accounted on
463 average for 2.23, 2.43, and 7.35 g $100g^{-1}$ fat, respectively.
464 Among goat milk FA, odd- and branched-chain fatty acids are also responsible
465 for the typical aroma of caprine milk and cheese (Alonso et al., 1999). These FA can
466 be almost exclusively found in dairy products from ruminants and have received
467 increasing attention by researchers in recent years due to their anticancer properties
468 (Oku and Yanagita, 2009; Parodi, 2009). Linear odd pentadecanoic (C15:0) and
469 heptadecanoic (C17:0) acids accounted for the majority of total detected odd- and
470 branched-chain fatty acids; their average concentrations were equal to 0.80 and 0.71 g
471 100 g^{-1} milk fat. Among branched-chain FA, the most abundant ones in *Fiurinà* milk
472 fat were the *iso* and *aiso* forms of pentadecanoic and heptadecanoic acids, the *iso*
473 form of hexadecanoic (C16:0) acid and the *aiso* form of octadecanoic (C18:0) acid;

474 such results confirm previous findings for goat milk fat (Massart-Leën et al., 1981;
475 Alonso et al., 1999; Žan et al., 2006).

476 Considering unsaturated FA, either FA of nutritional interest such as conjugated
477 linoleic acids (CLA) and omega-3 FA or presumably negative FA such as *trans* fatty
478 acids (TFA) can be found in milk and dairy products from ruminants. The acronym
479 CLA refers to a mixture of positional and geometric isomers of octadecadienoic acid,
480 with double bonds located in adjacent carbon atoms. Many beneficial biological
481 effects have been attributed to CLA in animal models of human diseases, including
482 anticarcinogenic, antidiabetic, antiaterogenic, and antinflammatory properties (Park,
483 2009). Appreciable amounts of CLA are usually reported in goat milk as well as in
484 other ruminant-derived food products. Results of FA analysis showed that total CLA
485 in *Fiurinà* milk was 0.64 g $100g^{-1}$ fat. In the applied chromatographic conditions the
486 most represented among CLA isomers, rumenic acid (C18:2 *c*9*t*11), coeluted with
487 other two isomers (CLA *t*7*c*9 and *t*8*c*10). As usually occurs in milk fat from
488 ruminants (Parodi, 2009), the sum of these three isomers accounted for more than
489 90% of total CLA.

490 Omega-3 FA have been shown to possess positive health effects in chronic
491 diseases including cancer, insulin resistance and cardiovascular diseases (Anderson
492 and Ma, 2009). Increasing levels of these FA in dairy food products are thus pursued.
493 The sum of omega-3 FA in *Fiurinà* milk was equal to 1.25 g $100g^{-1}$ fat. Remarkable
494 amounts of α-linolenic acid (C18:3 *c*9*c*12*c*15, ALA), the most abundant among
495 omega-3 FA in ruminant-derived food products, were found. Detected ALA levels, in
496 fact, averaged 1.02% of total detected FA; such value was more than doubled
497 compared to the mean value (0.42%) reviewed by Park et al. (2007) for goat milk fat.

498 In ruminant milk fat, the predominant TFA have a chain length of 18 carbon atoms.
499 Both mono- and diunsaturated TFA, especially the latter ones, have been reported to
500 increase risk factors for coronary hearth diseases (Baylin et al., 2003). In milk from
501 *Fiurinà* they accounted for 2.70 and 1.47 g 100 g^{-1} fat. The most abundant among
502 TFA in ruminant derived food products is vaccenic acid (Precht et al., 2001).
503 Differently from other TFA, vaccenic acid has been reported to exert protective
504 effects against cardiovascular diseases (Wang et al., 2012). In the applied
505 chromatographic conditions, vaccenic acid's peak coeluted with those of other *trans*-
506 octadecenoic isomers (C18:1 *t*6 - 10), showing a concentration equal to 1.98 g 100 g^{-1}
507 fat. Detected monounsaturated TFA with less or more than 18 carbon atoms were
508 C14:1 *t*, C16:1 *t*, C17:1 *t*, and C20:1 *t*. These FA were found only in low or very low
509 concentrations in *Fiurinà* milk fat, similarly to previous observations in cow and
510 sheep milk (Abilleira et al., 2009; Collomb et al., 2008).

511 The obtained results regarding the FA composition of *Fiurinà* milk are in
512 accordance with range values previously reported for goat milk in the literature (Park
513 et al., 2007). Of particular note is the remarkable ALA level found in *Fiurinà* milk,
514 which deserves positive considerations. Similar ALA amounts were previously
515 observed in milk from dairy goat breeds reared in the alpine environment and
516 managed according to extensive pasture-based systems (Žan et al., 2006).

517

518 ***Milk Caseins Polymorphism***

519 The extraction of DNA from milk samples gave DNA of varying quality and
520 quantity. The estimated concentration of the samples ranged from 4.39 to 71.57 ng/μL.
521 The 260/280 ratios ranged from 1.22 to 1.93 with 13 samples having a 260/280 ratio

522 inferior to 1.5 and the remaining a mean 260/280 ratio of 1.67. Despite the limited
523 purity and concentration of DNA, all the samples but one gave good PCR products.

524 A total of 19 alleles were found in the breed and 14 had a frequency higher than
525 0.05 (Table 9). In particular, 7 variants were found in the *CSN1S1*, and 4 in each of
526 the other 3 casein genes. Genetic equilibrium was generally found at each gene,
527 except for a significant deviation at *CSN2*, in which an excess of homozygotes CC
528 occurred, due to the linkage of this variant with the 0_1 allele at the *CSN1S1*.

529 It is well known that goat caseins are characterized by different expression
530 levels, distinguishing alleles responsible for strong, medium, weak or null casein
531 content in milk, depending on the casein fraction (Chiatti et al., 2007; Küpper et al.,
532 2010). The 4 strong alleles found at the *CSN1S1* had a total frequency of 0.135, even
533 lower than the frequency of the null allele 0 (0.198). The medium allele E and the
534 weak allele F occurred with the same frequency (0.333), giving a population with a
535 quite balanced proportion of medium-strong and weak-null alleles at the *CSN1S1*. A
536 null allele was found also at the *CSN2*, even if only at the heterozygous status in one
537 individual. The same distribution was found for the rare alleles *CSN1S1*A'* (0.010)
538 and *CSN1S1*B'* (0.010), thus far described only in German (Küpper et al., 2010) and
539 African breeds (Caroli et al., 2007), respectively.

540 Of the 240 haplotypes expected from the possible combinations of casein genes,
541 only 18 and 5 showed association frequencies higher than 0.008 and 0.05,
542 respectively. The predominant haplotype was the *CSN1S1*E-CSN2*A-CSN1S2*A-*
543 *CSN3*B*, whereas the ancestral one *B-A-A-B* (Caroli et al., 2006) was found only with
544 a frequency 0.029, due to the low frequency of the *CSN1S1*B* allele (0.063) in the
545 analyzed breed. Other two haplotypes were quite frequent: 0_1*-C-B-A* (0.194) and *F-*
546 C_1*-F-A* (0.171). Other two haplotypes carrying the weak *CSN1S1*F* allele associated

547 with *CSN2*C1* complete the list of the haplotypes occurred with a frequency higher
548 than 0.05: *F-C$_1$-F-B* (0.061) and *F-C$_1$-F-C* (0.051). The 5 haplotypes represent more
549 than 76% of the casein cluster variability in the *Fiurinà* breed. Interestingly the
550 *CSN1S1*F* allele was found always in association with the *CSN2*C$_1$* allele and the
551 association of the *CSN1S2*B* allele with the *CSN1S1*0$_1$* allele, previously described
552 in the Frisa Valtellinese breed (Caroli et al., 2006), was confirmed also in *Fiurinà*.
553 The individual heterozygous for the *CSN2*0'* allele carries the haplotypes *A-0'-A-*
554 *A/F-C$_1$-F-B* with a probability of 0.963, thus including the *CSN2*0'* allele in a
555 haplotype combination never described before.

556 The *Fiurinà* breed is characterized by an interesting and wide variability in the
557 casein cluster, represented by alleles and haplotypes spread in different breeds (i.e., *F-*
558 *C-F-A*, *F-C-F-B*, *E-A-A-B* haplotypes), rarely found in other breeds (i.e., *F-C-F-C* in
559 the White Shorthaired, as described by Sztankóová et al. in 2009 or F-C-C-B in the
560 Bunte Deutsche Edelziege, as described by Küpper et al. in 2010), and detected only
561 in the *Fiurinà* breed (i.e., *A-C-F-C'*, *E-A-C-B*, *F-C$_1$-F-C'* haplotypes). Haplotypes
562 frequencies found in *Fiurinà* were also compared with haplotypes frequencies of three
563 breeds reared in the Lombardy region (Frisa Valtellinese, Orobica, and Verzaschese),
564 one in the Tuscan region (Garfagnina), and the Italian cosmopolitan Camosciata delle
565 Alpi breed analyzed in previous studies (Caroli et al., 2006; Martini et al., 2010).
566 Only alleles with a frequency higher than 0.01 were considered for the haplotype
567 reconstruction and *CSN2*C* and *CSN2*C1* were considered together because in all
568 but the *Fiurinà* and Garfagnina breeds no test for the *CSN2*C1* was carried out. As it
569 can be seen in Figure 4, the Principal Component Analysis clearly separates *Fiurinà*
570 from all the other breeds. The first three principal components (Prin1, Prin2 and
571 Prin3) accounted for the 0.33, the 0.31 and the 0.16 of the variability, respectively.

572 Both the presence of rare and unique haplotypes in the *Fiurinà* breed and the results
573 of the Principal Component Analysis confirm the genetic uniqueness of this breed
574 found in the genetic diversity analysis carried out in the present study.

575 The balanced frequency of medium-strong and weak-null *CSN1S1* alleles in
576 *Fiurinà* could be exploited for different breeding strategies. Because almost all
577 *Fiurinà* milk is currently processed into cheese, the cheesemaking aptitude could be
578 improved by selecting haplotypes carrying medium-strong alleles. Otherwise, the
579 selection of haplotypes carrying weak-null alleles could be used for fresh milk
580 consumption. Indeed, even if further studies will be needed to clearly assess the
581 relationship between goat casein genotypes and milk protein tolerability, it has
582 already been proven that particular *CSN1S1* genotypes carrying null or weak alleles
583 can reduce the intolerance of allergic subjects in specific cases (Ballabio et al., 2011).
584
585

586 **CONCLUSIONS**

587 Despite the limited number of *Fiurinà* goats, the results of the present paper
588 support the increasing interest of local farmers towards this native goat population.
589 The genetic uniqueness of the *Fiurinà* breed was found with genetic diversity analysis
590 and also supported by the presence of rare and unique haplotypes discovered in
591 caseins polymorphism analysis. The adaptability to the native territories as well as its
592 ability of exploiting local feed resources, are promising aspects that can be improved
593 through an appropriate breeding selection. Moreover, the appreciable milk yields, the
594 average gross composition, and the good fatty acids profile, characterized by a
595 remarkable amount of α-linolenic acid, are interesting results for dairy products made
596 with *Fiurinà* milk.

The results of the present study constitute a preliminary but fundamental step in the rescuing process of this autochthonous goat breed. An important result in the success of this process was the recognition by Regional authority. In a recent modification of the Rural Development Programme (Regione Piemonte, 2012) the *Fiurinà* goat has been inserted in the list of the threatened breeds and breeder can now receive financial support for their *Fiurinà* heads.

The *in situ* preservation program of the *Fiurinà* goats can help the safeguard of this population and give real economic opportunities to local farmers, consequently promoting the native territories with their traditions.

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875

875 **Figure captions**

876 Figure 1. A small flock of *Fiurinà* goats.

877

878 Figure 2. Nucleotide substitutions detected in 10 *Fiurinà* goat mtDNA sequences
879 (481-bp fragment) compared to the reference sequence (GenBank NC_005044
880 abbreviated as REF). The positions are given with respect to the reference sequence.
881 Dots (.) denote identical sites.

882

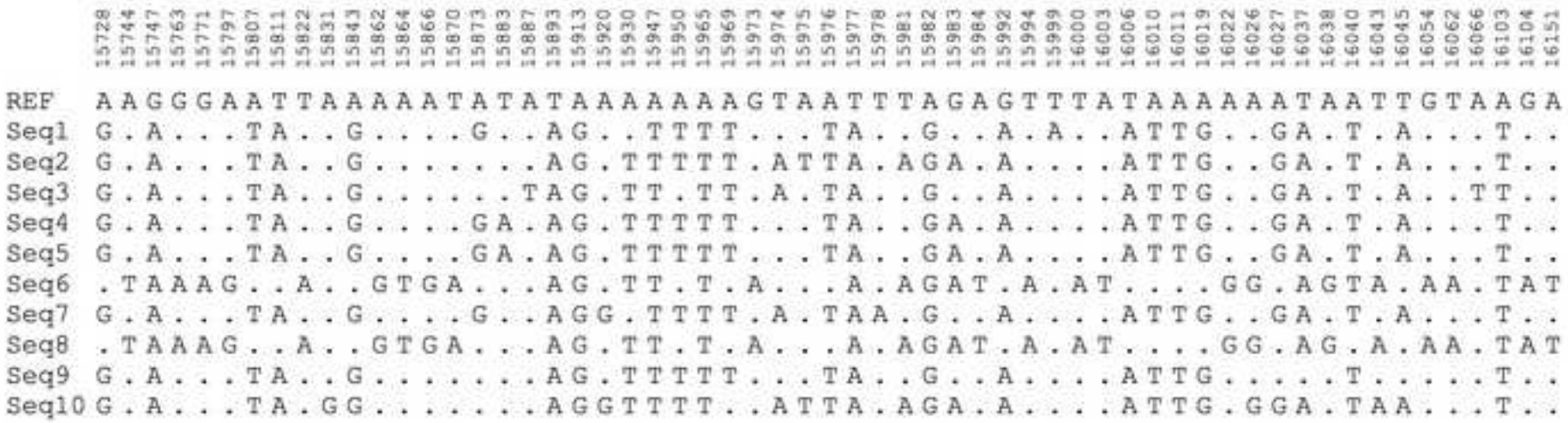
883 Figure 3. Neighbour-joining tree of domestic goat based on 10 mtDNA sequences of
884 the *Fiurinà* breed and on 22 reference mtDNA haplotypes (Ref. Seq.). Distances were
885 calculated using Kimura 2-parameter with 1,000 bootstrap replications.

886

887 Figure 4. Principal Component Analysis of the haplotypes frequencies in *Fiurinà* and
888 other five Italian goat breeds.

Figure 1 - color - on the Web only

Figure 2



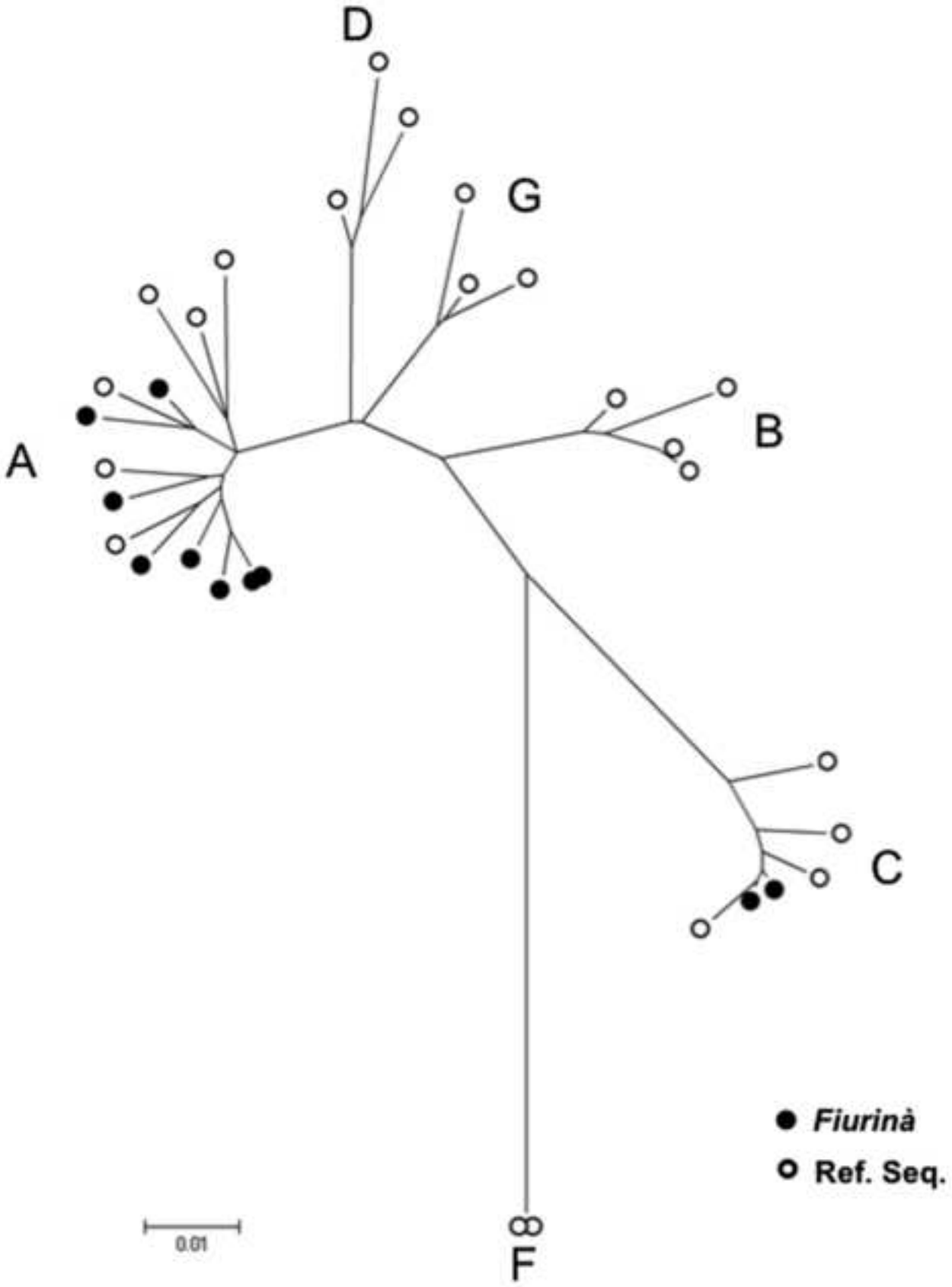


Figure 4

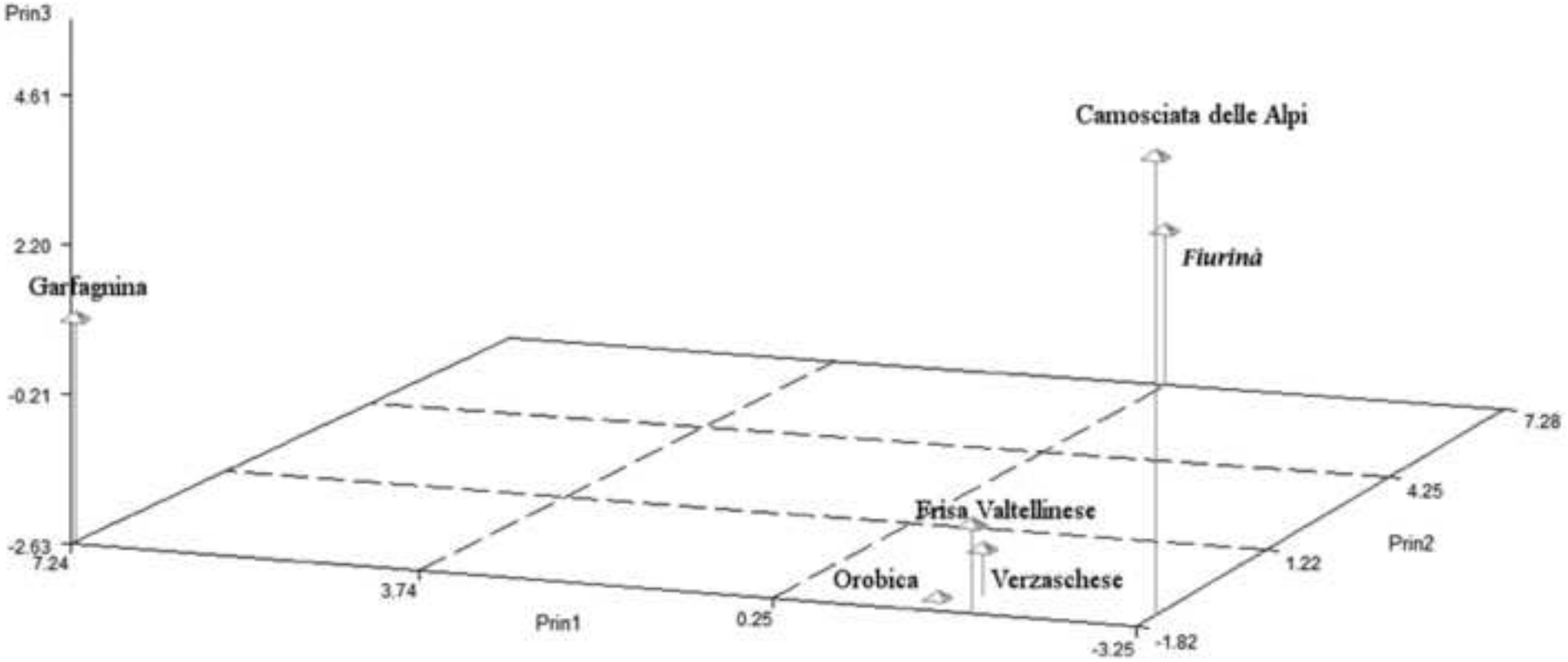

Prin3
4.61
2.20
-0.21
-2.63
Camosciata delle Alpi
Fiurinà
Garfagnina
Frisa Valtellinese
Orobica
Verzaschese
7.24
3.74
0.25
-3.25
Prin1
7.28
4.25
1.22
-1.82
Prin2

Table 1. Analyses applied to screen most of the genetic variability of goat caseins at the DNA level. A slash in the allele list indicates that they are not discernable with the method applied. The analyses available to separate part of the alleles grouping together within the first screening method are in the fourth column.

Gene[a]	DNA method[b]	Discernable alleles	Further analysis and discernable alleles
CSN1S1	PCR-SSCP[b] (Küpper et al., 2010)	A/0_1, A', B/E, B', F, N	AS[b]-PCR - B, E (Jansà-Pérèz et al., 1994) AS-PCR - A, 0_1 (Cosenza et al., 2003)
CSN2	PCR-SSCP (Chessa et al., 2008b)	A, C, A_1, C_1, E, 0, 0'	
CSN1S2	PCR-SSCP (Chessa et al., 2008a)	A/D/0/F, B, C, E	PCR-RFLP - A, D, 0 (Ramunno et al. 2001b) PCR-RFLP - A, F (Ramunno et al. 2001a)
CSN3	PCR-SSCP (Prinzenberg et al., 2005)	A, B/B', B", C, C', E, D/I/K/L, G, H, J, M	

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

[2]Methods: SSCP = Single Strand Conformation Polymorphism; AS = Allele Specific.

Table 2. Average morphometric data[a] of adult females in the *Fiurinà* breed and in other goat breeds reared in the same Italian region.

	Fiurinà	Camosciata delle Alpi[b]	Saanen[b]	Sempione[b]	Vallesana[b]
Height at withers (cm)	73±6.1	74	74	72	75
Rump height (cm)	75±4.8	77	77	74	78
Rump width (cm)	17±3.2	19	20	-	17
Trunk length (cm)	77±1.9	85	89	82	87
Chest girth (cm)	87±6.4	91	96	88	95
Chest height (cm)	34±7.8	34	35	-	-
Weight (kg)	50±12.9	70	60	60	70

[a]Standard deviations are reported only for *Fiurinà* breed since for the other considered breeds only mean values of morphometric data are available in the literature.

[b]Noè et al. (2005).

Table 3. Basic information, values, and significance for parameters[a] of microsatellites polymorphism observed in the *Fiurinà*, Sempione, and Vallesana breeds.

Breed	Approximate breed size	Sample size	H_O	H_E	AR	F_{IS}
Fiurinà	150	26	0.59	0.63	6.19	0.05
Sempione	155	22	0.66	0.69	6.41	-0.01
Vallesana	100	36	0.69	0.70	5.80	0.12 ***

[a]H_O = observed heterozygosity; H_E = expected heterozygosity; AR = allelic richness; F_{IS} = heterozygote deficiency coefficient.

Probability: ***$P < 0.001$.

Table 4. F-statistics F_{IS}, F_{ST}, F_{IT} and their P-values calculated for each microsatellite in the *Fiurinà*, Sempione, and Vallesana breeds.

Locus	F_{IS}	F_{ST}	F_{IT}
INRA005	-0.008	-0.003	-0.011
MAF65	0.002	0.016	0.018
INRA063	-0.006	0.002	-0.004
MCM527	0.187 ***	0.029	0.211 ***
ET10	-0.086	0.026	-0.057
SRCRSP5	-0.003	0.009	0.006
INRA023	-0.017	-0.001	-0.018
FCB20	0.140	0.025	0.162 ***
TGLA53	-0.021	0.021	-0.001
SRC247	-0.048	0.042 ***	0.088 *
CSRD247	0.320 ***	0.026	0.338 ***
ILST87	0.258 ***	0.119 ***	0.347 ***
Average	0.076 ***	0.029 ***	0.103 ***

Probability: *$P < 0.05$; ***$P < 0.001$.

Table 5. Reynolds's distance (above the diagonal) and pairwise comparisons using F_{ST} estimates (below the diagonal) among the *Fiurinà*, Sempione, and Vallesana breeds based on the microsatellites loci.

	Fiurinà	Sempione	Vallesana
Fiurinà	-	0.062	0.055
Sempione	0.041***	-	0.031
Vallesana	0.038***	0.013*	-

Probability: *$P < 0.05$; ***$P < 0.001$.

Table 6. Main constituents and somatic cell count in milk from *Fiurinà* goats.

	Mean	SD
Fat (g kg^{-1})	35.8	7.4
Solids-non-fat (g kg^{-1})	80.5	3.7
Lactose (g kg^{-1})	44.1	2.4
Protein (g kg^{-1})	29.9	3.0
Casein (g kg^{-1})	24.5	2.3
Urea (mg dL^{-1})	34.66	7.65
Somatic cell count[a] ($\times 10^3$ cells mL^{-1})	490.00	(217.00 – 917.25)

[a] Results are expressed as median and interquartile range.

Table 7. Individual fatty acids (g $100g^{-1}$ fat and % of total FAME[a]) in milk from *Fiurinà* goats.

	g $100g^{-1}$ fat		% total FAME	
	mean	SD	mean	SD
C4	2.29	0.32	2.91	0.41
C5	<0.01	<0.01	<0.01	<0.01
C6	2.23	0.27	2.85	0.46
C7	0.02	0.01	0.02	0.01
C8	2.43	0.40	3.12	0.62
C10	7.35	1.34	9.39	1.84
C10:1	0.20	0.06	0.26	0.08
C12	2.88	0.66	3.68	0.87
C13 *iso*	0.02	0.01	0.02	0.01
C13 *aiso*	0.02	0.01	0.03	0.02
C12:1 *c* + C13	0.10	0.03	0.13	0.04
C14 *iso*	0.07	0.04	0.09	0.05
C14	7.31	1.24	9.30	1.49
C15 *iso*	0.17	0.06	0.21	0.08
C14:1 *t*	<0.01	<0.01	<0.01	<0.01
C15 *aiso*	0.30	0.10	0.38	0.11
C14:1 *c*	0.07	0.03	0.09	0.04
C15	0.80	0.17	1.02	0.20
C16 *iso*	0.18	0.07	0.22	0.08
C16	19.02	3.10	24.10	3.14
C17 *iso*	0.37	0.14	0.47	0.16
C16:1 *t*	0.11	0.05	0.13	0.05
C17 *aiso*	0.61	0.13	0.77	0.13
C16:1 *c*	0.35	0.09	0.44	0.10
C17	0.71	0.15	0.89	0.15
C18 *iso*	<0.01	<0.01	<0.01	<0.01
C17:1 *t*	0.05	0.03	0.06	0.03
C18 *aiso*	0.27	0.11	0.34	0.14
C18	9.12	2.33	11.54	2.63
C18:1 *t*5	<0.01	<0.01	<0.01	<0.01
C18:1 *t*6 - 11	1.98	0.71	2.51	0.88
C18:1 *t*12 - 14 + *c*6 - 8	0.71	0.28	0.89	0.34
C18:1 *c*9	13.94	2.79	17.65	3.03
C18:1 *c*11	0.37	0.09	0.47	0.10
C18:1 *c*12	0.13	0.04	0.16	0.05
C18:1 *c*14 + *t*16	0.38	0.12	0.49	0.14
C19	0.11	0.03	0.13	0.04
C18:2 *t*,*t* - NMID + *t*9*t*12	0.11	0.04	0.14	0.05
C18:2 *c*9*t*13 + *t*8*c*12	0.05	0.03	0.06	0.03
C18:2 *c*9*t*12	0.22	0.09	0.28	0.11
C18:2 *c*,*c* - MID + *t*8*c*13	0.22	0.08	0.27	0.09
C18:2 *t*11*c*15 + *t*9*c*12	0.24	0.09	0.30	0.11
C18:2 *c*9*c*12 (LA)	1.17	0.32	1.47	0.35
C18:2 *c*9*c*15	0.02	0.02	0.02	0.02
C20	0.26	0.13	0.33	0.15
C20:1 *t*	0.01	0.01	0.01	0.01
C18:3 *c*6*c*9*c*12 (GLA)	0.01	0.01	0.01	0.01
C20:1 *c*5	0.01	0.01	0.02	0.01

C20:1 *c*9	0.03	0.02	0.04	0.03
C20:1 *c*11	0.03	0.02	0.03	0.02
C18:3 *c*9*c*12*c*15 (ALA)	0.82	0.26	1.02	0.28
CLA *c*9*t*11 + *t*7*c*9 + *t*8*c*10	0.61	0.25	0.77	0.31
CLA *t*11*c*13 + *c*9*c*11	0.02	0.02	0.02	0.02
CLA *t*9*t*11	0.01	0.01	0.01	0.01
C20:2 *c*,*c* n6	0.01	0.01	0.01	0.01
C22	0.04	0.03	0.05	0.03
C20:3 n6	0.01	0.01	0.01	0.01
C20:3 n3	0.01	0.01	0.01	0.01
C20:4 n6 (AA)	0.08	0.04	0.09	0.05
C20:5 n3 (EPA)	0.06	0.03	0.07	0.03
C22:5 n3 (DPA)	0.09	0.05	0.11	0.06
C22:6 n3 (DHA)	0.03	0.03	0.04	0.04

[a]FAME = fatty acid methyl esters; SD = standard deviation; *c* = *cis*; *t* = *trans*; NMID = non methylene interrupted diene; MID = methylene interrupted diene; LA = linoleic acid; GLA = γ-linoleic acid; ALA = α-linoleic acid; CLA = conjugated linoleic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

Table 8. Groups of fatty acids (g $100g^{-1}$ fat and % of total FAME[a]) in milk from *Fiurinà* goat.

	g $100g^{-1}$ fat		% total FAME	
	mean	SD	mean	SD
Σ short chain[b]	14.52	1.94	18.55	2.93
Σ medium chain[c]	32.39	4.44	41.10	4.53
Σ long chain[d]	31.85	5.82	40.30	5.85
Σ saturated[e]	56.59	4.88	71.88	3.88
Σ branched chain[f]	2.01	0.50	2.54	0.56
Σ monounsaturated[g]	18.47	3.22	23.39	3.34
Σ C18:1[h]	17.51	3.16	22.18	3.31
Σ C18:1 *trans*[i]	2.70	0.88	3.42	1.06
Σ polyunsaturated[j]	3.75	0.95	4.72	0.98
Σ C18:2[k]	2.65	0.66	3.35	0.70
Σ C18:2 *trans*[l]	1.47	0.47	1.86	0.55
Σ *trans* without CLA[m]	6.77	2.06	8.58	2.45
Σ n3 FA[n]	1.25	0.39	1.57	0.42
Σ n6 FA[o]	2.47	0.56	3.08	0.59
Σ n6 / Σ n3	2.08	0.61	2.68	0.91
Σ CLA[p]	0.64	0.27	0.81	0.33
Σ unsaturated[q]	22.21	3.90	28.12	3.88

[a] FAME = fatty acid methyl esters; SD = standard deviation; CLA = conjugated linoleic acid; FA = fatty acids.

[b] C4, C5, C6, C7, C8, C10, C10:1.

[c] C12, C13 *iso*, C13 *aiso*, C12:1 *c* + C13, C14 *iso*, C14, C15 *iso*, C14:1 *t*, C15 *aiso*, C14:1 *c*, C15, C16 *iso*, C16, C17 *iso*, C16:1 *t*, C17 *aiso*, C16:1 *c*.

[d] C17, C18 *iso*, C17:1 *t*, C18 *aiso*, C18, Σ C18:1, C19, Σ C18:2, C20, C20:1 *t*, C18:3 *c*6*c*9*c*12, C20:1 *c*5, C20:1 *c*9, C20:1 *c*11, C18:3 *c*9*c*12*c*15, C18:2 *c*9*t*11 + *t*7*c*9 + *t*8*c*10, C18:2 *t*11*c*13 + *c*9*c*11, C18:2 *t*9*t*11, C20:2 *c*,*c* n6, C22, C20:3 n6, C20:3 n3, C20:4 n6, C20:5 n3, C22:5 n3, C22:6 n3.

[e] C4, C5, C6, C7, C8, C10, C12, Σ branched chain, C14, C15, C16, C17, C18, C19, C20, C22.

[f] C13 *iso* + *aiso*, C14 *iso*, C15 *iso* + *aiso*, C16 *iso*, C17 *iso* + *aiso*, C18 *iso* + *aiso*.

[g] C10:1, C12:1 *c* + C13, C14:1 *ct*, C16:1 *ct*, C17:1 *t*, Σ C18:1, C20:1 *t*, C20:1 *c*5, C20:1 *c*9, C20:1 *c*11.

[h] C18:1 *t*5, *t*6 - 11, *t*12 - 14 + *c*6 - 8, *c*9, *c*11, *c*12, *c*14 + *t*16.

[i] C18:1 *t*5, *t*6 - 11, *t*12 - 14 + *c*6 - 8.

[j] Σ C18:2, C18:3 *c*6*c*9*c*12, C18:3 *c*9*c*12*c*15, C20:2 *c*,*c* n6, C20:3 n3, C20:3 n6, C20:4 n6, C20:5 n3, C22:5 n3, C22:6 n3.

[k] C18:2 *t*,*t* - NMID + *t*9*t*12, *c*9*t*13+*t*8*c*12, *c*9*t*12, *c*,*c* - MID + *t*8*c*13, *t*11*c*15 + *t*9*c*12, *c*9*c*12, *c*9*c*15, *c*9*t*11 + *t*7*c*9 + *t*8*c*10, *t*11*c*13 + *c*9*c*11, *t*9*t*11.

[l] C18:2 *t*,*t* - NMID + *t*9*t*12, *c*9*t*13 + *t*8*c*12, *c*9*t*12, *c*,*c* - MID + *t*8*c*13, *t*11*c*15 + *t*9*c*12, C18:2 *c*9*t*11 + *t*7*c*9 + *t*8*c*10, C18:2 *t*11*c*13 + *c*9*c*11, C18:2 *t*9*t*11.

[m] C14:1 *t*, C16:1 *t*, C17:1 *t*, Σ C18:1 *t* , Σ C18:2 *t* (without CLA *trans*), C20:1 *t*.

[n] C18:2 *t*11*c*15 + *t*9*c*12, C18:2 *c*9*c*15, C18:3 *c*9*c*12*c*15, C20:3 n3, C20:5 n3, C22:5 n3, C22:6 n3.

[o] C18:1 *t*12, C18:1 *c*12, C18:2 *t*,*t* - NMID + *t*9*t*12, C18:2 *c*9*t*12, C18:2 *t*11*c*15 + *t*9*c*12, C18:2 *c*9*c*12, C18:3 *c*6*c*9*c*12, C20:2 *c*,*c* n6, C20:3 n6, C20:4 n6.

[p] C18:2 *c*9*t*11 + *t*7*c*9 + *t*8*c*10, *t*11*c*13 + *c*9*c*11, *t*9*t*11.

[q] C10:1, C12:1 *c* + C13, C14:1 *ct*, C16:1 *ct*, C17:1 *t*, Σ C18:1, Σ C18:2, C20:1 *t*, C18:3 *c*6*c*9*c*12, C20:1 *c*5, C20:1 *c*9, C20:1 *c*11, C18:3 *c*9*c*12*c*15, C18:2 *c*9*t*11 + *t*7*c*9 + *t*8*c*10, C18:2 *t*11*c*13 + *c*9*c*11, C18:2 *t*9*t*11, C20:2 *c*,*c* n6, C20:3 n6, C20:3 n3, C20:4 n6, C20:5 n3, C22:5 n3, C22:6 n3.

Table 9. Casein allele and haplotype frequencies in the *Fiurinà* breed. Haplotype frequencies were calculated both under hypothesis of loci independence (H0) and taking association into account (H1) by the HAPLOTYPE procedure of SAS (SAS Institute Inc., 2008). Only haplotypes with H1 higher than 0.008 are shown.

Casein gene[a]	Allele	Frequency	*CSN1S1*	*CSN2*	*CSN1S2*	*CSN3*	H0	H1
CSN1S1	*A*	0.052	*E*	*A*	*A*	*B*	0.033	0.310
	A'	0.010	0_1	*C*	*B*	*A*	0.003	0.172
	B	0.063	*F*	C_1	*F*	*A*	0.017	0.171
	B'	0.010	*F*	C_1	*F*	*B*	0.022	0.061
	E	0.333	*F*	C_1	*F*	*C*	0.002	0.051
	F	0.333	*E*	*A*	*F*	*B*	0.028	0.034
	0_1	0.198	*B*	*A*	*A*	*B*	0.006	0.029
CSN2	*A*	0.406	*F*	C_1	*A*	*B*	0.027	0.023
	C	0.219	*A*	*C*	*A*	*B*	0.003	0.023
	C_1	0.365	0_1	C_1	*B*	*A*	0.005	0.022
	0'	0.010	*A*	*C*	*A*	*A*	0.002	0.020
CSN1S2	*A*	0.388	*B*	*A*	*C*	*B*	0.001	0.020
	B	0.204	*B*	*A*	*A*	*A*	0.004	0.013
	C	0.041	*F*	C_1	*F*	*C*'	0.001	0.012
	F	0.367	*E*	*A*	*C*	*B*	0.003	0.012
CSN3	*A*	0.388	*A*	*C*	*F*	*B*	0.002	0.010
	B	0.541	*F*	C_1	*C*	*B*	0.003	0.009
	C	0.051	*A*	*C*	*F*	*C*'	0.000	0.008
	C'	0.020						

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