
Bioactive Compounds in Goat Milk and Cheese: The Role of Feeding System and Breed

Salvatore Claps, Roberta Rossi, Adriana Di Trana,
Maria Antonietta di Napoli, Daniela Giorgio and
Lucia Sepe

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Abstract

This chapter provides an introductory overview of some bioactive compounds in goat milk, presenting a selection of key results from literature. The aim of the chapter is to review the effects of the feeding system and of the breed on goat milk and cheese fine quality in order to identify management options aimed at improving the nutraceutical characteristics of milk and dairy products. We will discuss a series of case studies focused on the assessment of the effects of feeding system and breed and their interaction on specific health-promoting bioactive compounds: (i) fatty acid (FA) profile, (ii) antioxidant compounds and (iii) oligosaccharides (OS). Experimental data will be discussed highlighting the potential role of local Mediterranean breeds for the production of functional dairy products.

Keywords: bioactive compounds, feeding system, goat, Mediterranean breeds, fatty acids, antioxidants, oligosaccharides, milk, cheese

1. Introduction: overview on the main bioactive compounds of goat milk

Bioactive compound, according to the National Cancer Institute (USA), is “one type of chemical food in small amounts in plants and certain foods (such as fruits, vegetables, nuts, oils, and whole grains). Bioactive compounds have actions in the body that may promote good health. They are being studied in the prevention of cancer, heart disease, and other diseases”. By [1], a bioactive compound is “a compound which has the capability and the ability to interact with one or more component(s) of the living tissue by presenting a wide range of probable effects”. The origin of these substances can be natural—terrestrial or aquatic, a plant, animal or other

source (e.g., microorganisms)—or synthetic. The term “bioactive compound” is not attributed to the nutrient contained in food or, more broadly, to the nutrients that are essential for a living organism, such as primary metabolites.

Controversies over except the essential elements of the definition of bioactive compounds arise for food (or source of nutrition in general), where food constituents include water, carbohydrate, proteins, lipids and fatty acids, fibres, vitamins, minerals and oligo-elements. Ref. [2] consider that bioactive peptides, many vitamins, fatty acids, flavonoids and phytosterol and the soluble and insoluble fibres are bioactive compounds. Examples of bioactive compound include lycopene, resveratrol, lignan, tannins and indoles.

In recent years, functional foods and bioactive components in foods have drawn a lot of attention and interest among food scientists, nutritionists, health professionals and general consumers. A functional food may be similar in appearance to a conventional food; it is consumed as a part of normal diet but has various physiological benefits and can reduce the risk of chronic diseases beyond basic nutritional functions.

Goat milk (GM) when compared to cow milk in terms of fatty acid (FA) profile shows a larger content of medium-chain fatty acids (MCFA) such as caproic (C6:0), caprylic (C8:0) and capric (C10:0), which can be considered bioactive compounds [3]. These three fatty acids that alone represent up to 15–18% of total FA in goat milk and not more than 9% in cow milk, due to their great energy giving facility, play a key dietary role in improving lipid metabolism, especially in patients suffering from various forms of malabsorption syndromes, typically developed after intestine resection, in rehabilitating premature and undernourished infants [4]. Dietary GM improves iron bioavailability favouring the recovery of haematological parameters [5]. GM contributes to restore bone demineralisation associated to anaemia by increasing the digestive and metabolic utilisation of calcium and phosphorus. Its consumption has beneficial effects on nutritive utilisation of iron and copper [6].

The role of polyunsaturated fatty acid (PUFA), and in particular conjugated linoleic acid (CLA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA), has received much attention of nutritionists in the last 10 years. The n-3 fatty acids (i) reduce total cholesterol and low-density lipoprotein cholesterol (LDL) levels but increase high-density lipoprotein (HDL) cholesterol, (ii) counteract hypertension, (iii) play a role in the regulation of hormonal secretion and (iv) are beneficial in the care of skin pathologies and are also useful in the therapy of arthritis and other inflammatory problems.

The acronym CLA is used to express the mixture of isomers of the linoleic fatty acid with double conjugated bonds, located, above all, on the atoms of carbons 9 and 11. Biological activity is mainly attributed to rumenic acid (C18:2 cis 9, trans 11), which represent about 90% of the total isomers present in the fat of ruminants [7]. The CLA in milk has two origins: from the rumen biohydrogenation of unsaturated fatty acids, present in substantial quantities in fresh forage, and from the synthesis in animal tissue, mainly the mammary gland and adipose tissue, starting with the vaccenic acids (VA) through the action of the delta 9-desaturase enzyme.

The amount of these biologically active molecules in milk and cheese fat from ruminants is affected by animal diets [8]. There have been studies about grazing based on shrub and woody lands affecting CLA and VA content in milk and cheese from sheep and goats [9] also with regard to specific forage species in the pasture [10]. Nevertheless, our knowledge on the effects of common Mediterranean forage species, agronomic management, forage conservation and breed on goat milk bioactive fractions is still limited.

Recently, another topic of interest has been the antioxidant content in milk and cheeses. In milk there are several antioxidant compounds which can be classified as enzymatic antioxidant and non-enzymatic antioxidant. Among antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidases have been demonstrated in milk. Non-enzymatic antioxidants, lactoferrin, vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols), carotenoids and polyphenols can be formed in the animal body or need to be supplied in the feed as essential nutrients [11, 12]. Several non-enzymatic antioxidants act as radical scavengers in the lipid phase, such as vitamin E, carotenoids and ubiquinol, whereas vitamin C acts in the water phase. Others can react in both the lipid and the water phase, such as some polyphenols (flavonoids), which operate both as radical scavengers and metal ion binders [12]. The parameters that are taken more into consideration are the beta-carotene and α -tocopherol content and the level of protective antioxidants. A molecule is recognised as an antioxidant when it is able to slow down, or hinder, oxidising processes against certain substances. A synthetic index of this capability is represented by the degree of antioxidant protection (DAP) [13]. The DAP is calculated as ratio between the amount antioxidant element (e.g., α -tocopherol) and the element to be protected against oxidation (cholesterol).

Increasing α -tocopherol in milk is important not only to enhance its nutritive value but also to prevent lipid oxidation which leads to rancidity of milk and dairy products; vitamin E supplementation is a standard practice in most farming systems. Milk tocopherol content depends on several factors such as breed, feed and stage of lactation; large differences exist among ruminant species and within species.

Very little is known on the effect of diet on the content of non-volatile phenolic compounds in milk or cheese. The results of a few recent studies demonstrate the accumulation of various phenolic compounds in the milk of grazing goats [14, 15]. High content of phenols in milk has shown to improve the quality of milk, such as its oxidative stability of the process' efficiency and quality of dairy products [16].

The interest towards drinking goat milk is increasing, due to the recognised nutritional properties of this milk in comparison with cow milk [17, 18]. A class of bioactive compounds recently rose to major interest, namely, the oligosaccharides [19], due to their beneficial effects on human health as intestinal inflammation [20] and on brain development and immunity in infants [21]. The content of goat oligosaccharides (OS) compared to other domestic ruminants milk is about 4–5 times higher than cow milk and up 10 times than sheep milk [22]. The scarce availability of those from human milk encouraged to deepen studies on these bio-compounds. The studies showed OS content and profile in goat milk most similar to breast milk in comparison to other farm mammals, in particular as far as fucosylated and sialylated OS to human milk oligosaccharides [23], as to suggest, by several authors, the use in the production of products for human nutrition, such as infant formulae.

This chapter provides an overview of the main bioactive compounds in milk and goat cheese (fatty acids, antioxidant and oligosaccharides) conveying data from significant case studies carried out at the experimental farm of Council for Agricultural Research-Unit for Extensive Husbandry (CREA-ZOE), located in Bella (Muro Lucano, Potenza), Basilicata region (Southern Italy).

2. Feeding strategies affecting the bioactive compounds in milk and cheese

2.1. Fatty acids affected by feeding regimen

Nutrition is a natural and low-cost way for farmers to rapidly and sharply modulate milk and cheese FA profile towards a healthy profile [24]. The composition of milk fat reflects to some extent the composition of the dietary fat, despite the hydrogenation and isomerisation process to which the FA may be subjected in the rumen. Forages, even though containing a relatively low level of lipids, are often the major source of beneficial unsaturated fatty acids in ruminant diets, and they also provide a low-cost approach to improve milk FA profile in comparison with diet supplementation strategies. In literature, several studies have focused on the impact of different diets on the main milk FA classes, and they also have examined the associations between feeding of various forages and FA composition of milk fat.

Among forages, legumes deserve a special attention due to the raising number of farmers in conversion to organic and low-input production system (i.e. the environmental role of legumes in cropping systems has been even enhanced in Europe by common agricultural policy (CAP) reform) but also to the need of reducing the dependence on the import of protein-rich feed material. Even if, for a given crop, substantial within-species variation occurs, altogether some legume forages such as white clover and birdsfoot trefoil can be considered a rich source of PUFA [25]. Birdsfoot trefoil PUFA content (19.4 g/kg DM) was found higher than in many other legumes, grasses and forbs [26], while white clover with an average ALA content of 16 mg/g DM was a richer source than other common forage legumes (alfalfa, trefoil and red clover) and grasses (orchard grass, fescue and timothy) [27]. Fresh grass is the one main source of ALA. It has been recognised that favourable changes in milk FA profile can be obtained by grazing or feeding fresh forages. Several studies have shown that milk from grazing goats is naturally enriched in fatty acids considered as favourable for human health in comparison to goats fed with high-concentrate diets [24, 28].

Goats unlike sheep are predominantly browsers; in Mediterranean shrublands browse can account for up to 60–80% of goat's diet; animals well adapted to tannin-rich woody forage sources can consume relatively large amounts of tannins without suffering any systemic toxicity [29]. While tannin content in forages is negatively correlated with voluntary intake, digestibility and nitrogen retention, a relatively low amount in ruminant diet can positively affect milk FA composition by protecting dietary PUFA against rumen biohydrogenation [30]. Many forage legumes such as clovers, vetches and Sulla (*Hedysarum coronarium*) are a rich source of polyphenols and especially tannin phenols (TP). Ref. [31] observed that condensed tannins (CT) in Sulla

at flowering were contributing to a higher concentration of linoleic and ALA in ewe's milk and a lower $\omega 6/\omega 3$ ratio. Refs. [32, 33] showed that red clover silage, which contained levels of ALA similar to that of grass silage, improved milk PUFA due to a high proportion of red clover ALA passing through the rumen. Polyphenol oxidase (PPO), which is the enzyme involved in the browning reaction of red clover leaves when cut or crushed and exposed to air, has been found to reduce protein and lipid degradations *in vitro* and potentially in the rumen [34].

Overall, this brief overview on the role of feeding regimen in modulating goat milk fatty acid profile shows that both farm-grown forages including legumes, as well as native pastures, can be considered an effective low-cost way to improve goat milk FA composition without compromising yield and opening new alleys towards a sustainable intensification of the extensive dairy goat system.

2.1.1. Case study: feeding and season on milk fatty acid profile

In this section we report a study carried out at the experimental farm of Council for Agricultural Research and Economics-Research Unit for the Extensive Animal Husbandry (CREA-ZOE) located in Bella (Basilicata region, Southern Italy) during winter, spring and summer seasons. In order to examine changes in milk FA profile under the effect of different feeding regimes, typical of Mediterranean extensive and semi-extensive goat production systems, four groups of Mediterranean Red goats were formed and allocated to different feeding systems: (G) grazing on a native pasture (8 h/day) without supplementation, (GS1) grazing supplemented with 550 g/d of maize and broad beans (CP 14% and NDF 18%, slowly degradable), (GS2) grazing supplemented with 550 g/d of barley and chickpeas (CP 14% and NDF 18%, rapidly degradable) and (H) housing and fed with hay produced with the grass from the same pasture plus 550 g/d of mixed grains (CP 15% and NDF 18%) [35–38]. Regarding lipid extraction method, briefly milk sample (10 ml) was homogenised (2 min) with CHCl_3 and MeOH mixture (2/1, v/v) and centrifuged (500 × g, 10 min). After removing the upper layer, the lower layer was filtered through a Buchner funnel, rinsed with CHCl_3 (30 ml) and then again filtered. The chloroform-lipid extract was dried over anhydrous Na_2SO_4 , rinsed with CHCl_3 (30 ml) and concentrated using a rotary evaporator at 30°C. The residue was stored at -80°C for lipid determination. Lipid extract was methylated adding hexane (1 ml) and 2 N methanolic KOH (0.05 ml). Gas chromatograph analysis was performed on a Varian model 3800 GC instrument fitted with an automatic sampler (CP 8410) for a multiple injection. Fatty acid methyl esters (FAME) were separated through a cyanopropyl polysiloxane (DB 23, J & W) fused silica capillary column (60 m × 0.25 mm i.d.). Operating conditions were a helium flow rate of 1.2 ml/min, a FID detector at 250°C and a split-splitless injector at 230°C with a split ratio 1:100. The column temperature was held at 60°C for 5 min after sample injection (1 μl), increased at 14°C/min to 165°C and at 2°C/min to 225°C and held at 225°C for 20 min. The individual fatty acid peaks were identified with reference to the retention times of standard of CLA isomers (cis-9, trans-11 97% and trans-10, cis-12 3%; Larodan, Malmö, Sweden) and a known mixture of standards (FAME, Sigma). Fatty acids were expressed as percentage of total FAME.

Milk produced by goat groups showed a wide variability in its FA composition linked to the characteristics of the ingested feed in each type of feeding system (**Figure 1**). In particular,

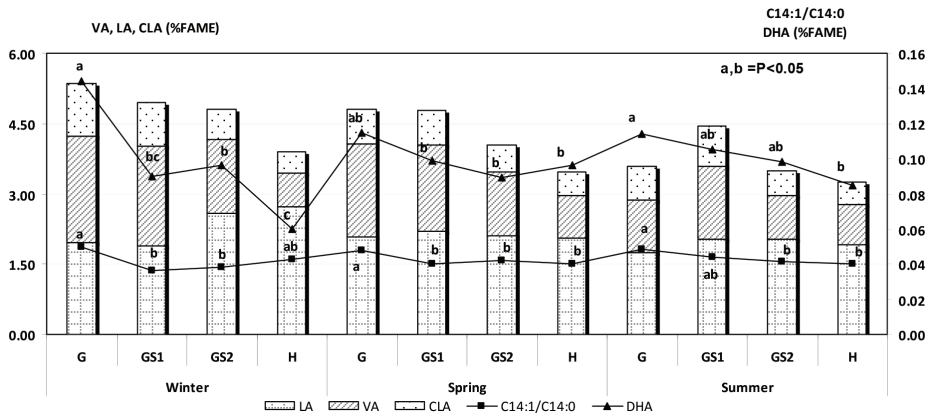


Figure 1. Effect of feeding system \times season interaction on vaccenic acid (VA), linoleic acid (LA), conjugated linoleic acid (CLA) and docosahexaenoic acid (DHA) content and on Δ^9 -desaturase activity (C14:1/C14:0) of milk of goats fed with (G) grazing on native pasture without supplementation, (GS1) grazing plus maize and broad beans (GS2) and grazing plus barley and chickpeas and (H) housed and fed with hay plus mixed grains (modified from Refs. [35–37]). a, b and c = $P < 0.05$.

G and GS1 groups produced milk with a higher content of CLA and VA compared to other groups. Indeed, it is noted that the consumption of high-concentrate diets, compared with high-forage ones, affects the extent of ruminal biohydrogenation with a consequent reduction of CLA and VA production. The high variability of CLA and VA levels in milk of G group, with the highest level reached in winter, could be ascribed to the seasonal changes in grass availability and in phenological stage of the plants. The similar pattern of VA and CLA observed in milk of goat rearing in different feeding systems confirms the positive relationship between these intermediate products of ruminal biohydrogenation. Feeding regimen and season also affected Δ^9 -desaturase activity (C14:1/C14:0), responsible of endogenous synthesis of CLA, with fresh grass being able to enhance this enzyme activity (**Figure 1**).

Grazing significantly increased the proportion of long-chain n-3 PUFA, such as DHA, and decreased the n-6/n-3 PUFA ratio in milk (**Figure 2**). The level of DHA reached interesting value in milk fat of grazing goats in winter probably because of the high content of its precursor (ALA) in the pasture. The ratio between n-3 PUFA and n-6 PUFA is an index commonly used to assess the nutritional value of fats [39]. Housing goats exhibited a higher n-6/n-3 PUFA ratio than other treatments, probably attributable to the high level of LA in milk (**Figure 1**), the main component of n-6 PUFA. The composition of concentrate mixture offered to H group appears to explain the highest content of LA found in milk fat.

The distribution of concentrates to grazing goats significantly affected milk FA profile. Under grazing condition, GS1 dietary treatment characterised by slowly degradable concentrate improved milk FA profile compared to GS2 group fed with rapidly degradable concentrate. Probably the supplementation received by GS1 group could have determined a rumen environment favourable to a less efficient biohydrogenation of substrate with consequent accumulation of intermediate products. Besides, the differences observed in milk FA composition between supplemented and non-supplemented grazing groups could be linked to the different herbage selections of supplemented grazing goats, as suggested in Ref. [40].

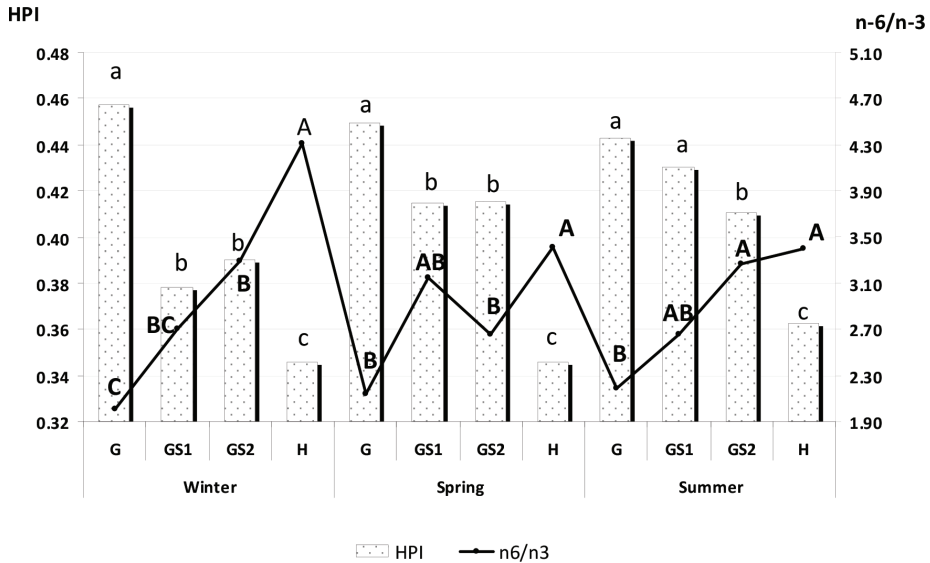


Figure 2. Effect of feeding system \times season interaction on n-6/n-3 and health-promoting index (HPI) value of milk of goats fed with (G) grazing on native pasture without supplementation, (GS1) grazing plus maize and broad beans and (GS2) grazing plus barley and chickpeas and (H) housed and fed with hay plus mixed grains [modified from Ref. [38]]. A, B and C = $P < 0.01$ for n6/n3, a, b and c = $P < 0.05$ for HPI.

The effect of different feeding systems on beneficial FA in milk is more evident using the health-promoting index (HPI, **Figure 2**), an index that expresses the health value of dietary fat, and it is calculated as follows: total unsaturated FA/[C12:0 + (4 \times C14:0) + C16:0] [41]. Dairy product with high HPI value is assumed to be more beneficial to human health. According to this index, pasture feeding allows the optimisation of the balance between detrimental and valuable fatty acids in goat milk, thus obtaining beneficial effects for consumer's health.

The results of this study show that milk from goats fed with pasture had higher amounts of nutritionally peculiar FA than milk from other feeding treatments. On the other hand, grazing supplementation with concentrates that better interact with the nutritive characteristics of pasture could represent a strategy to meet nutritional requirements of animals and sustain milk production without worsening its quality.

2.1.2. Case study: relationship between forage species and fatty acids of cheese

In this section we report a study carried out at the CREA-ZOE experimental farm in spring. Eight homogeneous groups of Red Syrian goats have been allocated to eight different feeding treatments. The housed goat groups received during 11 days a single forage species *ad libitum*, and they had access to water and salt blocks. Daily, the forages were cut and provided to the goat groups. Seven forages (*Avena sativa*, *Lolium perenne*, *Hordeum vulgare*, *Lotus corniculatus*, *Medicago sativa*, *Trifolium incarnatum*, *Vicia sativa*) were used at the phenological stage commonly used in Southern Italy for grazing and one (*Triticosecale*) as silage. After an adaptation period to the forage

supplied, the milk of each group was collected and processed into *Caciotta* cheese, a traditional goat cheese manufactured in Southern Italy, ripened for 20 days. Cheese samples (3 g) were finely grated, and lipid extraction and composition were performed as described in Section 2.1.1.

In this study, forage species had an effect on FA profile of *Caciotta* cheese [42]. As regards CLA (**Figure 3**), cheeses from goat groups fed with *T. incarnatum*, *Triticosecale* and *H. vulgare* showed higher values than those obtained from *A. sativa* and *L. corniculatus*. The lowest content of CLA and the highest content of ALA were detected in the cheese made from milk of goats fed with *V. sativa*. Cheeses from *L. corniculatus* and *M. sativa* displayed the same ALA content. The ALA showed a major variation among cheeses (range 1.04), while the CLA exhibited a smaller variation (range 0.349) [42–44].

Cheeses from legume groups showed significantly higher values of ALA compared with grass groups, whereas the content of ALA in cheese from *Triticosecale* silage was in trend with other fresh grasses. Green fodders are excellent source of ALA and are the most effective feeds in shifting the milk FA profile towards a healthy profile. Fortunately, the milk processing does not change substantially its FA profile [45]; it follows that the bioactive compounds, CLA and ALA, of dairy products are dependent of their content in the unprocessed raw milk [43, 46]. The effects of different forage species on ALA, VA and CLA content in cheeses could be connected to the high content of PUFA in green forage, with ALA being the most representative of this FA class (**Figure 4**), and to the role of secondary metabolites (polyphenols) and vegetable enzymes (polyphenol oxidase) present in forage species. These compounds have potential to interact with lipolysis and biohydrogenation of PUFA *in vitro*, in fact a negative relationship was found between tannic polyphenols/ALA content ratio and ALA biohydrogenation [47]. In our study, the highest content of ALA in cheese from goats fed with *V. sativa* could be linked to the higher level of tannic polyphenols in the forage as reported by Ref. [48].

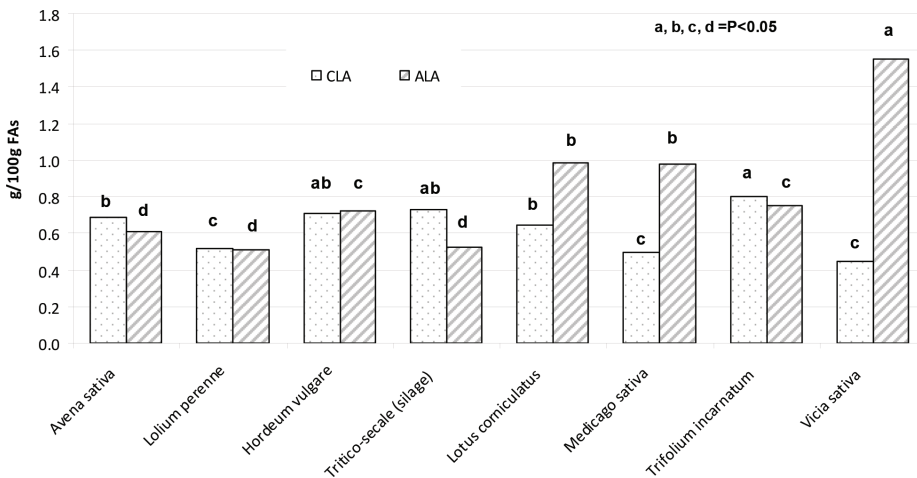


Figure 3. Comparison of conjugated linoleic acid (CLA) and α -linolenic acid (ALA) of *Caciotta* cheese made from milk of goats fed *ad libitum* with a single forage species (modified from Refs. [42–44]). a–d = $P < 0.05$.

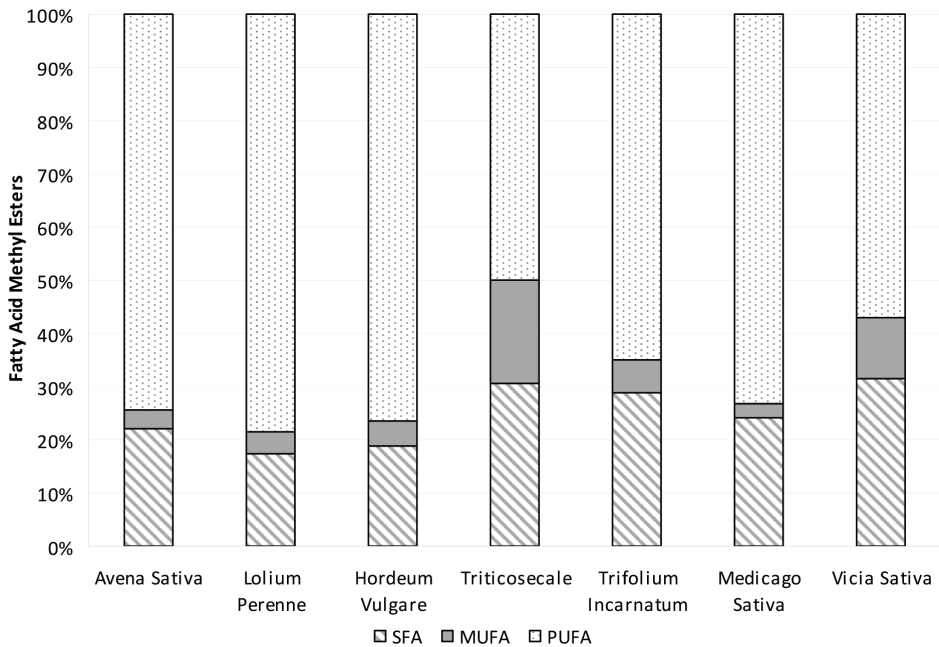


Figure 4. Comparison of percentage content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in fresh forage species (authors' own unpublished data).

The health-promoting index was calculated in order to have an immediate view of the bioactive compounds present in the cheese [41]. The forage species affected the HPI (**Figure 5**). The higher HPI values observed in *Caciotta* cheese from goat groups fed *H. vulgare* and *A. sativa* [43, 44] could be linked to the high level of PUFA found in these forages (**Figure 4**). The HPI observed in other cheeses is still higher compared to those found in milk from animals fed with dry fodder (see Section 2.1.1).

This case study shows that the single forage characterises the bioactive compounds' content in cheese; this result can be a strategy to guide, depending on farm fodder availability, the production of dairy products beneficial to human health.

2.2. Antioxidant compounds in goat milk

Measuring the total antioxidant capacity of milk and cheese helps to understand the relationships between the bioactive compounds present in milk and their ability to protect the substrate. Antioxidant activity can be enhanced by providing food as a source of antioxidant components [16]. The results of a few recent studies show the accumulation of various bioactive compounds biotransformed and/or as such in the milk and cheese of grazing goats or fed with a mixture of forage legume [18, 49]. The high value of total phenolic concentration with added nutritional and sensory values, without changing properties of the cheeses itself, was observed in cheeses made from goats fed with non-distilled thyme leaves, one of the aromatic

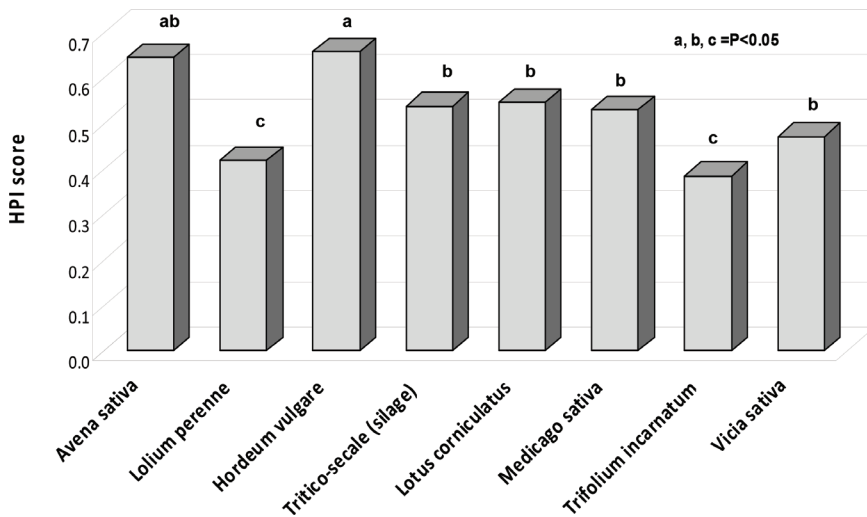


Figure 5. Comparison of health-promoting index (HPI) of *Caciotta* cheese made from milk of goats fed *ad libitum* with a single forage species (modified from Refs. [43, 44]).

plants widespread in the Mediterranean area [50]. The influence of high-polyphenol diet on cheese total polyphenol content and antioxidant capacity has been reported by Ref. [51]. They found higher level of polyphenolic compounds and antioxidant activity in cheese produced with milk from grazing goats, with a rich content of secondary metabolites, in comparison with cheese from goats kept in full indoor confinement and fed with Lucerne hay and concentrate. Grazing management represents a better option than indoor feeding to produce a healthy profile of bioactive compounds, providing an increase of total polyphenol, hydroxycinnamic acids and flavonoid concentrations. The feeding strategy involves not only polyphenols but also fat-soluble vitamins, especially those that play an important role as antioxidant (α -tocopherol and β -carotene). A positive relationship was observed between pasture-based rations rather than the hay-based rations for goats and levels of α -tocopherol and retinol in Rocamadour cheese, while β -carotene was not detected [52]. Ref. [49] found that grazing level high and medium, as percentage of net energy of requirement recovered from pasture, on Mediterranean shrublands and month of grazing also affect α -tocopherol content in goat milk without change of milk total antioxidant capacity.

Among forages, legumes are a rich source of polyphenols; large variability occurs among species; some ancient crops like common vetch (*V. sativa*) were found to contain threefold more polyphenol and five times more flavonoids than soybean [53]. Another important class of antioxidant compounds in legumes is represented by carotenoids and tocopherols; as many other compounds, their concentration is influenced by leaf proportion. Ref. [54] recommend a strategic approach to the choice of harvest date and wilting duration, since these can be a key tool for manipulating vitamins and FA composition in forages. Among forage legumes birdsfoot trefoil shows a relatively high content of lutein and contains almost threefold higher concentration of α -tocopherol content than yellow sweet clover and Lucerne (65 vs. 23 and

21 mg/kg dry matter, respectively) [26]. In forages, however, the complex mechanisms of interaction between pro- and antioxidant compounds must be taken into account [55]. While legumes might not be the richest/most effective source of α -tocopherol, it is the synergy between the phenolic acids, CT and anthocyanins that contribute to build up the so-called antioxidant network [56]. Altogether, the choice of natural feeding strategies for goats, without the use of expensive supplements, synthetic and/or encapsulated, could provide a way to encourage the consumer to the choice of dairy products obtained with natural resources and associated with beneficial health effects beyond its pure nutritional value.

2.2.1. Case study 1: borage and hawthorn and phenolic compounds in milk

Mediterranean pastures are highly variable in relation to the season, the proportion of grass plants decreases from 85 to 55% from winter to spring, while forbs increase from 25 to 65% from late spring to early summer. In early summer, goats graze mainly on forbs, some of which are used as medical plants by human. In order to highlight a relationship between non-volatile phenolic compounds in plant species and the same class of metabolites in milk or cheese, Ref. [14] examined the nuclear magnetic resonance (NMR) spectra of two green plants, borage (*Borago officinalis* L.) and hawthorn (*Crataegus oxyacantha* L.) (**Figure 6**) and milk (**Figure 7**) obtained from two groups of goats fed *ad libitum* with these plants during 18 days. A control group was fed *ad libitum* with natural hay and concentrate. Briefly, air-dried plants (500 g) were extracted with CHCl_3 and MeOH (10%, w/v) at room temperature. Chloroform extracts were fractionated on silica-gel column (80 × 4 cm) eluting with CHCl_3 and CHCl_3 -MeOH mixture of increasing polarity. Fractions were purified by RP-HPLC on a μ -Bondapak column eluting with H_2O -MeOH (1:1). Methanol extracts of plants were fractionated between BuOH and H_2O to give a butanol residue, which was chromatographed on Sephadex LH-20 eluting with MeOH. Fractions were purified by RP-HPLC as reported above. Milk sample (1 L) was lyophilised and then extracted, fractionated and purified as described for plant sample. The structure of the pure compounds isolated from samples was determined by analysis of ^1H , ^{13}C and ^{13}C DEPT NMR data (Bruker DRX 600 NMR Spectrometer; Bruker, Karlsruhe, Germany) and by comparison with literature data. Compound identification was also confirmed, when possible, by HPLC analyses with reference to the retention times of standards (Sigma-Aldrich Co., Milan).

The authors found a relationship between the antioxidant intake from borage and hawthorn and the levels of antioxidant metabolites in milk, flavonoids and terpenoids contained in these herbs that were found in milk. Quercetin and rutin were excreted in part without modification, while other compounds were structurally modified. No metabolite has been found in the control group milk. The different solvents, methanol or chloroform, used in the complex method of extraction for the plant material and milk have generated great differences in the recovered metabolites. For the purpose of a useful comparison of results from different experiments, the standardization of extraction methods appears to be desirable.

Thus, the hypothesis of the authors was that gastrointestinal microflora of goats can structurally modify plant metabolites through hydrolyses and/or other interactions that result in structurally less complex molecules in milk. This study demonstrates that the presence of phenolic compounds in milk depends on the animal feed.

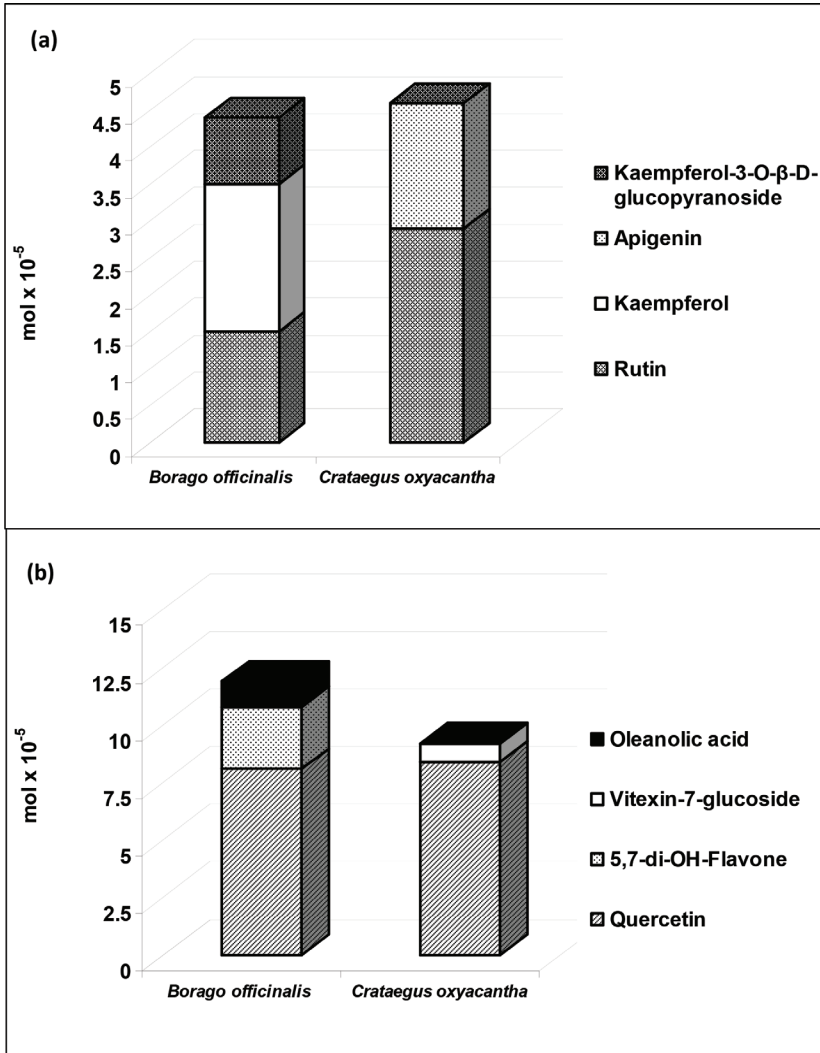


Figure 6. Metabolites found in plant of *Borago officinalis* and *Crataegus oxyacantha*. Plant extract with methanol (a) and chloroform (b) (modified from Ref. [14]).

2.2.2. Case study 2: oat and phenolic compounds

The wild species or aromatic plants in the pasture are less present in quantity than forage species. As forages represent a high proportion of ruminant diet, in order to observe the link between phenolic content of forage species and phenolic content of milk, whey and cheese, Ref. [15] planned an experiment with ten Mediterranean Red goats fed indoor with fresh *A. sativa* forage, in pureness, without any other supplementation. After 10 days of adaptation

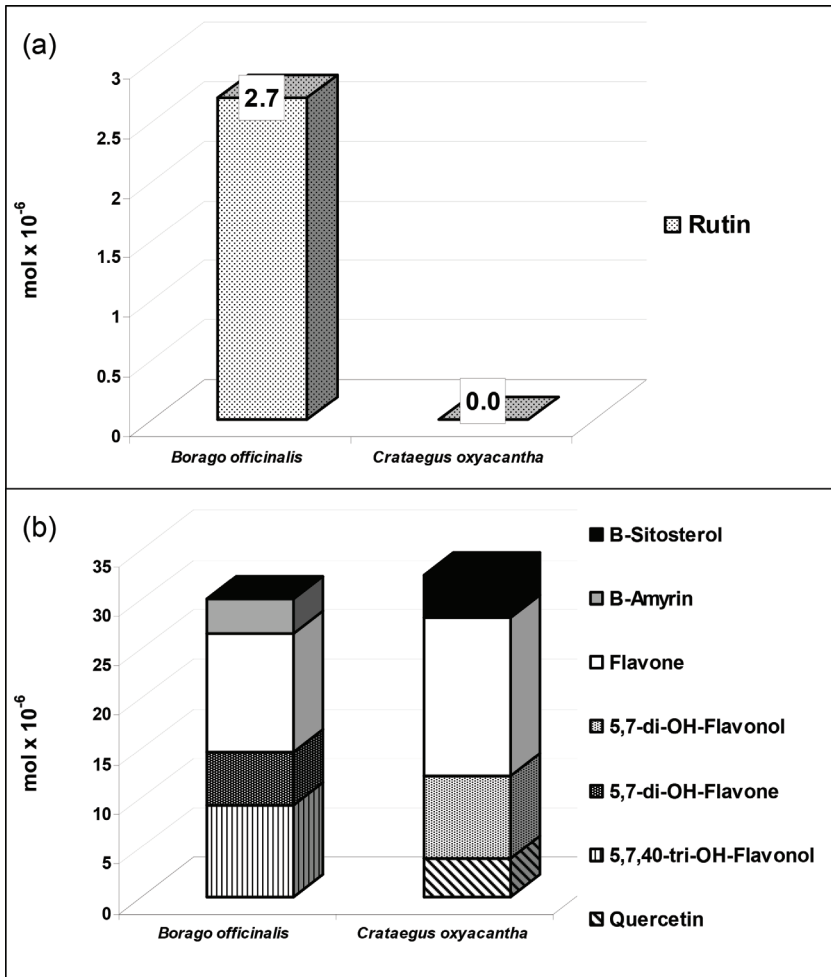


Figure 7. Plant metabolites found in milk from goats fed with *Borago officinalis* and *Crataegus oxyacantha*. Milk extract with methanol (a) and chloroform (b) (modified from Ref. [14]).

and 3 days of experiment and sample collection, phenolic compounds were extracted from herbage, milk, whey and cheeses by methods appropriate to the substrate and analysed by high-performance liquid chromatography (HPLC-DAD).

Ten Siriana goats were fed indoor with *A. sativa* in pureness, given fresh for 10 days of adaptation and 3 days of trial. In these 3 days, herbage (the part of plant effectively ingested) was daily collected, freeze-dried and ball milled. Milk and whey samples were collected on days 2 and 3, contextually with cheesemaking (*Caciotta* cheese) and stored at -20°C . Briefly, phenolic compounds were extracted from herbage (200 mg) with $\text{EtOH}/\text{H}_2\text{O}$ (80:20) at 90°C while from milk (10 ml) and whey (10 ml) by a precipitation in CH_3CN (22 ml)

and an overnight deconjugation using a glucuronidase-sulfatase enzyme mixture. Cheese was homogenised and centrifuged, and the supernatant was used for phenolic extraction as described for milk. Phenolic compounds of the extracts were analysed as UV-absorbing compounds using HPLC-DAD on a reverse phase column (LiChroCART® 125-4, Merck) eluted by 0.3 ml/min of a 0–100% gradient of acetonitrile in water, both containing 0.1% formic acid. The UV spectra were compared to those of standard compounds and classified into simple phenol, benzoic acid derivatives, cinnamic acid derivative and flavones groups. The *A. sativa* forage revealed an interesting metabolite profile, where cinnamic acid and flavones largely occur (mostly derived from apigenin and luteolin or chrysoeriol). These phenolic compounds affected milk and whey, even though in different measures: flavones disappeared, while simple phenols, benzoic acid derivatives and some unclassified phenols were identified (Figure 8).

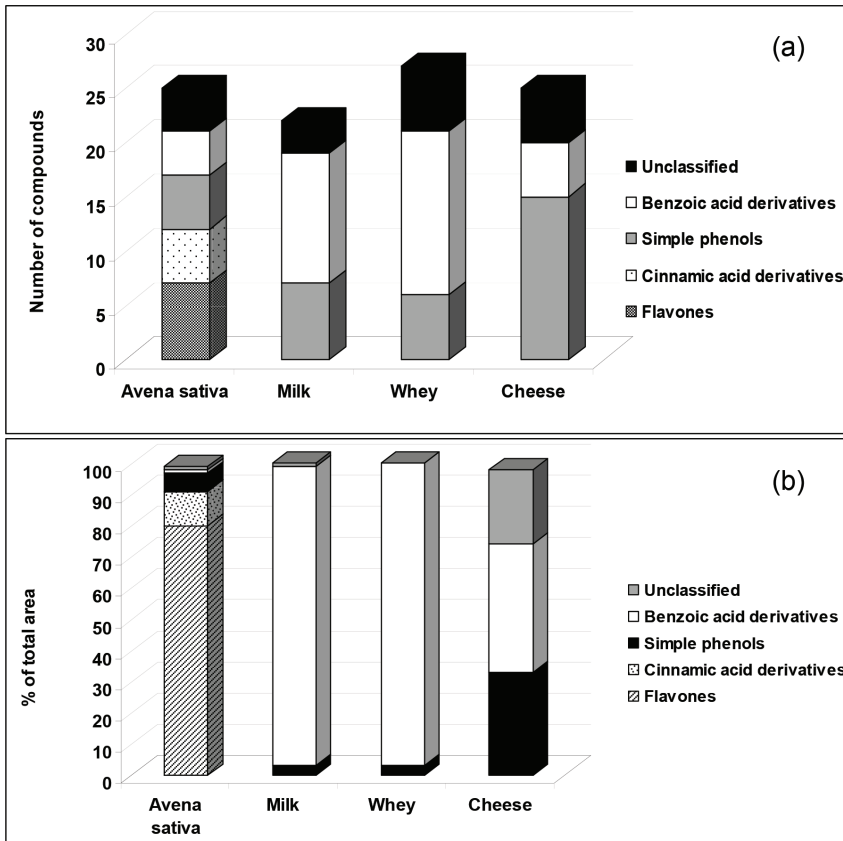


Figure 8. Phenolic compounds in *Avena sativa* forage and milk, whey and cheese from goats fed with fresh *Avena sativa* in pureness. (a) Data are expressed as abundance of compounds and (b) percentage of total picks by HPLC-DAD analyses (modified from Ref. [48]).

In cheese, although the largest amount of phenolic compounds was still by benzoic acid derivatives, there was a greater number of simple phenols and one of the indole derivatives found in milk. Nevertheless, phenolic compounds' profile of milk was much closer to whey profile than to cheese ones.

These preliminary results have allowed us to get an overview of the transfer of the plant metabolites directly or processed or degraded in the digestive tract, to the product. However, quantitative studies would be desirable to measure the partition of phenolic compounds in serum and cheese.

2.2.3. Case study 3: relationship between forage species and antioxidant compounds in milk

In goat feeding, forage plants such as grasses and legumes have an essential role, since they represent a high proportion of diet. Forages commonly used in Mediterranean area can be a natural source of bioactive compounds that can be transferred to animal products. In order to evaluate and compare the potential contribution of some grass and legume species, to increase the level of bioactive compounds and antioxidant capacity in milk, Refs. [48, 57] compared the total polyphenol intake of three grasses, *Festuca arundinacea*, *H. vulgare* and *Triticosecale*, and four legumes, *Pisum sativum*, *Trifolium alexandrinum*, *V. sativa* and *Vicia faba minor*, given to seven groups of Mediterranean Red goats without supplement for eleven days. The single forage was cut daily and given *ad libitum* indoor. After an adaptation period, forage samples and milk samples of each group were collected and analysed for polyphenolic compounds and total antioxidant capacity. Folin-Ciocalteu method as described by Ref. [60] was used to determine tannic and non-tannic polyphenol contents in forage samples, after the addition of insoluble matrix polyvinylpyrrolidone (PVPP) and total and free polyphenol contents in milk samples. Contents were expressed as gallic acid equivalents (GAE). Milk conjugate polyphenol content was obtained by difference between milk total and free polyphenol contents. Total antioxidant capacity (TAC) was measured using the ferric-reducing antioxidant power as indicated by Benzie and Strain method and was expressed as $\mu\text{M FeSO}_4$.

Among forages, *T. alexandrinum* and *F. arundinacea* were shown to enhance the milk-free polyphenol content (**Figure 9**) and the antioxidant capacity (**Figure 10**), respectively.

The phenological stage to which the fodder was used by goats may have contributed, as reported in the literature [47], to total polyphenol intake (**Figure 9**) and milk polyphenol content. As polyphenolic beneficial compounds occur largely in forages, it could be assumed their possible relationships and their transfer from diet, through some biotransformations or, as such, to milk according to De Feo et al.'s [14] study.

2.2.4. Case study 4: Sulla forage and phenolic compounds and antioxidant capacity

Among plant species that are used in ruminant feed in the Mediterranean area, Sulla (*Sulla coronarium* L.), which is a short-lived perennial legume, plays a key role in the cereal-based systems that are used in semiarid regions. This legume forage has widespread availability in Mediterranean areas, where it is greatly appreciated for the positive effects of its nutrient and CT contents on milk yield and composition, as demonstrated in both sheep and goats [58, 59].

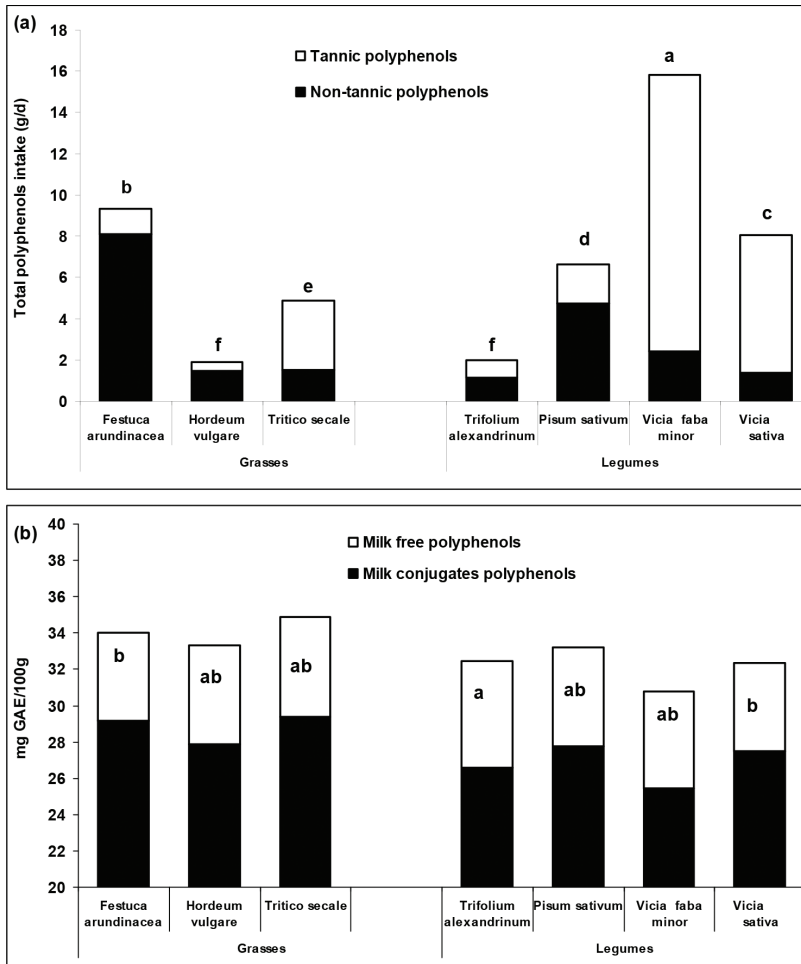


Figure 9. Comparison of total polyphenol intake (a) from goats fed *ad libitum* with a single species, in pureness, given fresh and milk total polyphenol content (b) from goats fed with the same forage species (modified from Ref. [48]). a, b, c, d, e, f = $P < 0.05$.

The results of a recent study [60] on three groups of *Girgentana* goats fed with *Sulla* fresh forage *ad libitum*, *Sulla* fresh forage *ad libitum* plus 800 g/d of barley meal and mixed hay *ad libitum* plus 800 g/d of barley meal indicate that *Sulla* fresh forage improves the plasma oxidative statuses of goats [61], milk total polyphenol (Table 1) content and the total antioxidant capacity of milk. Methods for milk total polyphenols and milk TAC assays are given in Section 2.2.3. Milk total polyphenol content seems closely related to its antioxidant activity. This fresh forage exerts antioxidant capacity due to its secondary compounds, which provide additional value in terms of oxidative status, and *Sulla* fresh forage seems to be a promising strategy for improving product quality.

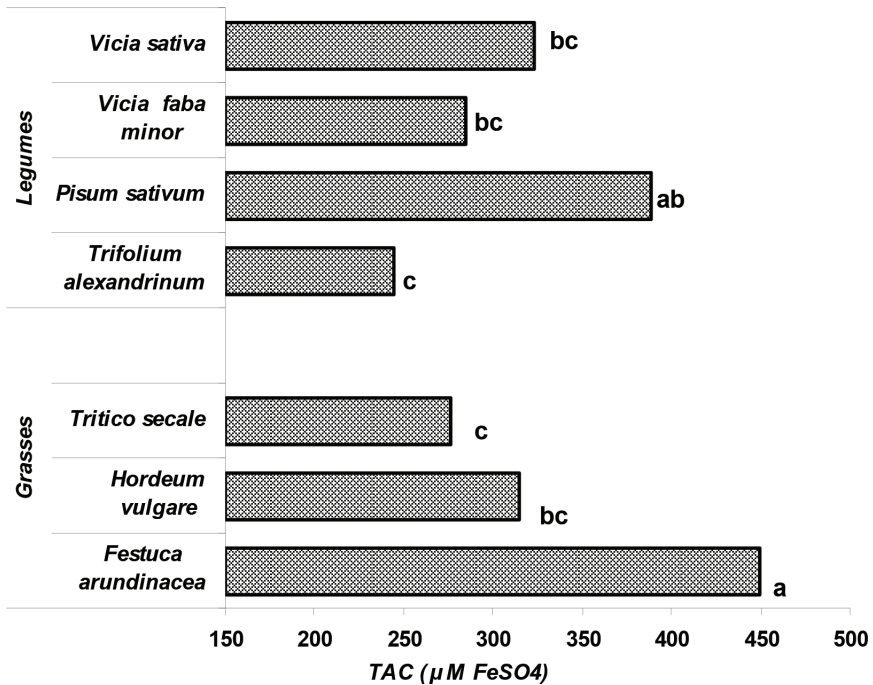


Figure 10. Comparison of total antioxidant capacity (TAC) in milk from goats fed *ad libitum* with a single species in pureness, given fresh (modified from Ref. [48]). a, b and c = $P < 0.05$.

	Feeding regimen		
	HB	SUL	SULB
Total polyphenol intake (g of GAE/d)	1.53 ^b	9.20 ^b	8.88 ^a
Non-tannic polyphenol intake (g of GAE/d)	1.26 ^b	3.72 ^a	3.24 ^a
Tannin intake (g of GAE/d)	0.26 ^b	5.48 ^a	5.64 ^a
Condensed tannin intake (g of DE/d)	3.5 ^c	47.2 ^a	35.6 ^b
Milk total polyphenols (g of GAE/d)	0.819 ^b	0.964 ^a	1.081 ^a
Milk-free polyphenols (μg/mL of GAE)	49.3 ^b	56.7 ^a	56.2 ^a
Total antioxidant capacity (log μmol/L)	2.38 ^f	2.43 ^e	2.47 ^e

HB = mixed hay plus 800 g/d of barley meal; SUL = Sulla fresh forage; SULB = Sulla fresh forage plus 800 g/d of barley meal. GAE = gallic acid equivalent. DE = delphinidin equivalent,

^{a-f} Values within a row without a common superscript letter are significantly different ($P \leq 0.05$).

Table 1. Daily intake of polyphenol compounds, milk content and total antioxidant capacity according to feeding regimen [60].

2.2.5. Case study 5: degree of antioxidant protection

In this case study, Ref. [13], in order to trace and identify milk and cheese from different feeding systems, proposed an interesting tool. Milk and cheese samples from ten feeding systems as grazing, grazing plus different types of supplement and indoor and zero grazing were studied to identify a tracing parameter correlated to the feeding system. In particular, α -tocopherol and cholesterol were measured in milk and cheese and were combined to calculate the degree of antioxidant protection (DAP). This tracing parameter was calculated as molar ratio between antioxidant compounds and a selected oxidation target. In dairy products from goats, only α -tocopherol was selected as the antioxidant because β -carotene is absent in goat's milk, and cholesterol was selected as oxidation target. All samples were analysed for α -tocopherol and cholesterol content. Briefly, all samples were hydrolysed in alkaline solution, and the extracted residue was dissolved in 2-propanol (1%) in n-hexane and analysed by the normal phase chromatographic method described in Ref. [13]. This index allows an evaluation of milk and cheese resistance to oxidative reactions, the main determinants of food quality and functionality for human nutrition. The DAP values (**Figure 11**) greater than 7.0×10^{-3} were found in grazing feeding systems, and values lower than 7.0×10^{-3} were found in indoor and zero grazing feeding systems, for milk and cheese.

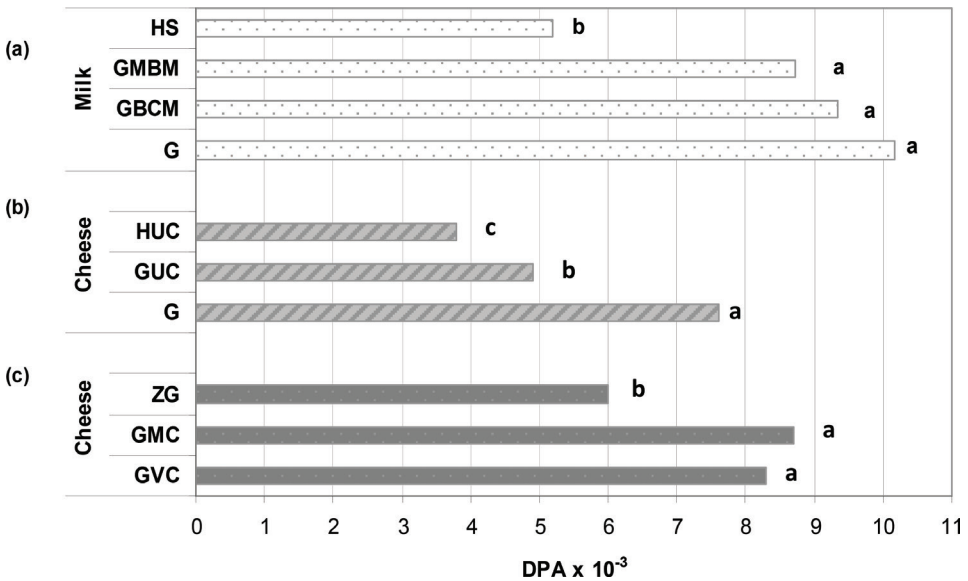


Figure 11. (a) Degree of antioxidant protection (DAP) of milk from goats fed with G = grazing, GBCM = grazing plus 0.6 kg/d mixed barley and chickpeas grain, GMBM = grazing plus 0.6 kg/d mixed corn and broad beans grain and HS = pasture hay *ad libitum* plus 0.6/kg/d of commercial concentrate. (b) DAP of *Caciotta* cheese from goats fed with G = grazing, GUC = grazing plus unlimited concentrate and HUC = hay plus unlimited concentrate. (c) DAP of *Caciotta* cheese from goats fed with GVC = grazing on valley pasture, GMC = grazing on mountain pasture and ZG = zero grazing (modified from Ref. [13]). a, b and c = $P < 0.05$.

These results show that cholesterol was highly protected against oxidative reactions when herbage was the only feed or was dominant in the goat diet. A strong positive correlation between herbage intake and DPA values allows to identify a linear regression: $y = 0.12x + 5.52$, where $y = \text{DPA} (\times 10^{-3})$ and $x =$ contribution of grazed herbage intake to the animal diet calculated as a percentage of the maximum intake of mature Maltese goats ($1100 \text{ g/d} = 100\%$ grazing). The DAP index equal to 7.0×10^{-3} was able to distinguish dairy products when the grazed herbage in the goats' diet exceeded 15%. The reliability of DAP to measure the antioxidant protection of cholesterol appeared more effective when the feeding system was based on grazing than when cut herbage or zero grazing was utilised indoors by animals.

3. The role of the breed on oligosaccharides: a special focus on the Mediterranean goats

Besides the feeding system, the breed plays a fundamental role in affecting the nutritional profile of goat milk and cheese. The breed may be considered the result of the adaptation of a species to a given environment, basically in order to go over the climate and feeding and water resource limits that might affect the reproduction and kidding. The goats are present in high mountains as far as in the internal lands and coastal regions; they are reared in technological farms but also in extensive, grazing systems in the Mediterranean area, an environment characterised by high variability, that was able to select very different breeds [62].

The so-called native breed has become able to optimise the resources in terms of water and feedstuff [63]. The breed's answer is expressed as phenotype, quantity and, overall, quality of production. The differences are both in micro and macronutrients, and they are affected by the environment directly or indirectly. In the first case, we can say that different breed means different feeding behaviour and thus milk yield and quality, since it is well known that feeding largely affects the milk composition [64]. Moreover, the genetic polymorphism may affect the milk features.

Within the same breed, in the same environment and diet, it is expectable to have very similar performances. Contrarily, especially for goat, significant differences have been found for quality but also quantity parameters. This variability has been explained, in part, by the genetic polymorphism of caseins that are α_{s2} -casein, β -casein and k-casein but in particular at the locus α_{s1} -casein, first discovered by Boulanger et al. [65]. It was found that goats carrying strong alleles (AA) for high α -s₁ casein present higher percentage of milk casein, fat, calcium, phosphorus and smaller micelles than the milk from goats with weak alleles (FF) [66, 67]. Several goat breeds have been characterised for this variability: the Vallesana, Roccaverano, Jonica, Garganica and Maltese breeds [68] and Alpine breed [69]. Spanish Malagueña goats with a high (HG) and low (LG) genetic capability for α_{s1} -casein synthesis were used to determine whether the different genotypes were related to differences in feed utilisation (13.6 vs. 17.7% crude protein content for diets 1 and 2, respectively). The findings have let to explain the differences in milk composition between the two genotype groups by the greater nitrogen and energy utilisation of HG vs. LG goats [70]. Moreover, the interaction genotype \times feeding system was studied (e.g., see Ref. [71] on Malagueña dairy goat breed).

The breed effect on milk oligosaccharide (OS) composition, and in particular sialyloligosaccharide (SOS) content, is scarcely studied. The milk from the Spanish Murciana-Granadina goat breed was found characterised by 25 OS [72], later [73] isolated 15 new oligosaccharide structures from fresh milk of Spanish goats, obtaining a virtually lactose and salt-free product, containing more than 80% of the original oligosaccharide content. Evenly, the effect of interaction of breed \times feeding is scarcely studied.

Within the management's strategies, the choice of the breed is a key element to weigh up towards the type of livestock, the available resources and the business plan, in terms of destination of the milk, namely, if destined to the market of drinking milk or to dairy production. Some speculations can be made whether optimizing production, rheological properties, and bioactive profile and content may be feasible with feeding management modulated in terms of energy and protein supply depending upon genotype. The following three case studies are presented, in order to partially cover this gap on Mediterranean goats.

3.1. Case studies 1 and 2: oligosaccharides in colostrum and milk

A study on the content of three SOSs, namely, 3'-sialyllactose (3'-SL), 6'-sialyllactose (6'-SL) and di-sialyllactose (DSL) in colostrum and milk, was carried out [22] with two Italian goat breeds, Garganica goat (a native breed from Gargano Mountain in Apulia region, Southern Italy) and the Maltese goat breed, native from Malta isle in the Mediterranean area. The animals were fed indoor, receiving hay (from polyphytic cultivated meadows) *ad libitum* and concentrate supplementation (400 and 600 g/h/d, respectively) at 14% crude protein, according to their milk yield (800 and 1200 g/day milk, respectively). The SOSs were isolated from individual colostrum and milk samples obtained in five periods (at kidding, 24 h, 7 days, 30 days and 90 days after delivery). Briefly, after centrifugation ($2000 \times g$, 4°C , 10 min) the supernatant lipid layer was removed, and the proteins were precipitated by addition of 0.5 volumes of $1.8 \text{ g } 100 \text{ mL}^{-1} \text{ Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ and 0.5 volumes of $2 \text{ g } 100 \text{ mL}^{-1} \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O}$. The blend was vortexed and centrifuged ($12,000 \times g$, 10 min, 4°C). The supernatant was removed and centrifuged again. The second supernatant was filtered with a $0.45 \mu\text{m}$ nylon filter prior to analysis by high-performance anion-exchange chromatography (HPAEC) on a Dionex PA100 column (Dionex, Sunnyvale, California, USA). Elution was monitored by pulsed amperometric detection (Dionex ED40) and the gradient controlled by a Varian ProStar pump system. Data were collected and analysed by Star Chromatography Workstation 6.41 (Varian, Inc. Walnut Creek, California, USA), and 6'-SL, 3'-SL and DSL external standards were used to generate standard curves for comparison.

The results showed a significant effect of breed and sampling time on SOS content. Garganica breed showed the highest values of 3'-SL and 6'-SL while Maltese breed the lowest content of DSL (**Figure 12**).

Also the interaction breed \times sampling time affected the SOS content in milk and colostrum; in particular, 3'-SL content was significantly higher in Garganica colostrum at 24 h after kidding and in milk at the 7th and 30th day. DSL was affected by interaction, showing higher values in Garganica's colostrum at parturition and Maltese's colostrum 24 h and milk. The content of the

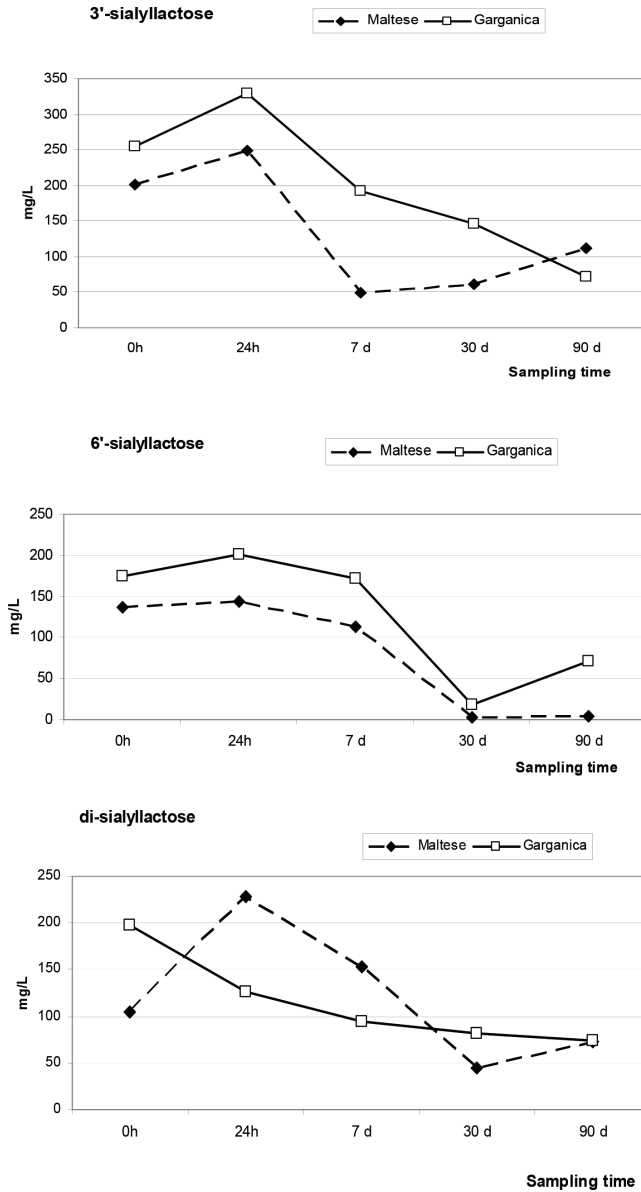


Figure 12. Effect of breed and sampling time on SOS concentration in milk [22].

three SOSs was higher than values found by Ref. [73] on Spanish goats, confirming the breed effect and, consequently, the importance of the choice of the breed among the management options aimed at improving the nutraceutical quality of milk, namely, the oligosaccharide content.

In a further study [74], Garganica goat milk SOSs were compared with Saanen goats' from colostrum time to the 90th day after parturition, in their 3rd parity. In the experiment, the Saanen and Garganica goats were fed indoor, receiving hay *ad libitum* (50% polyphytic meadow and 50% alfalfa hay) and 700 g/h/day DM and 450 g/day/h DM, respectively, of concentrate supplementation at 18.3% crude protein (wheat by-products, corn grain, soybean meal, molasses, supplemented with mineral mixtures). The results confirmed a breed effect on SOS contents in colostrum and milk also between Garganica and Saanen breed, where Garganica goats showed mean higher values for the three SOSs (see **Table 2**).

Moreover, a significant interaction breed \times sampling time was recorded for 3'-SL ($P < 0.001$) and 6'-SL ($P < 0.01$), while no significant interaction was found for DSL. The results may be considered under a genetic point of view. In fact, under equal feeding condition, the breeds expressed their OS synthesis potential, probably influenced by the genetic polymorphism in the locus CSN1S1 (α_{s1} -casein). On this matter, the Saanen goats were characterised by a high frequency of defective alleles (F and E) and low frequency of strong alleles (A and B) at α_{s1} -casein locus; contrarily Garganica goats had high frequency at strong alleles and low frequency at weak alleles (F) [75].

In a previous study, Ref. [76] have found in Alpine goats that the genotype (A/A or 0/0) affected the OS profile, even though not the total OS production. So, Claps et al. [74] speculated that an indirect link between goat breeds, about the allelic frequencies at the locus α_{s1} -casein in Saanen and Garganica goat breeds, might have affected the SOS content in milk and colostrum, where the Saanen breed, characterised by high frequencies of defective alleles [66], could have adversely affected the production of SOSs.

3.2. Case study 3: interaction of genotype with feeding regimen

The Mediterranean Red goat was characterised for the content of three SOSs considering the polymorphism at locus CSN1S1 and its interaction with feeding regimen [77]. Six goats, with genotype A/A (strong, α_{s1} -casein producers), and six goats with genotype F/F (weak) were fed with two diets in pellet, respectively, at 100% of energetic and 105% of protein requirements (M) and 70% of energetic and 75% of protein requirements (L). Milk samples at the 69th \pm 3 day of milking were analysed for the content of three sialyllactoses (see Section 3.1),

SOS	Garganica	Saanen	SE	Significance
3'-SL (mg/L)	195.5 a	124.8 b	12.7	***
6'-SL (mg/L)	129.7 a	15.4 b	5.5	***
DSL (mg/L)	104.0 a	79.9 b	7.3	*

Means within a row with different letters (a, b) differ at $P \leq 0.05$. SE = standard error.

* $P < 0.05$.

*** $P < 0.001$.

Table 2. Mean content in milk of 3'-sialyllactose (3'-SL), 6'-sialyllactose (6'-SL) and disialyllactose (DSL) from Garganica goat breed compared with Saanen, a cosmopolitan breed, at 30 days in milk (from Ref. [74]).

considering the genotype, the diet and their interaction. The results revealed that genotype and diet affected the 3'-SL content in milk ($P < 0.05$) (**Figure 13**), while their interaction expressed only a trend of variation ($P = 0.10$). The goats fed with undernourishing diet (D) showed a 3'-SL content 58.5% lower than M goats.

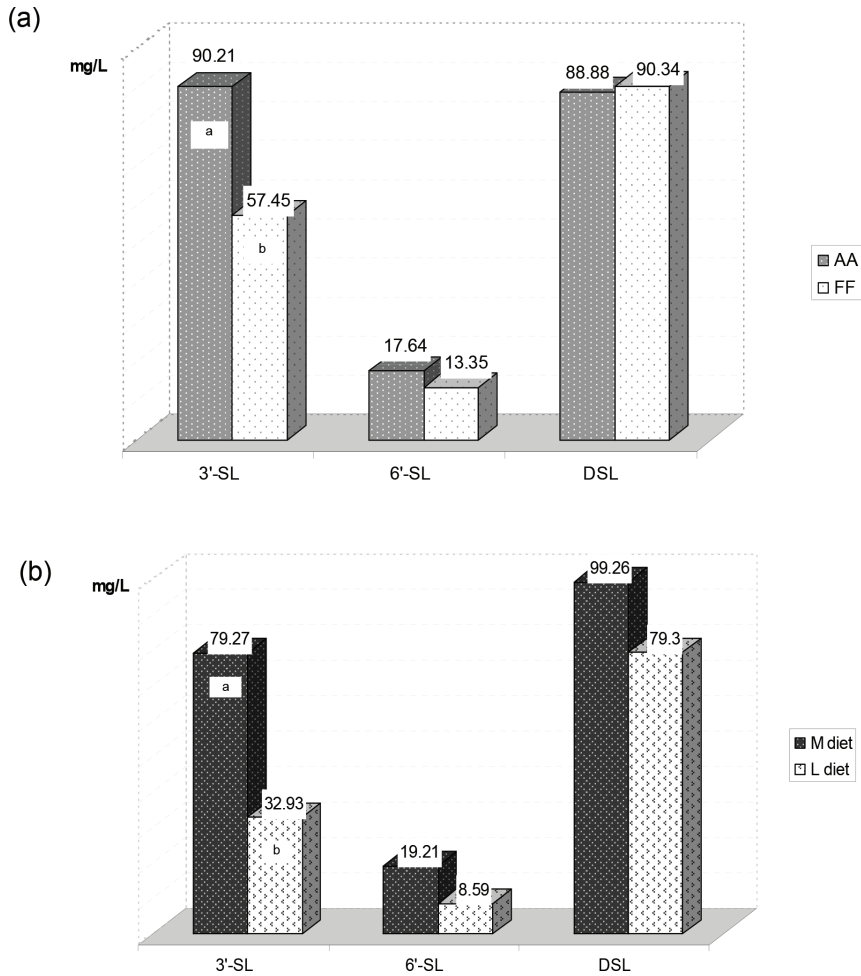


Figure 13. Effect of genotype (a) and diet (b) on three sialyloligosaccharides in Mediterranean Red goat milk (adapted from Ref. [77]).

The 6'-SL and DSL showed only a decreasing trend. This result might be related to the reduction of the expression of genes involved in the milk synthesis after a prolonged fasting [78]. Similarly, in human milk a decrease of OS was found in milk from undernourished women [11].

These results demonstrated that there is a different efficiency in diet utilisation and response in synthesis of metabolites such as oligosaccharides, depending on the genotype. Consequently, in systems that use selected animals, the diet may be formulated taking into account the genotype, in order to achieve certain qualitative profile of goat milk and increase the efficiency in feeding management.

4. Conclusion

The case studies discussed along with the literature review contribute to widen the farmers' basis for choosing the breed and the feeding regimen by including management decisions specifically aimed at improving milk and cheese nutraceutical properties. The management options considered hereby are mainly conceived for Mediterranean extensive and semi-intensive goat production systems but can easily lend themselves to other ruminant and non-ruminant production systems. This analysis can help farmers to improve milk nutraceutical composition in a sustainable and low-cost way by partially replacing expensive commercial concentrate with farm-grown forages and natural pasture grazing. Data on the nutraceutical profile of milk produced by Mediterranean breeds, as well as on the use of farm-grown forages, open new alleys towards the potential role of local breeds/forages for developing innovative and sustainable health-promoting dairy supply chains.

Author details

Salvatore Claps^{1*}, Roberta Rossi¹, Adriana Di Trana², Maria Antonietta di Napoli², Daniela Giorgio² and Lucia Sepe¹

*Address all correspondence to: salvatore.claps@crea.gov.it

1 CREA Research Centre for Animal Production and Aquaculture, Bella, Italy

2 School of Agricultural, Forest, Food and Environmental Science SAFE, University of Basilicata, Potenza, Italy

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