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DIPARTIMENTO DI SCIENZE DELLE
PRODUZIONI AGRARIE E AGROALIMENTARI

Doctoral thesis

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Genetic polymorphism of α_{s1}-casein.
Feeding strategies to optimize productive performances of
dairy goat with different genotype.

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General introduction

1.1 Introduction

Breeding system of dairy small ruminants exhibits great diversity ranging from extensive to intensive. Extensive or semi-extensive farms are very often located in less favourable zones which are unfertile and hard to be reached. In these conditions, the use of local and well adapted breeds is of fundamental importance because they can be reared in marginal areas thanks to their capability to fully exploit also very poor lands (Figure 1). This aspect is very important not only because allows animal breeding at relative low costs, but also because it avoids the abandon of these areas and preserves genetic variability. This aspect is of particular interest in the Mediterranean area where is concentrated about 25% of the total goat milk production. Almost all the goat milk here produced is transformed into local cheeses, which can greatly differ also among proximal regions. Therefore goat breeding helps also to maintain traditions and typical products which could be lost because new global economy. The issue of protected labels for traditional products is having always increasing attention because it can represent an important economic source for local breeders. That is why in the last decades there has been a proliferation of "labelled products" (PDO and PGI).

Figure 1 – Goat grazing in poor land

In the past before introduction of milk formulas, goat milk represented a valid substitution of mother's milk. In fact, thanks to the peculiar chemical composition (Table. 1) and physical characteristics, goat milk is more easily digestible than cow milk. In particular, its nutritional value is increased by the small size of fat globules and the richness in short- and medium chain fatty acids, while casein composition reduces its allergenic power.

Table 1 - Average chemical composition (%) of milk from different species

	Water	Fat	Protein	Lactose	Ash	Total solids
Human	87.4	4.0	1.2	6.9	0.2	12.3
Cow	87.8	3.6	3.3	4.6	0.7	12.3
Buffalo	80.6	9.0	5.1	4.5	0.8	19.4
Sheep	83.7	5.3	5.5	4.6	0.9	16.3
Goat	87.9	3.8	3.5	4.1	0.8	12.2
Horse	89.0	1.6	2.7	6.1	0.5	11.0
Rabbit	73.6	12.2	10.4	1.8	2.0	26.4

From Bittante 2007

The management of the most important factors affecting milk composition represents the basis for the production of milk with the best attitudes in function of its productive destination of goat milk (transformation or drinking milk). A brief overview on goat milk constituents, and main factors affecting milk composition is given in the next sections.

1.2 Lactation curve

Milk yield and composition are not constant during lactation. In the past, the creation of a model able to describe the temporal evolution of milk production have been one of the most important challenge for mathematical modelling applied to animal science. Several equations (reviewed in Macciotta et al., 2008) have been developed to this aim. Wood equation (1) is the most used model to describe the curve of lactation.

$$y(t) = at^{b}e^{-ct} \qquad (1)$$

In the pattern described by Wood model, milk production rapidly reaches a peak after which constantly decreases. Figure 2 describes lactation curves of several goats.

Though lactation is regulated by complex biological processes, only three parameters are presents in the equation; from one side this is an advantage because the few number of input makes the model very general, but on the other

Figure 2 – Goat lactation curve

From Gorjanc 1999

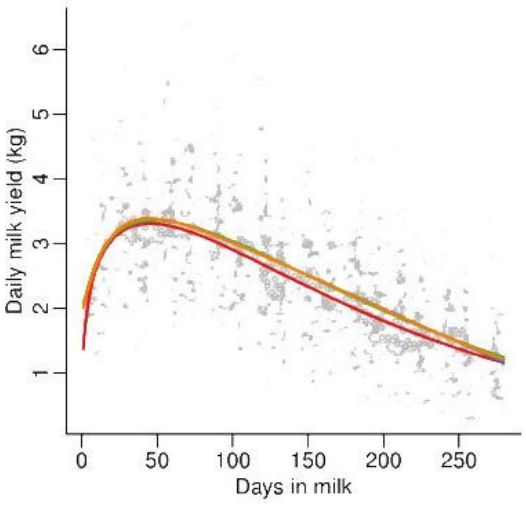


Table 2 – a, b, and c parameters of Wood function estimated in several goat breeds (Macciotta 2008)

Breed	A	B	C
Alpine, Saanen	2.316	0.230	-0.005
Derivata di Siria	1.388	0.163	-0.005
Murciano-Granadina	2.287	0.129	-0.029
Sarda	1.007	0.182	-0.007

side the same parameters must to be specifically estimated to fit the model for specific breeds (Table 2). Parameters *a*, *b* and *c* can be useful used to predict important information such as the time when peak of lactation will be reached, the persistency of lactation and the milk yield at peak.

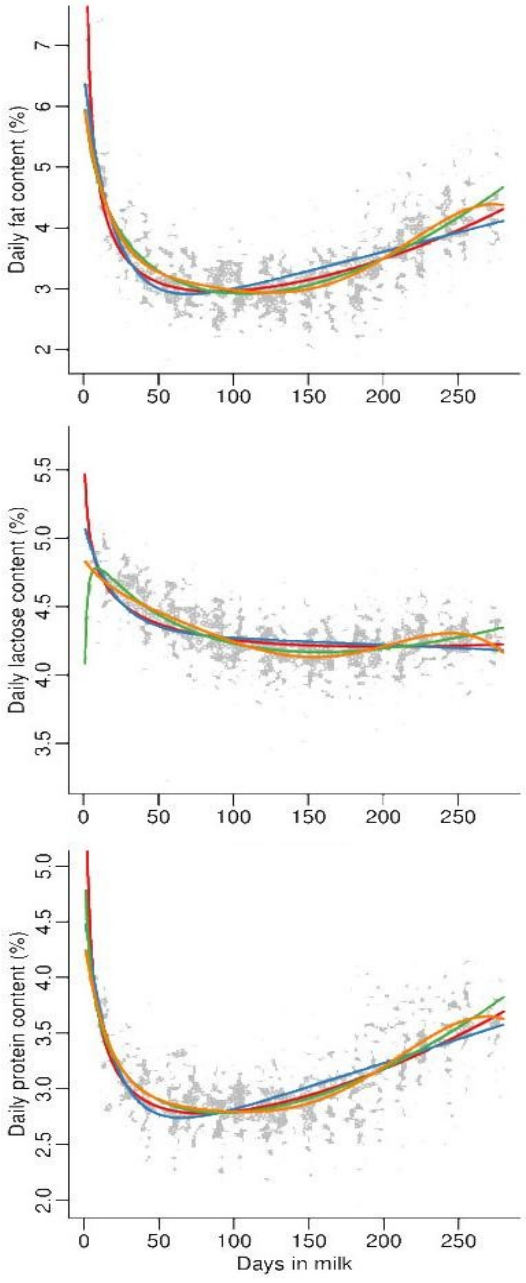
Figure 3 reports the pattern of fat, protein and lactose throughout lactation. It is evident how milk yield is negatively correlated to its quality. In particular, fat and protein contents are high in early lactation to rapidly fall until peak of lactation when they reach their minimum. After the peak of lactation, milk yield constantly decreases whereas fat and protein percentage raise. This "dilution effect" respond to the equation (2)

$$y = ax^{b} \qquad (2)$$

where *y* is fat or protein yield, *x* is the milk yield, *a* and *b* are equation coefficients. In goat, value of *a* and *b* is about 0.95 for non selected breeds, while their value is about 0.83 and 0.87 for fat and protein, respectively, in highly selected breeds (Pulina et al., 2003). This means that the effect of dilution is stronger in breed such as Saanen and Alpine. Lactose shows a

Figure 3 - Fat, protein and lactose curves

From Gorjanc 1999



different trend because it is transferred from blood to milk according to their osmotic pressure. Since osmotic pressure is more or less the same between the two biological fluid, lactose is secreted at the same rate as milk and is quite constant trough lactation.

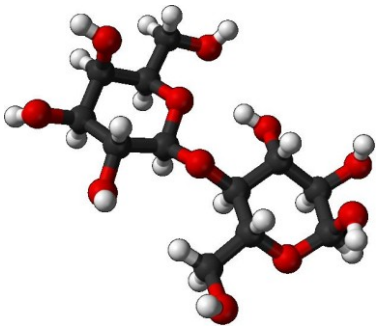
1.3 Goat milk composition

Goat milk composition is not dissimilar from cow milk in terms of total fat, protein and lactose content. The main difference lies in the "quality" of fat and protein. Indeed, they deeply differ for protein and fat composition. As compared to human milk, goat milk differs both for quantity and quality of constituents. In particular, goat milk is higher in protein whereas is lower in lactose content; moreover, protein composition greatly differs because human milk contains more whey protein than goat milk. Taken together, all these differences give cow, goat and human milk very different physical, chemical and nutritional characteristics.

1.3.1 Carbohydrates

Lactose represents almost the totality of the carbohydrates present in milk. This disaccharide derives from the condensation of galactose and glucose residues bound by a β-1→4 linkage (Figure 4). The two monosaccharides differ only for the arrangement of the -OH group in position 4.

Figure 4 – Structure of lactose



Lactose is biosynthesized in mammary glands only during lactation. Out of lactation, galactosyltransferase, the enzyme responsible for the condensation of galactose and glucose, catalyzes the biosynthesis of glycoproteins containing galactose. During lactation, galactosyltransferase and α-lactoalbumin (constituent of milk whey proteins) are bound together to form a complex called α-lactoalbumin-galactosyltransferase which is able to promote the biosynthesis of lactose (Larson and Smith, 1974). The role of the lactose in mammary gland is to maintain the osmotic equilibrium between blood and alveolar cells during milk

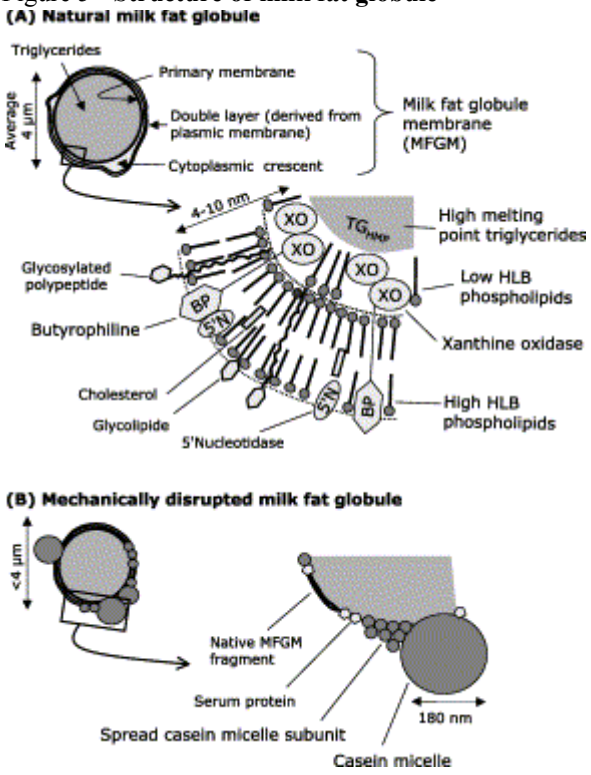
synthesis and secretion. Lactose provide the newborn with energy and with the substrate to build up central nervous system.

Carbohydrates other than lactose can be found in milk under different forms (mono- and oligosaccharides, glycoproteins, glycolipids and nucleotide sugars).

1.3.2 Lipids

Goat milk contains about 3.8% of lipids. Mono-, di- and triglycerides, also called free lipids, represent 98% of total fat, while bound lipids (glyco-, phospho- and neutral lipids) account about 2% of total. Milk lipids are secreted from mammary epithelial cells as fat globules which are primarily composed of a globule of triglycerides surrounded by a lipid bilayer membrane (Figure 5) similar to the apical membrane of the epithelial cells. This membrane helps to stabilize the fat globules in an emulsion within the aqueous environment of milk (Danthine et al., 2000; Ye et al., 2000). Lipids can naturally or after centrifugation rise to the top resulting in a cream layer because a lower buoyant density than water. Cow milk needs to be homogenized to reduce globules size and avoid this phenomenon. Homogenization is not necessary in goat milk because the size of fat globules is naturally smaller (3.5 *v.* 4.5 micrometers) and then better dispersed (Fahmi et al., 1956). Smaller fat globules provide lipases with a greater surface area so that they can undergo an enhanced digestive action, resulting more easily digestible by humans, but at the same time, globules with smaller size are incorporated at slower rates during casein coagulation lowering cheese-making properties.

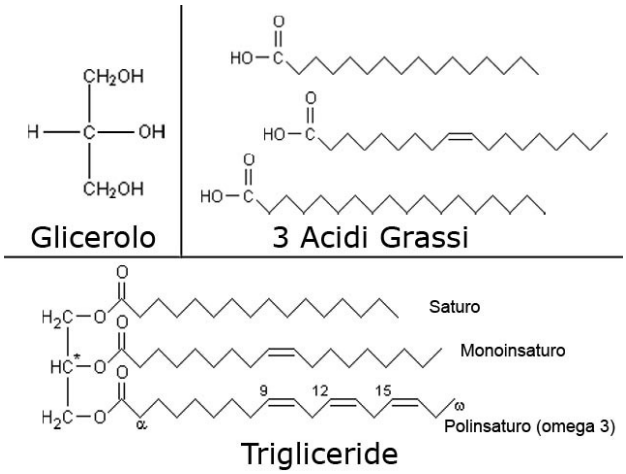
Figure 5 - **Structure of milk fat globule**



1.3.2.1 Biosynthesis of fatty acids

Triglycerides (Figure 6) are the most representative components of milk lipids. They are formed by the combination of glycerol with three molecules of fatty acid. The glycerol molecule has three hydroxyl (-OH) groups which form ester bonds with the carboxyl group (COOH) of three fatty acids, which are generally different among them.

Figure 6 – Structure of triglycerides



Fatty acids are usually classified according to the length of chain and the number of unsaturated bonds. Fatty acids with a length from 4 to 16 atoms of carbons are called short- and medium chain fatty acids (SMCFA), long chain fatty acids (LCFA) have a carbons chain longer then 16, among them very long chain fatty acids (VLCFA) have more than 22 atoms of carbons. Depending on the number of double bonds in the chain we can distinguish: saturated fatty acids (SFA), mono- (MUFA) and polyunsaturated (PUFA). In a triglyceride, the methyl end of the molecule is called ω; unsaturated fatty acids are classified as a ω-3, ω-6 and ω-9 according to the distance between the first double bond and ω (Figure 6). Fatty acids belonging to ω-3 and ω-6 classes are defined essential because they cannot be synthesized by mammary glands, therefore they have a big importance on human health.

Origin of milk fatty acids is both endogenous and exogenous. Endogenous fatty acids are *de novo* biosynthesized in mammary gland by the progressive addiction of acetyl-CoA to volatile fatty acids (acetate and 3-hydroxybutyrate). The main enzymes involved in this process are acetyl-CoA carboxylase and fatty acid synthetase (FAS), which is responsible for the progressive elongation of fatty acids until a maximum length of 16 atoms of carbon. The presence of short- and medium-chain fatty acids (C4:0 to C16:0) in milk derives from an altered specificity of FAS; in ruminants this

enzyme exhibits a transacylase with both loading and releasing activity for acyl chains with a length from two to 12 atoms of carbons (Chilliard et al., 2000). The process of elongation can involve not only volatile fatty acids, but also medium chain fatty acids which are uptaken from blood plasma and elongated until 16 atom of carbons. Though palmitic acids (C16:0) is synthesized in the mammary gland, half of C16:0 is of dietary origin. Enzymatic pool of mammary glands is not able to convert C16:0 to C18:0 and the regulation of elongation process at cellular level have yet to be identified. Because of the pathway for biosynthesis, acyl chain is typically linear and with an even number of carbon atoms.

Goat milk fat is rich in medium chain triglycerides. Caproic (C6:0), caprylic (C8:0) and capric acid (C10:0) are so called because preferentially found in goat milk, where they account up to 20% of total fatty acids (Park et al., 2007) and give the typical "goaty" flavour to the milk. Haenlein (2004) reviewing the importance of goat milk in human nutrition, described the importance of MCFA as medical treatments for a wide range of clinical disorders. For example, thanks to the digestive process, different from that used for long chain fatty acids, MCFA can be absorbed and reach the liver and the tissues without any reesterification or storage in adipose tissues. Therefore, goat milk, in comparison to the more consumed cow milk, represents a valid source of direct energy. MCFA are *de novo* synthesized, therefore they are not greatly affect by diet and some of them are synthesized at certain fixed relative rates. In particular, C12:0 (lauric acid) to C10:0 ratio is quite constant in goat milk and it has been used to detect the addiction of cow milk into goat milk. Also C4:0 and C6:0 are either unchanged by diet or lipid mobilization because they are partially synthesized by a metabolic pathway not dependent on acetyl-CoA carboxylase (Palmquist et al., 1980).

Fatty acids of exogenous origin represent up to 60% of milk fat. They are not synthesized by mammary, but arise from preformed fatty acids that can be found in blood plasma under form of lipoproteins and non-esterified fatty acids after digestion and gut absorption or as a consequence of mobilization of body lipid reserves. Lipoprotein lipase allows the hydrolysis of glycerides which became available for uptake by mammary gland and to be included in fat globules. Long chain and odd- and branched chain fatty (OBCFA) acids are typically of exogenous origin, deriving from

forages or supplements given to the animal and from metabolism of bacteria living in the rumen, respectively. That is why a huge amount of literature is available on the effect of diet on goat and ruminants milk fatty acid composition (Sanz-Sampelayo et al., 2007; Chilliard etl., 2004 and 2007).

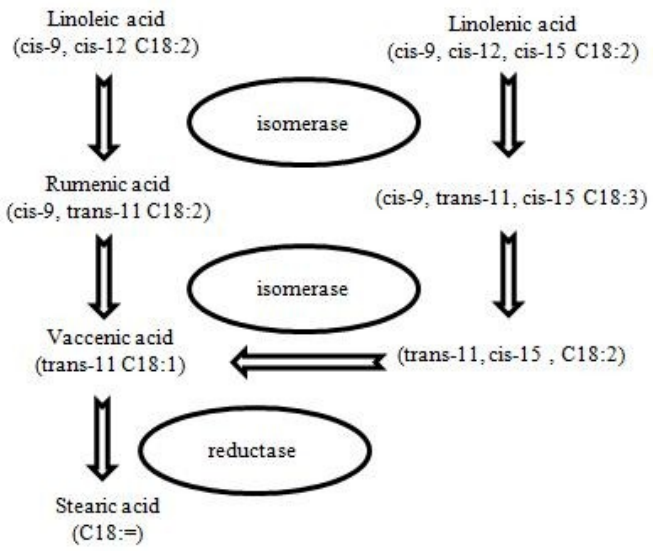
1.3.2.2 Rumen microflora and lipids metabolism

Fat of animal origin is, generally, a concern for customers because it is rich in saturated fatty acids which are responsible for the onset of cardiovascular diseases, whereas PUFA are considered to be protective for human health. For these reasons, on 1994, Department of Health of United Kingdom indicated ideal values for PUFA:SFA and ω-6:ω-3 ratios (≥0.45 and ≤ 4, respectively), referring these values not to the single foods but to the dietary regimen (Department of Health UK, 1994). However, concerns for SFA and healthy properties for PUFA should be reconsidered taking into account that among saturated fatty acids only C12-C16 are thought to have atherogenic effect when consumed in excessive amounts (Knopp and Retzlaf 2004), that stearic acid (C18:0) is not atherogenic (Dabadie et al., 2005) and that some *trans*-isomers of linoleic acid (C18:2) are suspected to be very harmful. The control of fat composition has been a challenge for animal food scientists and still is. About half of milk fatty acids arise from diet (PUFA above all), therefore it is easily understandable how greatly the diet can affect milk fatty acid composition.

An enormous number of study have been carried out in order to make animal diets able to provide PUFA to be transferred into milk. Unfortunately, not all the dietary PUFA arrive unchanged to milk. Polyunsaturated fatty acids present in animal feeding (forages, cereals and oil seeds), are toxic for bacteria living in the rumen. Immediately after their ingestion, they are massively (from 60 to 90%) transformed to stearic acid (C18:0) by cellulolytic rumen microflora with the aim to detoxify them. The process of detoxification is called biohydrogenation because PUFA with a *cis*-12 double bond, are progressively saturated to C18:0 by the addiction of hydrogen atoms (Figure 7). The first step of biohydrogenation is the isomerization of the *cis*-12 double bond to *trans*-11position. Non-esterified linoleic (*cis*-9, *cis*-12 C18:2) and α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3) are the main substrates of this microbial isomerase

because it can act only on *cis*-12 unsaturated fatty acids with free carboxylic function. After isomerization, *cis*-9 bond is hydrogenated by a microbial reductase; eventually, also double bond in *trans*-11 position is reduced to stearic acid.

Figure 7 – Biohydrogenation process



The main effect of biohydrogenation is to worsen milk fat quality by the increase of the proportion of unhealthy fatty acids, indeed it is responsible for the greater percentage of SFA regardless the level of unsaturated fatty acids of the diet provided to the animals. Nevertheless, during the first step, when linoleic acid is the target of biohydrogenation, an intermediate (*cis*-9, *trans*-11conjugatetd linoleic acid (CLA)) with positive effect on human health is produced. As well as all the other intermediates of biohydrogenation, CLA can escape the rumen before undergoing the complete saturation and be transferred to the milk. Since 1979 when its beneficial properties have been discovered in meat juice (Pariza et al., 1979), an increasing number of publications demonstrated the role of CLA in the prevention of certain forms of cancer and on 1996, it was defined as <<*the only fatty acid shown unequivocally to inhibit carcinegenesis in experimental animals*>> in the report on "Carcinogenes in the Human Diet". CLA can inhibit cancer development at different stages (McGuire and McGuire, 2000), but can also reduce cholesterol level in blood (Lee et al., 1994) and prevent diabetes (Houseknecht et al., 1998; Chin et al., 1994). It is unlikely that only one molecule is responsible for all the biological activities, indeed

CLA is not just a single molecule, but it is a series of isomers among which, *cis-9, trans-11*-CLA is the most represented (Pariza et al., 2000) and the only whose activity has been demonstrated; however it is supposable that individual isomers have specific effects.

Rumen biohydrogenation does not represent the only way to produce CLA. In the reality, the quantity of milk CLA arising from rumen is smaller as compared to that arising from tissues. Several works demonstrated a linear relation between concentrations of *trans*-11 C18:1 isomer and *cis*-9, *trans*-11 CLA concentrations in milk fat across a wide range of diets (Baumann et al., 1999). On the basis of these findings, Griinari et al. (1997) suggested that a portion of CLA was of endogenous synthesis. The mammary gland is the site of endogenous synthesis of *cis*-9, *trans*-11 CLA for lactating ruminants. In the udder the action of Δ^9-desaturase enzyme introduces a *cis*-double bond between carbons 9 and 10 of fatty acids. Even if stearoyl-CoA and palmitoyl-CoA are the major substrates for Δ^9-desaturase, a wide range of saturated and unsaturated acyl CoA can serve as substrates, including *trans*-11 octadecenoic acid (Enoch et al., 1976; Mahfouz et al., 1980, Pollard et al., 1980). *Trans*-11 octadecenoic acid escaped biohydrogenation is converted to CLA in the udder providing about 70% of milk CLA.

Rumen microflora metabolism is also responsible for the presence of odd and branched chain fatty acids in ruminants milk. OBCFA are constituents of bacterial membranes (Kaneda, 1991; Mackie et al., 1991) and include: *iso* tetradecanoic acid (*iso* C14:0), pentadecanoic acid (C15:0), 13-methyltetradecanoic acid (*iso* C15:0), 12-methyltetradecanoic acid (*anteiso* C15:0), *iso* hexadecanoic acid (*iso* C16:0), heptadecanoic acid (C17:0), 15-methylhexadecanoic acid (*iso* C17:0), 14-methylhexadecanoic acid (*anteiso* C17:0). Linear odd-chain fatty acids are formed when propionyl-CoA, instead of acetyl-CoA, is used as primer, while branched chain fatty acids are formed by the elongation of isobutiryl-CoA or 2-methylbutiryl-CoA (Kaneda, 1991). Together with SMCFA, they are responsible for the sharp and persistent odor of goat milk. However, the interest in OBCFA arise from their anticarcinogenic effects on cancer cells (Wongtangtintharn et al., 2004) comparable to that of conjugated linoleic acid.

1.3.2.3 Effect of diet on fat synthesis and composition

Milk composition of ruminants is function of intrinsic and extrinsic factors. Though important, intrinsic factors such as breed, genotype, pregnancy etc., can give their effect over long terms, while extrinsic factors, mainly diet, largely affect milk production and composition in the short period.

Forage to concentrate ratio is one of the most important factors affecting milk yield and composition as well as the nature of concentrate. Rumen microflora of grazing goats is mainly represented by cellulolytic bacteria which produce acetic and butyric acid as a result of their metabolism. In certain periods, when grazing is not enough to cover energy requirements of lactating animals, it is necessary to supplement them with concentrate. In principle, providing concentrates that are rich in nonstructural carbohydrates, a higher proportion of concentrate in the diet and smaller-sized particles of fiber or fiber given in pelleted form, represent circumstances that cause a decreasing of cellulolytic in favor of amylolytic rumen microflora with a reduction in the formation of acetate and butyrate. Being these latter volatile fatty acids the main precursors of the fatty acids synthesized in the mammary gland, the animal's milk will have a lower fat content (Sutton, 1976). In addiction, propionic acid produced by amylolytic bacteria is precursor of glucose, which is transformed in to fat in the liver and stored in the adipose tissue causing a further depression of milk and fat secretion. The changes in the rumen microflora lead also to the production of specific (such as *trans*10-C18:1 or trans-10, cis-12 CLA) (Antongiovanni et al., 2004), and this could contribute to a decrease in milk fat yield and content (Griinari and Bauman, 2003). However, in general, no significant effect are observed when concentrate proportion does not exceed 50%, while negative effects are observed when concentrate represents more than 60% of diet.

As regrads goats, it seems that they are more sensitive to the energy intake than to forage:concetrate ratio (Mowlem et al., 1985). Indeed, when goats are provided a diet which in cows causes the so-called low-fat milk syndrome, the changing in milk fat content is negligible. Sauvant et al. (1987) found that the energy status of the animals is more important than the relative proportion of the diet constituents even when forage:concentrate ratio exceeds 20:80. Similar results were achieved by Schmidely et

al. (1999), who, after feeding goats with concentrates differing in the nature of carbohydrates, concluded that milk fat and protein concentrations were more affected by the animal energy balance. These results were confirmed by Mele et al. (2005), who found no changes in milk yield and fat content comparing high-concentrate *vs* low-concentrate diet at similar energy level, and by Chilliard et al. (2006a) who found a significant increase of milk and fat yield after an increase of energy level of diet. The influence of energy status of the goat on the quantity and composition of milk produced could depend on the fact that as compared to cows, ruminal turnover is faster in small ruminants so that they have less time to digest dietary constituents which would require longer time. This minor efficiency of ruminal digestion causes a less availability of energy deriving from volatile fatty acids produced in the rumen that can be compensated by goat only when they are in a good status of energy. Indeed when energy intake is kept constant and cover energy requirements of goats, goat milk fat secretion seems to be more dependent on the nature of forage (Rouel et al., 2000).

Even if goat are less sensitive to the forage:concentrate ratio than other ruminants, a high proportion of concentrate in the diet of goats cannot be a rule, but have to be used with care and when it is strictly necessary. The consumption of rapidly fermentable carbohydrates contemporary to a deficiency of fiber in the diet, could lead to the phenomenon of fat-protein inversion. In these conditions, goats produce a milk in which protein is greater than fat percentage. This situation is typical of the Mediterranean area, where especially in summer, the availability of green vegetation is scarce and goat are supplemented with concentrate to cover energy requirements.

1.4 Proteins

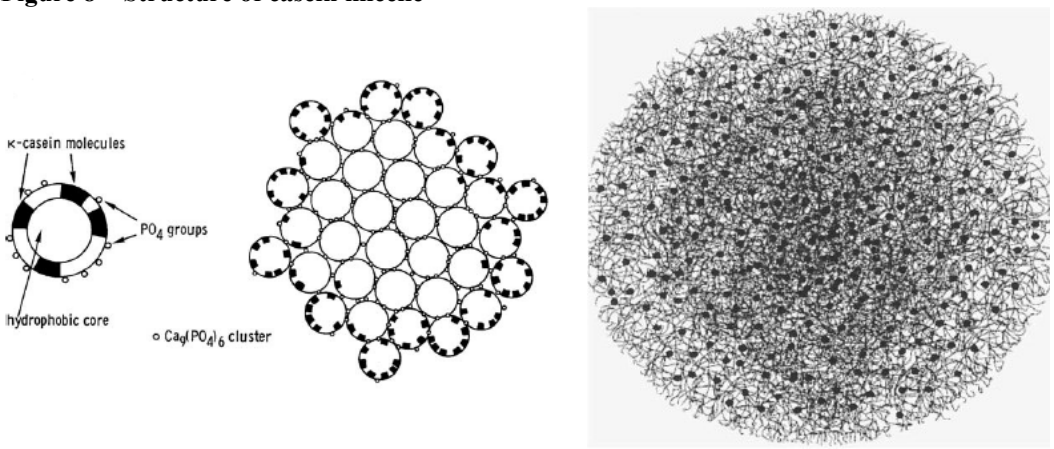
The average protein content in goat milk is about 3.5%, however this percentage can widely vary, also within species, according to several factors such as breed, stage of lactation, genetic polymorphism, feeding etc.

The principal proteins of goat milk are α-lactalbumin (α-Lac), β-lactoglobulin (β-Lg), immunoglobulins (Ig), lactoferrin (Lf), α_{s1}-casein (α_{s1}-CN), α_{s2}-casein (α_{s2}-CN), κ-casein (κ-CN) and β-casein (β-CN) and other minor proteins and ezymes. They can be subdivided in whey protein (α-La, β-Lg, Ig, Lf), so called because they remain in the

serum after the precipitation of the caseins (α_{s1}-CN, α_{s2}-CN, κ-CN, β-CN). In milk it is also present a non-protein nitrogen (NPN) component which includes ammonia, urea, creatinine, creatin and uric acid. Though goat milk protein components are the same of cow and sheep milk, goat milk results in lower renneting properties. This is due to the higher percentage of NPN (5% *vs* 9% for cow and goat, respectively) and a less level of casein nitrogen (73% vs 78% for cow and goat, respectively) (Guo, 2003).

Whey proteins represent a consistent proportion (~20%) of total nitrogen in goat milk. Nevertheless, the interest of research is mainly focused on the casein component because it represents the biggest part of milk protein (~80%), but also because goat caseins show a complex qualitative and quantitative variability resulting from several genetic polymorphisms and post-translational modifications that causes important effects on quality, composition and cheese making properties of goat milk.

Figure 8 – Structure of casein micelle



The α_{s1}-, α_{s2}-, and β-caseins are called calcium sensitive because they are precipitated by calcium binding to their phosphoserine residues. Differently from calcium sensitive caseins, κ-casein is the only soluble in calcium, but also interacts with the other caseins to form stable colloidal particles named micelle. About 95% of caseins are organized in micelles. For many years the most accepted theory of the structure of the casein micelle (Holt et al., 1992) described them as a spherical aggregates of the caseins (submicelles) held together by calcium–phosphate linkages (Figure 8). A major debate in the early studies of casein micelles was the whereabouts of the colloid stabilizing protein, κ-casein. It has been generally accepted that the majority of the κ-casein must reside on the surface of the casein micelles and that the other caseins

might also occur there as well, while the inner centre of the micelles and submicelles is virtually free of κ-casein. Hydrophilic C-terminal region of κ-casein is thought to forming a layer similar to "hair" responsible for the stabilization of casein micelles called glycomacropeptides (GMP). Indeed the removing of this layer by hydrolysis causes precipitation and coagulation of casein micelles.

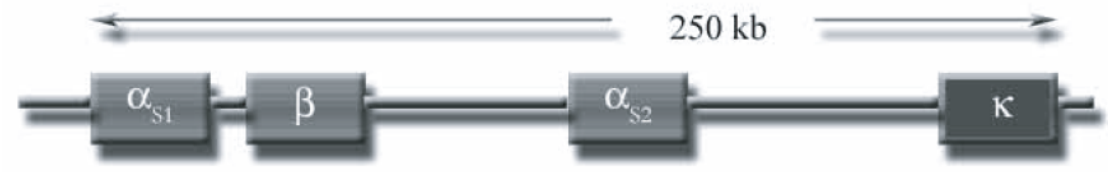
In recent years the classical theory on the micelle structure has been challenged by concepts arising from the study of the casein–calcium–phosphate interactions, the micelles themselves and physical chemical studies of the individual proteins at interfaces. However, the emerging theories agrees with the role of calcium–phosphate and κ-casein (Horne, 1998; De Kruif and Holt, 2003). The description of the new models lies outside the aim of this manuscript.

The micelle structure of goat milk differs from that of cow milk being the diameter of goat micelle higher than in cow milk and this leads to worse renneting properties.

1.4.1 Genetic polymorphism of goat caseins

In goats, as in cattle, casein genes are organized as a cluster (Figure 9), as first reported by Grosclaude et al. (1978). In order, α_{s1}-casein, β-casein, α_{s2}-casein, and κ-casein (Ferretti et al., 1990; Threadgill and Womack, 1990; Rijnkels et al., 1997) span 250 kb on the chromosome 6.

Figure 9 – Structure of the casein genes



The presence of each casein fraction in milk is genetically determined by co-dominant alleles. Several allelic variants associated to normal, intermediate or null content of the relative protein in milk have been reported for each casein fraction. Differences in primary structure arise from single nucleotide polymorphism, insertion/deletion and differential splicing patterns. These modification can directly affect several characteristic of the active form of the protein such as electric charge, size, shape,

hydrophobic properties or can influence the sequence of the promoter or the stop codon reducing or enhancing transcription rate of the gene. Similarly to all the other proteins, caseins undergo post-translation modifications, such us phosphorylation and glycosylation, which increase caseins heterogeneity in milk.

The β-CN is the most abundant casein fraction representing up to 50% of total caseins. Two main phosphorylation levels (5 and 6P) occur with comparable relative concentration, but also 3 and 4P have been reported. It has been considered to be monomorphic for long time. However, up to date five variants have been reported for β-CN. Three of them A, B (Mahe´ and Grosclaude, 1993) and C (Neveu et al., 2002) associated with a normal β-casein content in milk, with the last two allele differing for a single amino acid substitution (Ala177 → Val177) from the A variant. Furthermore, two null alleles (0 and 0') have been identified, both characterized by mutations responsible for premature stop codons in exon 7 (Ramunno et al., 1995) probably responsible for non-functional messengers and for the absence of β -casein in milk (Martin and Addeo, 1995).

Caprine κ-CN represents about 15% of total casein. Differently from the calcium sensitive caseins, it is glycosylated, hydrophilic and shows a lower degree of phosphorylation. The first two variants of caprine κ-casein were identified by isoelectrofocusing (Di Luccia et al., 1990) and successively confirmed both at the protein and DNA level by Caroli et al. (2001). So far, a total of 13 polymorphic sites were identified in the domestic goat (Jann et al., 2004), allowing the identification of 14 alleles corresponding to 11 protein variants. All these variants can be associated to two level of production. Variants of group A with isoelectric point of 5.29 (A, B, B', B'', C, C', F G, H, I, L) are associated with a higher presence in milk as compared to group B (D, E, K, M) which has isoelectric point equal to 5.66.

The α_{s2}-CN represents about 10% of total caseins, and is the only casein fraction which present cistein-cistein bond along the polypeptidic chain. At least eight alleles have been identified (A, B, C, D, E, F G, 0) at α_{s2}-CN locus. All the variants, except D and 0 (Ramunno et al., 2001) are associated to normal level of protein synthesis.

Variants A, B, C variants differ by single aminoacid substitution (Martin and Addeo,1995). At least four phosphorylation levels of α_{s2}-CN are detectable in milk.. Moreover, thought goat milk is generally considered to have low allergenic power, the amount of α_{s2}-CN was associated with allergenic properties. In particular, variants A, B, C, E and F showed higher allergenic potency, as compared to D and 0 (Marletta et al., 2004).

Among casein polymorphisms, polymorphism at α_{s1}-casein locus is of particular interest and deserves to be discussed apart.

1.4.2 Genetic polymorphism of α_{s1}-casein and milk composition

In the goat species, α_{s1}-casein locus is characterized by the most extensive and investigated polymorphism. In the last decades several studies have been carried out to individuate the allelic variants and their relation to goat milk composition and technological properties. The α_{s1}-casein locus spreads a transcriptional unit of 16.7 kb and consists of 19 exons. So far, at least 18 different alleles (for review see Neveu et al., 2002 and Moioli et al., 2007) have been found at this locus (Figure). All the variants can be subdivided into four categories (strong, intermediate, weak and null) as a function of the quantity of α_{s1}-CN in milk.

Among strong alleles (A, B_1, B_2, B_3, B_4, $B^{'}$, C, H, L and M), B_1 contains 199 amino acids residues and is the closest to the bovine and ovine homologous and it is considered as the original one in the goat species; therefore its productive level (3.6 g/L per allele) is taken as the "normal" reference level. The primary structure of the other strong variants contains 199 residues, but differs from B_1 for amino acid substitutions by single point mutations. Only M variants does not depend on amino acid substitution, but on the substitution and loss of phosphate group from allele A.

Intermediate alleles (E and I) produce about 1.1 g/L each. E variant shares structure with B_4, but it has a reduce protein synthesis due to the insertion of a 458 bp sequence in position 124. I variant shares structure with A variant and at the moment no

information is available on the factors responsible for the lower content of α_{s1}-CN in milk.

Weak alleles (D, F, G) contribute with 0.45 g/l of α_{s1}-casein per allele. F variant shows a deletion of 37 amino acid residues arising from an outsplicing of exons 9, 10 and 11 probably due to a single base deletion occurring in the first unspliced exon (exon 9) [15]. In G variant shows a deletion of 13 amino acid residues generated by an exon-skipping event (exon 4) triggered by the G→A transition at the first position in the intron 4 donor splice site [18]. The consequence is the loss of the hydrophobic sequence in the N-terminal part of the protein.

Null alleles (01, 02 and N) have been also found and are responsible for the apparent absence of this fraction in milk. Null allele 01 is characterized by a large deletion of about 8.5 kb starting from the place 181, while 02 is made by a large uncharacterized insertion. The N variant is characterized by the deletion of cytosine at the 23th nucletoide of exon 9 resulting in a premature stop codon at 12th exon.

Enne et al., 1997 in a study on the gene frequencies of the different variants at αs1-casein locus found that null alleles tend were predominant in Northern Italy breeds, while strong alleles were found at the highest frequency in breeds of Southern Italy, lastly breeds intermediate alleles were present in larger extent in Alpine and Saanen breed

1.4.3 Polymorphism at αs1-casein locus on milk characteristics

The genetic polymorphism of α_{s1}-casein causes a big range of variation (0-30%) in the presence of this protein in goat milk, moreover several works found that strong genotypes at this locus are associated with higher total protein and casein content in goat milk. If the direct effect on protein and casein content is obvious, less evidences are available to clarify whether have some direct effects also on the biosynthesis of the other caseins.

Ambrosoli et al. (1988) in a study on the correlation between polymorphism at α_{s1}-casein and milk components, found that milks with high levels of α_{s1}-CN had higher

total solids, phosphorus and lower pH than milks with low levels of α_{s1}-CN, moreover size of casein micelles were smaller in milk obtained from goat carrying strong alleles (Remeuf et al. 1993). Taken all together, these characteristics improve cheese-making properties of milk. Indeed, Clarck and Sherbon (2000) founds that coagulation time (the point at which coagulation is first notable) and coagulation rate (measure of how quickly the curd firms once coagulation has begun) were respectively shorter and higher in milk from goat with strong alleles. However, cheese organoleptic features are negatively correlated to the presence of α_{s1}-CN in milk. It has been shown that lipolysis, one of the processes involved in the formation o flavour, is reduce in high protein milk. A surprisingly relation have been found between the genotype at α_{s1}-CN locus and the biosynthesis of fat. Results published by Barbieri et al. (1995) suggest that fat content is higher in milk from goat with strong alleles as compared to milk from goat with deficient α_{s1}-casein biosynthesis, while milk yield seems to be unaffected by this factor (Chilliard et al., 2006).

It is likely that perturbations occur during secretion of milk components. In the reality, recently, Ollier et al. (2008) demonstrated that weak variants at α_{s1}-casein locus negatively affect gene expression of GPAM and FAS, which are two important genes implicated in the first step of triacylglycerols biosynthesis (Coleman et al., 2000) and in the endogenous biosynthesis of short and medium chain fatty acids (Smith, 1994) respectively. Moreover, at cellular level, Chanat et al. (1999) observed that in the mammary epithelial cells the rate of transport to the Golgi apparatus of caseins other than α_{s1}-casein was strongly reduced in goats carrying defective alleles. As a consequence of the accumulation of immature proteins and caseins, the endoplasmatic reticulm of weak genotype animals was remarkably enlarged in comparison to strong genotype goats, while the endoplasmatic reticulum of goats with intermediate alleles was only moderately distended. From one side this could explain the lower biosynthesis rate of caseins, but at the same time this dysfunction could disturbs the whole secretion process, including that of lipids as hypothesized by Neveu et al (2002).

In the main part of Europe, farmers have the interest in the selection of goat with high content of casein in order to increase the economical profits by rising cheese yield. Nevertheless, the breeding of goats carrying weak or null alleles could be justifiable if the aim is the production of goat milk to be used as a substitute of human milk. Indeed, compared to cow milk, goat milk CN is more similar to human milk and can contain only traces of the allergenic α_{s1}-casein resulting less allergenic. Indeed, in trial on pigs, Bevilacqua et al. (2000) found a 40% reduction in the allergic reaction when pigs were fed with milk deficient in α_{s1}-casein, concluding that goat milk with low or null content of as1 casein is less allergenic than other goat milk. Moreover both the smaller quantity of protein and fat could result in a more digestible food for humans (Ambrosoli et al., 1988).

1.4.4 Rumen metabolism and protein biosynthesis

Differently from milk fat, which can partially derive from ingested diet, milk protein are entirely synthesized in the mammary gland. However, also in this case rumen metabolism plays a major role. Indeed milk protein synthesis depends on amino acids taken up by the mammary gland and the amount of these amino acids depends on the amounts of microbial cells and by-pass protein deriving from the rumen.

Ruminant tissues require the same amino acids as most simple stomached animals; however, ruminants can survive on non-protein diets that are virtually free of amino acids. The absence of an absolute requirement for dietary amino acids does not lies in the fact that ruminants are able to *de novo* synthesize amino acids, but in the ability of microflora to do that. Indeed even when the diet contains little non protein nitrogen, 50 to 80% of the N reaching the small intestine is likely to be of microbial origin (Hogan et al., 1975).

Rumen microflora need a source of dietary nitrogen and of carbohydrates to build up amino acids. The proportion in which these nutrients are given to the animal and their characteristics greatly affect the rate of microbial protein biosynthesis. If the energy is limited, microorganisms degrade feed protein to ammonia to produce energy, but they cannot uptake the ammonia to build new amino acid and protein (Nocek and Russel, 1988). The ammonia escaped the rumen is not included in microbial cells (Nolan et

al., 1975), but is detoxified in the kidney and in the liver loss under form of urea in milk and urine. Milk urea is then considered a good indicator of the efficiency of the utilization of dietary nitrogen.

Quantity and quality of dietary nitrogen and carbohydrates source have to be chosen also bearing in mind productive requirements of the animals. In this sense, goats with different genetic aptitude to produce milk protein could use dietary nutrients in different ways or could have different productive requirements. Investigating this aspect, Schmidely et al. (2002) found differences in the utilization of dietary protein, calcium and phosphorus provided to the goats carrying different alleles (strong *vs* weak) at α_{s1}-casein.

1.5 Aim of the work

In the previous sections an overview on milk composition and on the principal factors which influence the level of the different constituents of milk has been given. Among them particular interest have been focused on the effects of diet and α_{s1}-casein genetic polymorphism; in the biggest part of the published researches made in order to assess the effect of these two factors on milk composition they are discussed separately. Besides to cover a lack of scientific knowledge on this topic, we think that to study the effect of interaction between dietary and genetic factors on milking performances can be of great utility for the development of new feeding strategies for dairy goat. Indeed, the design of specific diets based on the productive potential also for dairy goats can be an economical advantage for the farmers and can reduce the environmental impact of animal breeding.

Therefore, the aim of this study was to investigate the impact of different feeding practices on the performances of dairy goats differing in genotype at α_{s1}-casein locus. Specifically, we investigated:

- whether a different genetic aptitude for producing casein can affect diet selection and milk composition in lactating goats, by making available to them feeds with differing chemical nutritive;
- how goats, selected according to different α_{s1}-casein genotype, could reply to diets with different energy levels at similar protein content;
- the effect of the diet, genotype and diet × genotype interaction on milk fatty acid and casein composition.

2

Diet selection and milk production and composition in Girgentana goats with different *a*s1-casein genotype

Avondo M., Pagano R.I., Guastella A.M., Criscione A., Di Gloria M., Valenti B., Piccione G., Pennsisi P. (2009). Diet selection and milk production and composition in Girgentana goats with different αs_1-casein genotype. *Journal of Dairy Research*. *76:202-209.*

Abstract

In goats, as1-casein polymorphism is related to different rates of protein synthesis. Two genetic variants, A and F, have been identified as strong and weak alleles based on a production of 3.5 and 0.45 g/l of as1-casein per allele. The aim of the trial was to test whether goats can select their diet as a function of their genetic aptitude to produce milk at different casein levels and whether this selection can influence milk production or composition. Two groups of 8 animals, homozygous for strong (AA) or weak (FF) alleles were housed in individual pens. Using a manger subdivided into five separate containers, the goats were offered daily for 3 weeks: 1.5 kg of alfalfa pelleted hay, 0.7 kg of whole barley, 0.7 kg of whole maize, 0.7 kg of whole faba bean and 0.7 kg of pelleted sunflower cake. Total dry matter intake was similar between groups and resulted in nutrient inputs much higher than requirements. On average, goats selected 86% of maize plus barley and only 46% of faba bean plus sunflower. Indeed, AA goats selected less faba bean compared with FF goats (37.2 v. 56 . 7% of the available amount; P=0.01); during week 2 and week 3 they significantly increased maize selection (respectively for week 2 and week 3: 94.9 and 99.1% v. 85 . 3 and 87.3%) thus increasing the ratio between the highenergy feeds and the high-protein feeds (2.41 v. 1.81, P=0.023). As for true protein, the high soluble fraction (B1) and the indigestible fraction (C) were lower in the diet selected by AA goats (respectively in AA and FF groups: B1, 7.85 v. 9.23% CP, P<0.01; C, 6.07 v. 6.30% CP, P<0.001); these diet characteristics can be associated with lower losses of protein. Milk production, being similar in AA and FF groups when goats were fed with a mixed diet, significantly increased in AA group, when free-choice feeding was given (mean productions: 1198 v. 800 g/d, P<0.01). Casein content was higher in AA group than in FF group (2.70 v. 2.40%, P<0.01) whereas milk urea was higher in FF group (59.7 v. 48 . 8 mg/dl, P<0.01). In conclusion, when the animals were free to select their diet, their higher genetic aptitude to produce casein seemed to adjust their energy and protein dietary input in qualitative terms, thus leading to an increase in milk production and a decrease in milk urea. These results seem to demonstrate that interactions probably occurred between genetic polymorphism at the as1-casein locus, diet selection and the efficiency of nutrient transformation into milk.

Introduction

The genotype of individuals greatly affects the milk concentration of casein: goats with strong (AA) and weak (FF) genetic profiles will produce milk with as1-casein content of around 7 g/l and 0.9 g/l respectively (Martin et al. 1999). Milk protein synthesis depends on amino acids taken up by the mammary gland. The amount of amino acids available for this synthesis depends on the amounts of microbial cells and by-pass protein deriving from the rumen. There should be a link between the genotype that determines different casein levels in milk and the efficiency of use of the available nutrients. Very few studies have been conducted on the interactions between polymorphism at the alpha-s1 casein locus and nutrition. De la Torre et al. (2008) report that a greater efficiency in nitrose and energy utilization of goats with strong alleles v. weak alleles may explain the differences in milk composition between the two genetic groups. Moreover, this efficiency is strongly influenced by the characteristics of the diet. It has been demonstrated that food preferences can depend on physiological state (Kyriazakis et al. 1999; Villalba & Provenza, 1999). Studies on goats highlighted that lactation stage affects feed selection in extensive (Mellado et al. 2005) and intensive feeding systems (Fedele et al. 2002). It seems that ruminants are able to relate the sensory properties of a feed to the post-ingestive feedback signals learned from experience (Provenza et al. 1995). In particular, different studies have shown that foods that meet requirements for energy and nitrogen are preferred (Villalba & Provenza, 1997a, b). The aim of this research was to assess whether a different genetic aptitude for producing casein can affect diet selection and milk composition in lactating goats, by making available to them feeds with differing chemicalnutritive characteristics.

Materials and Methods

Animals and feeding management

Sixteen Girgentana goats (3rd and 4th lactation), homogeneous for milk production (0.8±0.1 kg/d), days of lactation (110±15 d) and body weight (37.6±5.1 kg) were selected from a flock of 120 goats deriving from three farms located in different areas of Sicily. Animals were divided into two groups, eight homozygous for strong (AA) and eight homozygous for weak (FF) alleles, as characterized by isoelectric focusing

(IEF) in ultrathin polyacrylamide gels according to Erhardt et al. (1998) and allele specific-polymerase chain reactions (AS-PCR) at the CSN1S1 locus for the strong A and weak F alleles according to Leroux et al. (1992). Moreover, the absence of null allele at as2- and b-casein loci was ascertained with PCR-RFLP (Ramunno et al. 2001) and AS-PCR (Rando et al. 1996) reactions, respectively. Prior to starting the study and during the trials the health status was evaluated based on behaviour, rectal temperature, heart rate, quality of respiration, cough, nasal discharge, eye discharge, faecal consistency, haematological and haematochemical profiles, and somatic cells count (SCC). All the animals were housed in individual pens with mangers subdivided into five separate containers. Goats were offered alfalfa pelleted hay, two sources of starch (whole barley and whole maize) and two sources of protein (whole faba bean and pelleted sunflower cake). In each pen water and salt were always available. The preexperimental period consisted of a 7-d period, during which the animals received a mixed ration of 1.5 kg of hay and 0.2 kg of each concentrate; a 10-d period during which goats received, separately, 1.5 kg of hay and a quantity of each concentrate gradually increasing from 0.2 to 0.7 kg. Taking into account that intakes tended to increase for each increment of concentrates supplied, we did not increase further the amount supplied, to avoid risks of metabolic diseases. The adaptation period lasted 7 d during which the animals received the experimental diet consisting of 1.5 kg of hay and 0.7 kg for each concentrate feed. The experimental period lasted 3 weeks (5–26 May).

Table 1 - Chemical composition of the available feeds.

Feeds	Alfalfa hay	Barley	Maize	Faba Bean	Sunflawer cake
Dry Matter %	93.3	89.6	86.4	86.1	89.4
Crude Protein, %DM	15.0	10.9	9.2	27.0	31.9
Neutral detergent fibre % DM	52.6	22.8	11.4	20.4	44.3
Acid detergent fibre % DM	35.1	6.4	2.1	14.1	31.3
Water-soluble carbohydrates % DM	7.1	2.5	1.5	4.7	4.0
Starch	1.9	49.0	65.2	46.8	0.7
Protein Franctions % CP					
A	28.2	6.5	11.4	15.6	22.3
B1	2.2	11.2	5.7	23.8	7.1
B2	37.1	55.9	60.9	43.7	48.4
B3	24.5	22.5	14.3	12.2	16.0
C	8.0	3.9	7.7	4.7	6.2

Data collection and analysis

Individual intake of each feed was measured daily, on the basis of residuals. Every three days individual milk production was recorded and milk samples were individually collected from the morning and evening milking. Three samples for each feed were analysed for dry matter (DM), crude protein (CP) (AOAC, 1990), structural carbohydrates (Van Soest et al. 1991), water-soluble carbohydrates (WSC) by a modified anthrone method (Deriaz, 1961), starch by an enzymic procedure (Megazyme International Ireland Ltd., Bray, Co. Wicklow), protein fractions according to Licitra et al. (1996).

Milk samples, consisting of proportional volumes of morning and evening milk, were analysed for lactose, fat, protein and SCC by an infrared method (Combi-foss 6000, Foss Electric, Hillerød, Denmark). Total nitrose (TN), non-protein nitrogen (NPN) and non-casein nitrose (NCN) were determined by FIL-IDF standard procedures (1964). From these nitrogen fractions, total protein (TN*6.38) and casein [(TN–(NCN*0.994))*6.38] were calculated. Milk urea content was determined using a differential pH meter (CL10, Eurochem, Savona, Italy).

Blood samples were collected, from all subjects, every three days before feeding, by jugular venipuncture, using vacutainer tubes (Terumo Corporation, Tokyo, Japan) with K3-EDTA. Blood samples were clotted at room temperature for 1 h and centrifuged at 1360 *g* for 10 min; sera were separated and stored at –20 *8*C until analysed. Sera were analysed with commercially available kits by means of a u.v. spectrophotometer (model Slim SEAC, Firenze, Italy). Serum concentrations of the following parameters were determined: albumin, total protein, total cholesterol, bilirubin, urea, glucose, NEFA, triglycerides, glutamate oxalacetate transaminases (GOT) and glutamate pyruvate transaminases (GPT).

Statistical analysis

Pre-experimental data for DM intake, milk production and composition were analysed using a one-way ANOVA. Individual data for intake, diet selection, diet composition, milk production and composition, and blood parameters were analysed using the GLM procedure for repeated measures of SPSS (SPSS for Windows, SPSS Inc., Chicago IL, USA). Milk production (means of the 3 weeks) was used as a covariate in fat, protein,

lactose, casein and urea analysis. As covariance was never significant (P>0.05) it was not included in the statistical model.

Results

The health status of goats, checked during the trial, was good: no clinical or subclinical signs of disease were observed. Values of rectal temperature, heart rate, respiratory rate, digestive function and SCC were within the normal range. The haematochemical trends obtained in the two groups were within the physiological range for the goat (Kramer & Hoffmann, 1997). Moreover the monitoring of GOT and GPT in all subjects showed the absence of hepatic diseases. No statistical differences were found in blood parameters, between groups (data not reported).

Table 1 shows the chemical composition of the five feeds. Cereal grains, maize and barley contained different levels of carbohydrates [respectively: starch, 65.2 and 49.0% DM; neutral detergent fibre (NDF), 11.4 and 22.8% DM]. Sunflower cake and faba bean were characterized by a high protein content and a different carbohydrate composition (respectively: starch, 0.7 and 46.8% DM; NDF, 44.3 and 20.4% DM); moreover, true protein in faba bean was more rapidly degradable than in sunflower, as demonstrated by the higher levels of the soluble fraction (B1), and the lower level of the neutral-detergent insoluble nitrogen (B3).

Table 2 – Composition of the selectes diet (% dry matter, DMI) and feeds chosen (% of available feeds)

	Genotype		Signficance (P)			EMS
	AA	FF	Genotype (G)	Week (W)	G×W	
Feed chosen % available feeds						
Hay	69.3	66.0	0.237	<0.001	0.825	39.4
Barley	78.9	87.4	0.202	<0.001	0.044	72.8
Maize	92.4	85.4	0.209	<0.001	0.041	65.0
Faba bean	37.2	56.7	0.010	<0.001	0.828	122.2
Sunflower cake	44.6	47.2	0.639	<0.001	0.002	135.2
Energy feed to protein ratio †	2.36	1.84	0.023	<0.001	0.012	0.20
Composition of the selected diet						
Crude Protein, %DM	15.7	16.5	0.068	<0.001	0.011	0.40
Neutral detergent fibre % DM	33.5	32.8	0.217	<0.001	0.468	2.02
Acid detergent fibre % DM	19.7	19.3	0.354	<0.001	0.490	0.48
Water-soluble carbohydrates % DM	4.4	4.4	0.263	<0.001	0.115	0.01
Starch	27.1	27.6	0.425	<0.001	0.173	1.91
Protein Franctions % CP						
A	18.6	18.2	0.090	<0.001	0.049	0.15
B1	7.9	9.2	0.002	0.059	<0.001	0.04
B2	46.1	45.8	0.183	0.004	0.251	0.11
B3	19.3	18.8	0.019	<0.001	0.019	0.07
C	6.1	6.3	<0.001	<0.001	<0.001	0.01

† Maize plus barley intake / faba bean plus sunflowerintake (g DM/d)

Table 2 shows results for feed choice and composition of the selected diets. On average goats from both groups showed a strong preference for maize, barley and hay, whereas they consumed less high-protein feeds. The genotype significantly influenced feeding behaviour: selection activity towards faba bean was significantly lower in AA group. Other significant grouprweek interaction effects are highlighted in Fig. 1: during week 1 AA goats elected less sunflower and barley, whereas from week 2 they selected more maize, compared with FF group. As a consequence of this selective activity the resulting energyrich to protein-rich feeds ratio was significantly higher in AA group. Despite the differences in feed selection, the CP and carbohydrate contents of the selected diets were surprisingly similar in both groups. As for true protein, the soluble fraction (B1) and the indigestible fraction (C) were significantly lower in the diet selected by AA group.

Table 3 – Dry matter intake, milk production and composition and casein yield

	Pre-experimental period (mixed ration)			Experimental period (free-choice feeding)					
						Signficance (P)			
	AA†	FF†	P	AA	FF	Genotype (G)	Week (W)	G×W	EMS
Dry matter intake, g/d	1.81	1.77	0.869	2.53	2.62	0.133	<0.001	0.191	0.02
Milk production, g/d	747.1	749.6	0.982	1197.9	800.5	0.001	0.009	0.020	2880.3
Fat, %	3.31	3.23	0.868	2.42	2.84	<0.001	<0.001	0.058	0.03
Protein, %	3.51	3.06	0.008	3.45	3.17	0.005	<0.001	0.062	0.01
Lactose, %	4.61	4.65	0.660	4.38	4.55	0.009	<0.001	0.663	0.01
Urea, mg/dl	32.7	39.8	<0.001	48.8	59.7	0.008	0.413	0.267	5.60
Casein, %	2.8	2.4	<0.001	2.7	2.4	0.006	<0.001	0.235	0.01
Casein g/d	21.3	18.3	0.299	32.3	19.2	<0.001	0.012	0.026	2.16

† Genetic variants, see text for details

Table 3 shows results for DM intake, milk yield and composition. Total DM intake was not affected by genotype. AA goats, starting from the adaptation period, increate their milk production reaching values significantly higher than FF goats (Fig. 2). Percentages of protein and casein, as expected for the genotypes studied, were significantly higher in the group with strong alleles (AA), whereas lactose was higher in the group with weak alleles (FF). As a consequence of the increased milk production, AA goats achieved a mean casein production 68% higher than FF goats. Milk urea was significantly higher in the FF goats, since the pre-experimental period (Fig. 3). Fat percentages, noticeably lower compared with those recorded during the pre-experimental period, were significantly lower in AA goats.

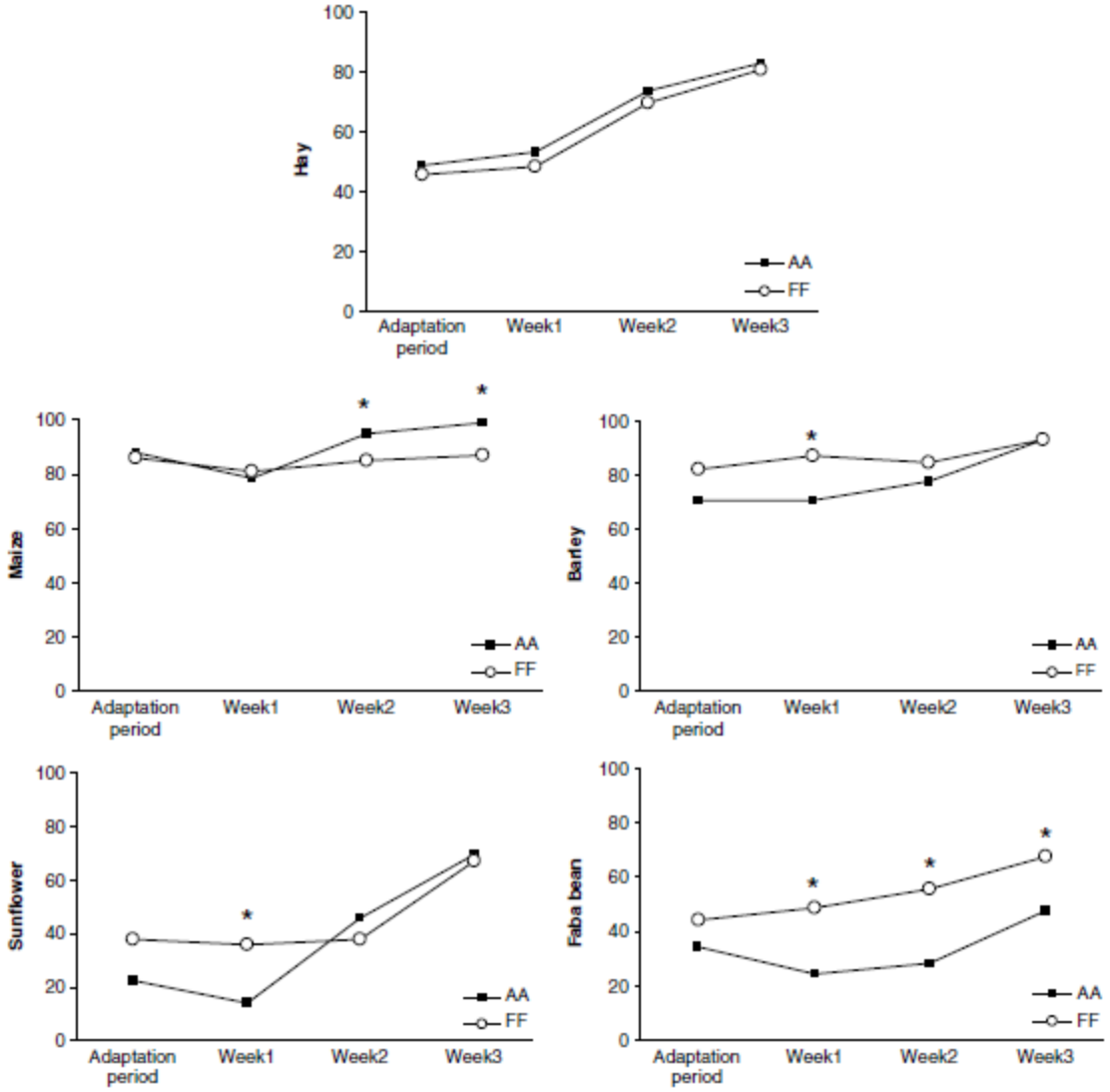


Figure 1 – Hay, maize, barley, sunflower and faba bean selection (% of each available DM amont) in AA (■) and FF (○) goats; * P<0.05

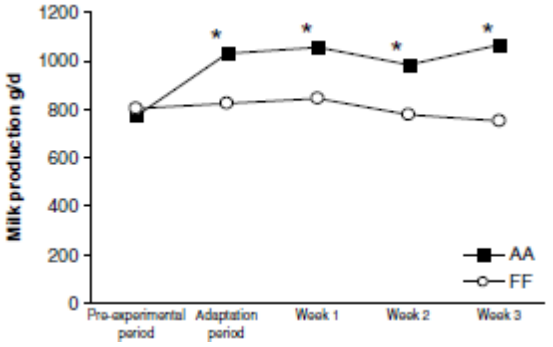


Figure 2 - Milk production (g/d) in in AA (■) and FF (○) goats; * P<0.05

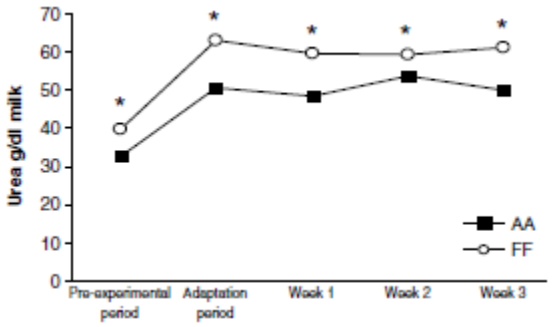


Figure 3 - Milk urea (mg/dl) in in AA (■) and FF (○) goats; * P<0.05

Discussion

In our experimental conditions, with the animals left free to choose their diet, intake was particularly high, on average equal to 170 g DM/kg metabolic body weight. According to INRA recommendations (Morand-Fehr & Sauvant, 1988), similar levels of DM and protein intake correspond to the requirements of a goat weighing 70 kg (compared with about 40 kg in our experimental conditions) and producing 4 l/d (compared with about 1 l). Despite this high nutrient input, goats did not show any clinical signs of metabolic disorders and blood parameters were within the normal range of values. Feeding behaviour shown by goats, when free to choose their diet, can probably explain this response: all the goats, during the experimental period, strongly increased selection towards hay and sunflower, the most fibrous feeds (Fig. 1); this behaviour seems to support the hypothesis that ruminants are able to select their diet in order to attenuate ruminal acidosis (Cooper et al. 1996; Phy & Provenza, 1998; Abijaoude´ et al. 2000a; Keunen et al. 2002). Moreover, goats selected on average 83 and 89% of the available barley and maize but only 47% and 46% of faba bean and sunflower, preferring the high-energy and low-protein feeds over the high-energy and high-protein feeds. This selective behaviour suggests that goats increased Energy intake without paying the metabolic price of an excessive protein intake. The capability of ruminants to respond to nutritional imbalances by modifying their feeding behaviour has been shown by Villalba & Provenza (1996, 1997a) and it seems linked to the perception of postingestive feedback signals related to the sensory properties of foods; in fact the animals can learn the negative or positive nutritional consequence of foods from their own experience (Provenza, 1995; Duncan et al. 2006). We cannot exclude the possibility that a supply of a lowprotein roughage might have resulted in a reduction of protein intake, also limiting the high energy input. However, using a poor quality roughage, which is generally associated with low palatability (Greenhalgh & Reid, 1971) would bring with it the risk of this aspect, more than nutritional and metabolic motivations linked to genotype, exercising a confounding effect on selective behaviour. As regards genotype effect, there are very few reports on the relationships between as1-casein polymorphism and nutrition. Schmidely et al. (2002) and de la Torre et al. (2008), in controlled feeding conditions, found greater intakes and a better diet efficiency in goats with strong alleles. In our

experiment we did not find any intake differences between groups. However, the genotype significantly influenced feeding behaviour: selection activity towards faba bean was significantly higher in FF group.

Moreover, during week 1 AA goats selected less sunflower and barley, whereas from week 2 until the end of the trial they selected more maize, compared with FF group. On average, this behaviour resulted in a higher ratio of energyfeeds to protein-feeds in AA goats (Table 2). In this way AA goats probably improved the efficiency of microbial protein synthesis and increased the availability of amino acids for mammary protein synthesis. In fact, it has been widely demonstrated that milk protein concentration is positively influenced by energy concentration of the diet (Nocek & Russel, 1988; Coulon et al. 2001; Pulina et al. 2008).

Surprisingly the different feeding behaviours in the two genotypes did not result in different contents of CP and carbohydrates in the selected diets. It should be taken into account, however, that even foods formulated to be isocaloric or isonitrogenous may differ functionally (Atwood et al. 2006) so creating nutritional characteristics beyond the scope of gross analysis. For example, in our experiment, AA goats consumed lower highly soluble (B1) and insoluble (C) true protein fractions, compared with FF goats, probably obtaining lower losses of protein, in terms of NH3-N and in terms of totally indigestible protein (Licitra et al. 1996). For starch-rich feeds, maize being less degradable than barley (Sauvant, 1997; Hadjipanayiotou, 2004) and with a higher proportion of ruminal escape starch, is supposed to affect the rumen fermentation pattern (Casper & Schingoethe, 1989) and microbial protein synthesis (Offner et al. 2003) and to be more efficiently used for milk production compared with barley (Nocek & Tamminga, 1991). At the start of the pre-experimental period the goats, fed with a mixed ration of 1500 g of pelleted hay and 200 g of each feed used during the trial, were not able to select their diet. Under those conditions, goats produced similar amounts of milk. However, starting from the free-choice adaptation period, milk production tended to increase significantly in AA group, even though the goats were well beyond the peak of lactation. To our knowledge there are no reports in the literature of similar productive differences between AA and FF genetic types. Schmidely et al. (2002) report that, when feeding practice is monitored, AA and FF goats have similar milk production, concluding that a direct effect of the genotype for

as1-casein on milk production is unlikely. As our goats, at the beginning of the trial, were homogeneous in terms of milk production and lactation stage, it is possible that, during the experimental period, giving the animals the opportunity to select their diet, the different feeding behaviour between groups induced different efficiencies of nutrient utilization for milk secretion; it seems to suggest an indirect effect of genotype on milk yield by way of the difference in selective activity.

Total milk protein content was obviously closely related to the casein content. On the basis of the classification of Martin et al. (1999) AA goats should have produced milk with 0.6% more casein than FF goats. In our study, milk casein content was only 0.3 percentage points higher in group AA. This result may be related to the 'dilution' effect on casein content of the higher milk production in AA group. Moreover, Caravaca et al. (2008) recently highlighted that breed-specific genetic and/or environmental factors can modulate the impact of the as1-casein gene polymorphism on its synthesis rate. Important differences between groups were seen for milk urea levels, which were significantly higher in FF goats. High concentrations of milk urea in ruminants are a consequence of excessive dietary CP (Broderick, 2003; Cannas, 2004) or an inadequate balance between protein and non-structural carbohydrate sources, both in quantitative terms and in rumen degradability (Hristov & Ropp, 2003; Moharrery, 2004) indicating a low efficiency of microbial synthesis. In the present study, urea levels were already higher in FF group during the pre-experimental period, when the animals were fed with the same diet, similarly to the finding of Schmidely et al. (2002); this difference tended to increase during free-choice feeding. In fact FF goats, selecting a diet with a worse combination of energy and protein feeds, compared with AA group, probably reached a lower efficiency of transfer of nitrose into milk protein. Moreover, a diet higher in highly soluble protein and lower in rumen-escape protein, was characterized by higher protein losses and lower milk protein levels.

Milk fat content, in both groups, was low in comparison with previous results (Todaro et al. 2005; Avondo et al. 2008) on Girgentana goats and was lower than protein content. This phenomenon of inversion of fat and protein percentages is not rare in goats (Pulina et al. 2008) during spring at mid lactation (Abijaoudé et al. 2000b; Bocquier et al. 2000; Morand-Fehr et al. 2000); it can be related t the

combined effects of lactation stage, day length (these conditions are consistent with our experimental conditions) and nutritional factors (Kawas et al. 1991; Santini et al. 1992; Chilliard et al. 2003) such as the low content of roughage in the diet selected by our goats, on average equal to 36% of DM. In this regard, according to INRA guidelines for goats, to maintain a good level of milk fat, forage should never be less than 40% of the ration (Morand-Fehr & Sauvant, 1988). The percentage of fat, which was similar in the two genetic groups before the experiment started, decreased significantly thereafter in the AA group. Such a finding has not been reported previously; indeed, the opposite is reported (Grosclaude et al. 1994; Barbieri et al. 1995; Chilliard et al. 2006). As milk fat percentage is closely linked to production levels, as suggested for protein, it is more likely that the lower percentage observed in AA goats was the result of a 'dilution' effect, rather than a direct result of genetic origin. During the 3-week experimental period goats of both groups increased body weight by about 2 kg. Taking into account that, even consuming similar amounts of nutrients, AA goats ate more energy feeds and produced more milk but with a lower level of fat, it could be hypothesized that a different partitioning of energy between tissue deposition and milk synthesis occurred (Kawas et al. 1991) as a consequence of the different proportion of feeds in the selected diet.

Conclusions

In conclusion, goats ate much more protein and energy than their apparent requirements; however, the genetic aptitude to produce higher casein levels induced AA goats to improve their energy and protein input, achieving an unexpected increase in the yield of milk, which was richer in protein and lower in urea. These results seem to demonstrate that interactions probably occurred between genetic polymorphism at the as1-casein locus, diet selection and the efficiency of nutrient transfer into milk. Further research is needed to understand the role of endocrinal and metabolic mechanisms involved in these interactions.

3

The role of polymorphism at αs1-casein *locus* on milk fatty acid composition in Girgentana goat

Valenti B., Pagano R.I., Pennsisi P., Avondo M.. (2009). The role of polymorphism at αs_1-casein locus on milk fatty acid composition in Girgentana goat. *Italian Journal of Animal Science. 8 Supplement 2; p. 441-443*.

Abstract

Sixteen lactating Girgentana goats were used to evaluate the effect of polymorphism at αs1-casein locus on milk fatty acids composition. Animals, homogeneous for milk production, days of lactation and body weight, were divided into two groups: eight homozygous for strong allele (AA group) and eight homozygous for weak allele (FF group). The experimental diet, identical for the two groups, consisted of alfalfa hay (1.5 kg), whole barley, whole maize, pelleted sunflower and whole faba bean (0.5 kg each). In spite of identical selected diets, also in terms of fatty acids, milk fatty acid composition resulted different between the two groups. In particular, except for C8:0, short and medium chain fatty acids and odd chain fatty acids resulted in higher percentage in the AA group. Taking in account that the difference reported in our experiment concerns above all de novo synthesized fatty acids, our results seem to confirm the hypothesis that polymorphism at αs1-casein locus can influence milk fatty acid composition in goats.

Introduction

αs1-casein polymorphism has been recognized as one of the major responsible for the casein content variation in goat milk (Leroux et al., 1992) and its technological properties. Most of the 17 alleles detected at this locus are associated with four levels of αs1-casein (CSN1S1) in milk ranging from 0 (CSN1S1 01, 02, N) to 3.6 g/L (CSN1S1 A, B1, B2, B3, B4, C, H, L) per allele (Martin et al., 1999). As compared to weak alleles, milk from goats with strong alleles at this locus, shows a greater total milk protein content, better cheese making properties, a higher fat milk concentration and seems to have a different fatty acid composition (Chilliard et al., 2006). Data on the effect of αs1-casein locus polymorphism on goat milk fatty acid composition are lacking and sometimes in disagreement.

The aim of this research is to provide new data to better understand the influence of polymorphism at αs1-casein locus on fatty acid profile in goat milk.

Material and methods

The experiment lasted 3 weeks. Sixteen lactating Girgentana goats, homogeneous for milk production (1.94±0.24 kg/d), days of lactation (110±15 d) and body weight

(37.6±5.1 kg) were divided into two groups: eight homozygous at αs1-casein locus for strong allele (AA group) and eight homozygous for weak allele (FF group). All the animals, housed in individual pens, were given, separately, alfalfa hay (1.5 kg), whole barley, whole maize, pelleted sunflower and whole faba bean (0.5 kg each). Goats were hand-milked twice per day. Daily, individual intakes of each feed were recorded and samples were taken for the chemical analyses. Twice a week, milk productions were recorded and samples were collected from each animal. Feeds were analyzed for dry matter, crude protein (AOAC, 1990), NDF (Van Soest et al., 1991), water soluble carbohydrates (WSC) (Deriaz, 1961), starch (Megazyme International Ireland Ltd.), and fatty acids profile (Palmquist & Jenkins, 2003).

Milk samples were analyzed for fat and protein by infrared method (Combi-foss 6000, Foss Electric, Hillerød, Denmark) and for fatty acid profile (Chouinard et al., 1999). Total nitrogen (TN), non-protein nitrogen (NPN), and non-casein nitrogen (NCN) were determined by FIL-IDF standard procedures (1964). From these nitrogen fractions, total protein (TN*6.38) and casein ((TN–(NCN*0.994))*6.38) were calculated. Data were analyzed using the GLM procedure for repeated measures of SPSS (SPSS for windows, SPSS Inc., Chicago, IL).

Results and conclusions

No significant differences were reported between the two groups for DM intake, choice within the 5 feeds, crude protein and carbohydrates content of the selected diets.

As expected, casein level resulted significantly higher in AA goats. Consistently with Chilliard et al. (2006), but in disagreement with Avondo et al. (2009), genotype did not affect milk yield. In contrast with Chilliard et al. (2006) and Schmidely et al. (2002), fat percentage did not show differences between groups (Table 1); moreover, both genotypes had lower milk fat than usually reported for Girgentana goats (Avondo et al., 2008) in all probability because of the diet rich in concentrates offered to the animals (Slater et al., 2000).

In our case the phenomenon of fat-protein inversion occurred, and probably this masked the higher proportion of fat that would be expected for the AA group.

Table 1 - Milk production and chemical composition				
	Genotype		P	SE
	AA	FF		
Milk yield (g/d)	925.3	801.2	ns	3693.72
Fat %	2.5	2.7	ns	0.10
Protein %	3.6	3.2	***	0.01
Casein %	2.8	2.4	***	0.01

ns: not-significant; *: P<0.05; ** P<0.01; ***: P<0.001

Milk fatty acid composition resulted affected by genotype. Among all the fatty acids investigated, ten showed statistically significant differences between the two groups (Table 2).

Table 2– Effect of genotype on milk fatty acid composition (% of total fatty acids)				
	Genotype		P	SE
	AA	FF		
C8	2.89	3.12	*	0.14
C9	0.32	0.25	*	0.01
C11	0.47	0.29	*	0.02
C12	7.66	5.92	***	0.32
C12:1	0.35	0.21	***	0.01
C13	0.31	0.21	*	0.01
C14	14.56	13.55	*	0.86
C15 anteiso	0.37	0.24	**	0.01
C15	1.62	1.15	**	0.18
C16	24.14	26.40	*	3.78
C16:1	0.92	0.74	*	0.01

*: P<0.05; ** P<0.01; ***: P<0.001

These findings are partially in contrast with Schmidely et al. (2002) who reported differences only for C14:0, which was higher in FF group, and for odd fatty acids C15:0 and C17:0, that similarly to our findings, were higher in the AA group. However, in that case, as suggested by Chilliard et al. (2006), the results might have been due to the negative energy balance of the AA goats. Except for C8:0, fatty acid profile obtained from Alpine lactating goats differing for the genotype at the at αs1-

casein locus (Chilliard et al., 2006), showed a trend similar to ours, but a wider range of fatty acids resulted affected by genotype; in fact, they found that also long chain fatty acids were higher in the AA group. However, it is not possible to exclude that the higher presence of long chain fatty acids in AA milk arose from a different fatty acid profile consumed with diet because it was not determined for each group. Taking in account that, in our conditions, the selected diets were identical also in terms of fatty acids, the differences found in the milk fatty acid profiles of the two groups seem to have a genetic origin. In this direction, it is noteworthy that Ollier et al. (2008) showed that weak alleles at αs1-casein locus negatively affect the gene expression of FANS of lactating mammary gland. This gene encodes the fatty acid synthase, which is a multifunctional protein that catalyzes the mammary de novo synthesis of fatty acids. Moreover, also the activity of this protein turned out to be lower in animals with weak genotype, and this could explain why the biosynthesis of milk short and medium-chain fatty acids and odd and branched chain fatty acids resulted lower in the FF group. In conclusion, this study seems to confirm that polymorphism at αs1-casein locus can play a role on goat milk fatty acid composition, in particular for the de novo synthesized fatty acids. However, on account of the particular experimental conditions, further studies with different feeding system are needed to better assess the role of the genetic αs1-casein polymorphism on milk fatty acid composition.

4

Effect of CSN1S1 genotype and its interaction with diet Energy level on milk production and quality in Girgentana goats fed ad libitum

Pagano R.I., Pennisi P., Valenti B., Lanza M., Di Trana A., Di Gregorio P., De Angelis A., Avondo M. (2010). Effects of CSN1S1 genotype and its interaction with diet energy level on milk production and quality in Girgentana goats fed *ad libitum*. *Journal of Dairy Research. 77:245-251.*

Abstract

A study was carried out to evaluate how the energy level of the diet can affect milk production and quality in Girgentana lactating goats in relation to polymorphism at α_{s1}-casein (CSN1S1) genotype locus. Twenty-seven goats, homogeneous for milk production (1.5±0.3 kg/d), days of lactation (90±10 d) and body weight (35.8±5.5 kg) were selected on the basis of their CSN1S1 genotype, as follows: nine goats homozygous for strong (AA) alleles, nine goats homozygous for weak alleles (FF) and nine goats heterozygous (AF). The goats were used in a 3 x 3 factorial arrangement of treatments, with three genotypes (AA, FF, AF) and three diets at different energy levels (100%, 65% and 30% of hay inclusion). The experiment consisted of three simultaneous 3 x 3 Latin squares for the three genotypes, with one square for each level of hay inclusion in the diet. All the animals were housed in individual pens. Each experimental period lasted 23 d and consisted of 15 d for adaptation and 8 d for data and samples collection, during which the goats received the scheduled diet *ad libitum*. The animals were fed three different diets designed to have the same crude protein content (about 15%) but different energy levels: a pelleted alfalfa hay (H100) and two feeds including 65% (H65) and 30% (H30) of alfalfa hay (respectively 1099, 1386 and 1590 kcal NE for lactation/kg DM). All the diets were ground and pelleted (6 mm diameter). AA goats were more productive than AF and FF goats (respectively: 1419 v. 1145 and 1014 g/d; P=0.002). Indeed the interaction energy level × genotype was significant (P=0.018): in fact AA goats showed their milk increase only when fed with concentrates. Differences in protein and in casein levels between the three genotypes were in line with results expected from the different allele contribution to α_{s1}-casein synthesis. Milk urea levels were significantly lower in AA goats compared to AF and FF genotypes (respectively 32.7 v. 40.4 and 40.4 mg/dl; P=0.049) and significantly lower when goats were fed with 65H and 30H diets than 100H diet (respectively 37.4 and 34.3 v. 41.7; P<0.001). Indeed, a significant interaction genotype × diet (P=0.043) occurred for milk urea which was significantly lower in AA goats but only when fed with concentrates (65H and 30H). Blood concentrations of energy indicators (glucose, non-esterified fatty acids and beta-hydroxybutyric acid) were not influenced by genotype. The results confirm that strong

alleles are associated with a greater efficiency of feed utilization and seem to show that a high energy level of the diet can further improve this efficiency.

Introduction

The marked genetic polymorphism at the α_{s1}-casein locus affects casein content of goat milk (Martin et al., 1999). Moreover it has been revealed that different α_{s1}-casein allelic variants can affect some milk parameters such as fat content (Grosclaude et al., 1994; Chilliard et al. 2006), urea level (Schmidely et al. 2002., Bonanno et al., 2007; Avondo et al., 2009), fatty acid profile (Chilliard et al., 2006).

Most of these parameters are also strongly influenced by nutrition. However, reports in the literature on the relationships between nutrition and milk protein genotype and their effects on milk characteristics are few and often controversial. Ollier et al., (2007) evaluated the genes whose expression is bound to dietary characteristics in lactating goats and they found a lower expression level of genes associated with α_{s1}-casein, α_{s2}-casein and β-casein synthesis, after withholding food for 48 h compared with feeding *ad libitum*. Mackle et al. (1999) investigated the effects of undernutrition on milk composition in cows characterized by different β-lactoglobulin phenotypes and suggested that the advantage of using animals with strong protein genotype could be counterbalanced by a low nutrient supply. In contrast, Auldist et al. (2000), focusing on the effects of different amount of pasture allowance on milk composition from cows of different β-lactoglobulin phenotypes found no interactions between nutrition and protein phenotype.

Only few studies have been carried out on the effect of the diet on milk production and composition, in goats at different α_{s1}-casein genotypes. In general, it has been shown that strong alleles are associated with a greater efficiency of N utilization, compared to weak alleles (Schmidely et al., 2002; De la Torre et al., 2008; 2009).

To our knowledge no investigation has been made on the effect of dietary energy levels on the performance of goats of different α_{s1}-casein genotype. However, in a previous free-choice feeding trial (Avondo et al., 2009), we highlight that goats carrying strong alleles voluntary selected a diet with a higher percentage of energy-rich feeds, compared to goats with weak alleles, thus increasing their milk and casein

production. The aim of the present study was to test how Girgentana goats, selected according to different α_{s1}-casein genotype and reared intensively in stall, respond to complete pelleted diets with different energy levels but of similar protein content.

Materials and methods

Animals and experimental design

Twenty-seven Girgentana goats in their 2nd to 4th lactation, homogeneous for milk production (1.5±0.3 kg/d), days of lactation (90±10 d) and body weight (35.8±5.5 kg) were selected on the basis of their genotype at α_{s1}-casein locus, as follows: nine goats homozygous for strong (AA) alleles, nine goats homozygous for weak alleles (FF) and nine goats heterozygous (AF). Moreover, all the goats were selected taking into account CSN2 and CSNS2 genotype. In particular all the goats were characterized by strong alleles at the two loci. Goat DNA samples were obtained from hair bulbs according to Bowling et al. 1993. The genotypes of individuals at the CSN1S1, CSN2, and CSN1S2 were determined by means of PCR analyses (Jansà Pérez et al., 1994; Ramunno et al., 1995; Ramunno et al., 2000, Ramunno et al., 2001; Ramunno et al., 2002 and Cosenza et al., 2003).

Goats in each genetic group derived from two different farms. The goats were used in a 3 × 3 factorial arrangement of treatments, with three genotypes (AA, AF, FF) and three diets at different energy levels (100%, 65% and 30% of hay inclusion). The experiment consisted of three simultaneous 3 x 3 Latin squares for the three genotypes (AA, FF, AF), with one square for each level of hay inclusion in the diet. All the animals, managed according to the guidelines of the Animal Ethics Committee of the University of Catania, were housed in individual pens where goats had access to water and salt blocks. The pre-experimental period consisted of a 12-d period during which the animals received a mix of the three experimental diets *ad libitum*. The experimental period lasted 69 days, from 17 February to 26 April. Each experimental period lasted 23 d and consisted of 15 d for adaptation and 8 d for data and samples collection during which the goats received the scheduled diet *ad libitum*.

The animals were fed three different diets designed to have the same protein content but different energy levels: a pelleted alfalfa hay (100% H) and two pelleted feeds

including 65% (65 H) and 30% (30H) of alfalfa hay (Table 1). All ingredients were ground and pelleted (6 mm diameter).

Sample collection and analysis

Individual intakes were measured daily, on the basis of residuals. Individual milk production and milk samples were collected from the morning and evening milking three times for each 8-days collection period. Three samples for each pelleted diet were analysed for dry matter (DM), crude protein (CP), fat (AOAC, 1990), structural carbohydrates (Van Soest et al. 1991), water-soluble carbohydrates (WSC) by a modified anthrone method (Deriaz, 1961), starch by an enzymic procedure (Megazyme International Ireland Ltd., Bray, Co. Wicklow). Milk samples, consisting of proportional volumes of morning and evening milk, were analysed for lactose, fat, protein and SCC by an infrared method (Combi-foss 6000, Foss Electric, Hillerød, Denmark). Total nitrogen (TN) and non-casein nitrogen (NCN) were determined by FIL-IDF standard procedures (Internation Dairy Federation, 1964). From these nitrogen fractions, total protein (TN*6.38) and casein [(TN–(NCN*0.994))*6.38] were calculated. Milk urea content was determined using a differential pH meter (CL10, Eurochem, Savona, Italy).

Body condition (BCS), scored as reported by Santucci & Maestrini (1985), was measured at the start and the end of the trial.

Blood samples (8 ml) were taken from all goats at the end of pre-experimental period and at the end of each experimental periods by jugular venepuncture using Vacutainer tubes containing lithium heparin (Becton, Dickinson and Co.) and immediately placed on ice. Within 1 h of the bleeding, blood samples were centrifuged at 1400 ***g*** f at 4° C or 20 min and plasma was harvested and stored at -20 °C until assayed. A TARGA model 2000 (Technology Advanced Random Generation Analyser, Biotecnica Instruments, Roma, Italy) automated analyzer was used to determine glucose, cholesterol, triglycerides, urea, total protein and albumin (Mercury, Riardo, Italy) in plasma samples. Non-esterified fatty acids (NEFA) and beta-hydroxy butyric acid (BHBA) were analyzed by using respectively FA 115 and Ranbut commercial kits (Randox Laboratories, Crumlin, Antrim, UK).

Statistical analysis

Individual data for intake, milk production and composition were analysed using the GLM procedure for repeated measures of SPSS (SPSS for Windows, SPSS Inc., Chicago IL, USA). The model included genotype, diets, blocks, periods and genotype x diet. Pre-experimental data of milk production and dry matter intake (DMI) were used as covariates respectively for milk production and composition and for DMI analysis. Plasma concentration of the metabolites were analysed by means of GLM procedure and analysis included main effect of as1 casein genotype (FF, AF, AA), diet (100H, 65H, 30H) and interaction genotype x diet. Data from the pre-experimental period were used as a covariate in plasma parameters analysis. When covariance was not significant (P>0.05) it was not included in the statistical model. Difference between means were tested by least significant differences (LSD). Pearson's correlation coefficients were calculated between the parameters measured in this study

Results

Table 1 shows the diets ingredients and chemical composition. As planned when formulating the diets, CP content was similar far all the pelleted feeds, whilst the diets differed markedly in their content of structural carbohydrates, starch and energy.

Table 1 - Ingredients and chemical composition of the diet .

	100H	65H	30H
Ingredients, % of fresh weight			
Pelleted alfalfa hay	98.0	65.0	30.0
Maize	-	16.0	35.0
Barley	-	8.0	17.0
Soybean meal	-	3.0	5.0
Carob pulp	-	3.0	5.0
Corn gluten meal	-	3.0	6.0
Vitamin-mineral premix	2.0	2.0	2.0
Chemical composition			
Dry matter (DM) %	87.7	85.4	84.3
Crude protein % DM	15.2	15.2	15.7
	29.8	22.7	12.5
Neutral detergent fibre % DM	54.6	44.0	27.5
Acid detergent fibre % DM	36.5	24.4	11.9
Lignin % DM	13.3	6.4	4.6
Crude lipids % DM	2.0	2.4	2.7
Ash % DM	11.1	10.1	8.1
Water-soluble carbohydrates % DM	7.1	6.8	5.9
Starch % DM	1.9	19.5	40.7
NEl† kcal/kg DM	1099.2	1386.3	1589.7

† Net energy for lactation (Cornad et al., 1984)

Table 2 reports data on intake, milk yield and composition. DMI was not affected by genotype, but was significantly influenced by hay inclusion in the diet (P<0.001), being lower when animals were fed 30H diet compared to 100H and 65H diets.

Table 2 – Least squares means of daily intake, milk yield and composition

	α_{s1}-casein genotype (G)			% Hay (H)			Significance (P)			SEM
	FF	AF	AA	100H	65H	30H	G	H	GxH	
DM Intake g/d	2512.2	2502.7	2481.3	2518.8^{a}	2530.8^{a}	2446.6^{b}	0.165	<0.001	0.247	7.69
EN intake kcal/d	3406.4	3394.8	3364.7	2768.5^{a}	3507.7^{b}	3889.8^{c}	0.203	<0.001	0.246	56.2
CP intake g/d	386.1	381.3	384.7	382.9	384.1	385.2	0.164	0.633	0.252	1.00
Milk yield g/d	1014.5^{a}	1144.6^{a}	1419.4^{b}	971.6^{a}	1313.8^{b}	1293.0^{b}	0.002	<0.001	0.018	50.9
Fat%	3.26^{a}	3.13^{a}	3.85^{b}	3.80^{b}	3.19 ^{a}	3.25^{a}	<0.001	<0.001	0.260	0.08
Protein%	3.29^{a}	3.66 ^{b}	3.94^{c}	3.62^{ab}	3.55^{a}	3.72^{b}	<0.001	0.044	0.386	0.04
Lactose%	4.44 ^{b}	4.36^{ab}	4.33^{a}	4.32^{a}	4.36^{ab}	4.45^{b}	0.006	0.006	0.454	0.02
Casein%	2.51^{a}	2.84^{b}	3.08^{c}	2.76^{a}	2.75^{a}	2.92^{b}	<0.001	0.004	0.578	0.04
Urea mg/dl	40.4^{b}	40.4^{b}	32.7^{a}	41.7^{b}	37.4^{a}	34.3^{a}	0.049	<0.001	0.043	0.88
Casein g/d	25.4^{a}	32.2^{b}	43.5^{c}	26.7^{a}	36.3^{b}	38.2^{b}	<0.001	<0.001	0.013	1.54
Casein% Protein	76.1	77.4	78.4	76.1^{a}	77.2^{b}	78.6^{c}	0.106	<0.001	0.98	0.37

^{a,b,c} values within row without a common superscript letter are significantly different (P<0.005)

Genotype and diet energy level significantly influenced milk yield: on average AA goats produced more than AF and FF goats (P=0.002) and concentrate diets (65H and 30H) increased milk production over that seen with the hay diet (100H) (P<0.001). Moreover a significant interaction genotype × diet was also found for milk production and casein production (respectively P=0.018 and P=0.013), as highlighted in figures 1A and 1B. When increasing the energy input, by reducing hay inclusion in the diet at 65% and 30%, goats carrying strong alleles showed milk production increases of 55% and 53%, respectively, compared to increases of 14% and 17% in goats carrying weak alleles; in heterozygous goats were intermediate (34% and 27%).

Genotype significantly influenced milk composition and, as expected, the protein and casein percentages (respectively P=0.001; P=0.001) were higher in AA than FF goats with intermediate values in AF goats. Milk fat content was higher in AA goats (P=0.001) than in the other groups, whereas lactose and urea were significantly lower in AA goats (respectively P=0.006; P=0.049).

Hay inclusion in the diet significantly affected milk composition. Fat and urea decreased (respectively P=0.001; P=0.001) when concentrate was included in the diet, whereas protein, lactose and casein contents were higher (respectively P=0.044; P=0.006; P=0.004) when goats were fed with the 30H diet.

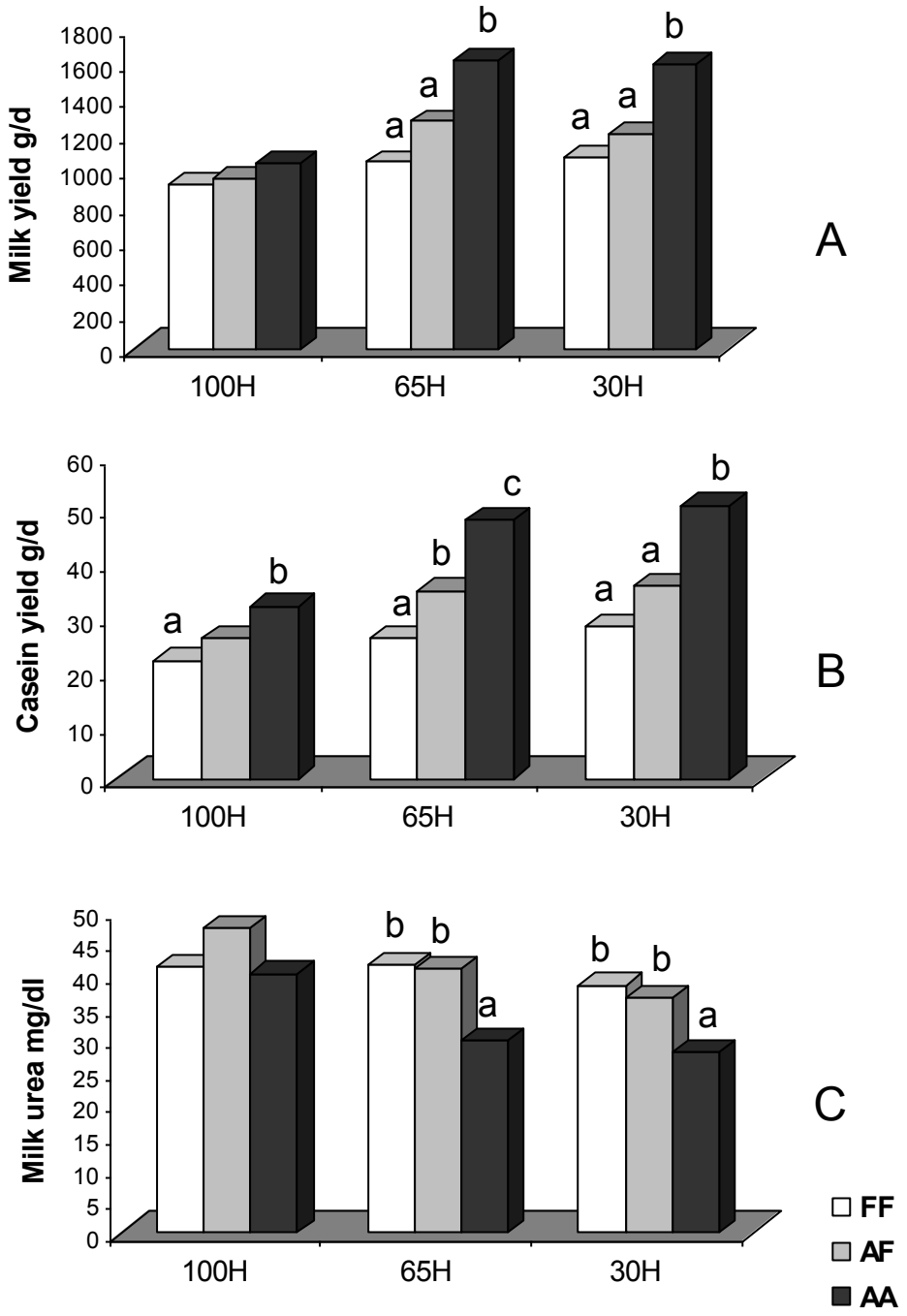


Figure 1 – Interaction between genotype (AA, AF, FF) and diet (100H, 65H, 30H) for milk yield (A), casein (B) and milk urea (C). Values within diets with different superscript letters are significantly different.

A significant genotype × diet interaction occurred for milk urea, which was significantly lower (P=0.043) in AA goats only when fed with concentrates (65H and 30H) (figure 1C).

A significant effect of feeding regimen was observed on plasma concentration of BHBA (P<0.023), and cholesterol (P<0.001) and plasma urea (P<0.045); conversely, values of other parameters were not affected by the dietary treatments (Tables 3). Concentration of BHBA was higher in the 65H group than in the 100H goats. A similar trend was notices for cholesterol level. Plasma concentration of urea increased in 100H and 65H groups compared to 30H group.

There was no significant effect of α_{s1}-casein genotype on plasma metabolite (glucose, NEFA, BHBA and urea). Plasma albumin concentrations was not affected by the α_{s1}-casein genotype.

	a_{s1}-casein genotype (G)			% Hay (H)			Significance (P)			SEM
	FF	AF	AA	100H	65H	30H	G	H	G×H	
Glucose mmol/L	2.88	2.71	2.71	2.77	2.70	2.82	0.209	0.574	0.271	0.075
NEFA mmol/L	0.117	0.13	0.145	0.137	0.122	0.133	0.812	0.923	0.372	0.028
β-Hydroxybutyric acid	0.406	0.324	0.291	0.241^{b}	0.436^{a}	0.348^{ab}	0.249	0.023	0.525	0.047
Cholesterol mmol/L	1.73	1.72	1.66	1.48^{b}	1.79^{a}	1.84^{a}	0.731	0.001	0.992	0.070
Triglycerides mmol/L	0.138	0.144	0.149	0.142	0.153	0.137	0.852	0.591	0.885	0.013
Urea mmol/L	8.58	8.02	8.03	8.68^{a}	8.55^{a}	7.66^{b}	0.147	0.045	0.599	0.310
Total protein g/L	75.31	77.30	75.14	74.82	77.57	75.34	0.513	0.405	0.449	1.510
Albumin g/L	27.00	26.35	27.15	26.81	27.07	26.64	0.100	0.507	0.217	0.295

Discussion

In our experimental conditions mean intake data resulted much higher, compared with goats at similar production levels but in different feeding conditions such as pasture (Bonanno et al., 2007; Avondo et al., 2008) or roughages and concentrates (Havrevoll et al., 1995) probably because of the different physical properties of the diets (forbes, 1995). In fact, intakes were similar to those observed previously in goats under a free-choice feeding system based on whole grains and pelletted hay (Avondo et al., 2009). DMI significantly decreased with the high energy diet (H30) possibly because of the lower DM percentage of this diet, compared to 100H and 65H diets.

As already reported by Schmidely et al. (2002) and by Avondo et al. (2009), total DMI was not affected by genotype.

As expected, milk production significantly increased when goats were fed with concentrate diets compared with 100% hay. This increase occurred despite the high intake levels reached by goats even when fed only with hay, which allowed an energy input as high as 2768 kcal net energy for lactation (NEl)/d; this energy input is higher than energy requirements indicated by INRA (Morand-Fehr & Sauvant, 1988) for heavier goats with a milk production of 2 kg/d. this findings illustrates the importance of energy source, starch-rich feeds v. roughage, on efficiency of milk synthesis, which induced the goats to increase their production when fed with concentrate feeds.

Genotype showed an important effect on milk yield in that AA goats were more productive than AF and FF goats. Moreover an interesting energy level × genotype interaction was evident: in fact AA goats showed their milk increase only when fed with concentrates (figure 1A). The increase in milk production reached its maximum level at 65% of hay inclusion whereas no further increase was found on further reducing hay inclusion (H30). Previous studies on goats at different α_{s1}-casein genotype do not report significant differences in milk production between strong, weak or intermediate alleles (Schmidely et al. 2002, Caravaca et al. 2009; De la Torre et al., 2009). De la Torre et al. (2009), however, obtained different responses from goats with different CSN1S1 genotype when fed with two CP levels: when fed with a 13.6% CP diet, goats with strong alleles (HG) showed a tendency (not significant) to produce more milk than goats with weak and intermediate alleles (LG), whereas when fed with a 17.7% CP diet, LG goats increased their production but HG goats milk production remained unchanged. The authors hypothized that HG goats achieved their maximum capacity for milk protein production with the low protein diet and, for this reason, no further increase in milk production was obtained on increasing CP level further.

In accordance with present results, in a previous study on Girgentana goats in a free-choice feeding system (Avondo et al., 2009) we found that goats with strong alleles (AA) were more productive compared than goats with weak alleles (FF); in fact, as a consequence of feeds selection, AA goats voluntarily consumed a diet with a higher energy to protein feeds ratio, compared to FF goats, which probably caused the production increase. Indeed, in the same trial, when the goats were fed with a mixture of the five feeds, no difference in milk production was noted between genotypes, even though a very high energy and protein input was reached. On the basis of those results,

we hypothesized an indirect effect of genotype on milk yield by way of the difference in selective activity.

Milk quality was strongly affected by feeding and genotype. As expected, owing to the structural carbohydrates levels in the diets, fat increased with hay percentage. As already reported (Schmidely et al., 2002; De la Torre et al. 2009) milk fat content was higher in goats with high genetic capability. No dilution effect of the higher milk production was noted in this group. The goats were genetically homogeneus for α_{s2}-casein and β-casein; this leads us to hypothesize that the differences in protein and in casein levels between the three genetic groups were in line with expected results as each allele contributes to α_{s1}-casein synthesis, taking into account that strong and weak alleles are associated, respectively, to 3.5, and 0.45 g/l of α_{s1}-casein synthesized (Mahé et al., 1993; Martin et al., 1999; Sacchi et al., 2005; Marletta et al., 2007). On average high energy diet (30H) caused a significant increase in casein and protein synthesis, in line with results from Morand-Fehr et al. (2000). This result might be associated to an improved efficiency of microbial protein synthesis due to the higher availability of non structural carbohydrate in the rumen (Koenig et al., 2003; Broderick, 2003) and to a consequent higher availability of milk proteins precursors to the mammary galnd.

No interaction genotype × diet was evident for milk casein and protein content. However, in keeping with the different milk production between genotypes, a significant genotype × diet interaction was seen for yield (g/d) of casein. In fact, AA goats fed respectively with 100H, 65H and 30H diets produced 45%, 84% and 80% more casein than FF goats (figure 1B).

On average, milk urea levels were significantly lower in AA goats compared with the other genotypes, confirming previous findings for goats of different α_{s1}-casein genotype (Schmidely et al. 2002., Bonanno et al., 2007; Avondo et al., 2009). In particular, the significant genotype × diet interaction (P=0.043) reflects the fact that milk urea was significantly lower in AA goats, compared with AF and FF goats, only when fed with concentrates (65H and 30H) (figure 1C). It is evident that only in this genetic group did the greater energy availability improv the efficiency of milk protein synthesis, thus reducing nitrogen losses.

All the goats used in this study were clinically healthy and the parameters reported represent “normal” values for goats. Blood concentrations of energy indicators

(glucose, NEFA and BHBA) and urea were not influenced by genotype, as reported by Chilliard et al. (2006). Concentrations of NEFA of 0.20–0.21 mmol/l have been suggested for lactating does at zero energy balance (Dunshea & Bell, 1989). In the present experiment, NEFA (Table 3) were below the critical values suggesting that goats were not mobilizing body fat reserves and animals were in the anabolic phase (McNamara, 1991). These results are in accordance with lactation phase (>90 d) and body condition score variations, measured from the start to the end of the trial, which were positive in all groups (respectively in AA, AF and FF goats: body weight variations , 4.9, +4.2 and 5.2 kg, P=0.351; BCS variations, 0.82, +0.90 and +0.99, P=0.188).

In all groups, cholesterol values were within the reference range and close to the lower physiological limit for caprine species (Kaneko et al., 1997). On average, the higher cholesterol content observed with 65H and 30H diets was linked to the increase of energy input as suggested by the positive correlation (r=0.94; P=0.04) between cholesterol and energy intake. Moreover cholesterol and BHBA, which are synthesized from the same precursor (Acetyl-CoA), showed the same trend and this is consistent with the positive correlation between the parameters (r =s0.76; P<0.07).

Urea levels in blood and milk during the experiment showed a similar trend (r=0.79; P<0.05), and the lowest values were recorded in the group 30H. The high starch percentage and the low fibre percentage in the 30H experimental diet (Table 1) might increase propionate production in the rumen (Petit & Tremblay, 1995), which could spare amino acids for gluconeogenesis (Sloan & Rowlinson, 1987) and increase the availability of amino acids for milk protein synthesis.

Albumin is the most abundant plasma protein in animal blood and it is produced in the liver. This variable is not a valid marker of nutritional status; rather it is a marker of hepatic functionality (Kaneko et al., 1997). In all groups, the albumin levels were close to the lower limit indicated for caprines; Di Trana et al. (1994) observed similar values of plasma albumin in Maltese goats, on 71-106 days from delivery, which were fed pasture plus a free choice of four types of grain.

Conclusions

The present results support the hypothesis that an interaction exists between α_{s1}-casein polymorphism and dietary energy level. It has been demonstrated that a high energy input improves the efficiency of transformation of the diet into milk and casein yield in goats carrying strong alleles, whereas it does not exert noticeable effects in goats carrying weak alleles. This could imply a need for new feeding recommendations for goats in relation to CSN1S1 genotype.

5

Polymorphism at α_{s1}-casein locus. Effect of Genotype x Diet interaction on milk fatty acid composition in Girgentana goat.

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Abstract

Eighteen Girgentana lactating goats, nine homozygous for strong alleles (AA) and nine homozygous for weak alleles (FF) at α_{s1}-casein locus, were used to evaluate the effect of genotype × diet interaction on goat milk fatty acid composition. Animals were divided in two groups. First group consisted of 5AA and 4FF, the second one consisted of 4AA and 5FF animals. The experimental groups were used in a 2×2 factorial arrangement of treatments, with two genotypes (AA,FF) and two diets (D100 and D65) at different energy level (1099 and 1386 kcal NEl/kg), obtained with 100% and 65% of pelleted alfalfa hay inclusion, respectively. All the animals were housed in individual pens. The genotype × diet interaction was significant ($P < 0.05$) for 11 different milk fatty acids. In particular, C8:0, C10:0, C12:0, C14:0 increased when FF animals shifted from D100 to D65, while the same fatty acids did not significantly change in AA animals; moreover, percentage of palmitic acid (C16:0) was significantly lower in animals with strong genotype when fed the high energy diet. Shifting from D100 to D65, long chain fatty acids (>C18) significantly increased in AA and decreased in FF goat milk. In conclusion, goats homozygous for weak and strong alleles at α_{s1}-casein locus seem to respond in a different way when fed diets with different energy levels; in particular, receiving a high energy diet, AA goats did not show any remarkable effect on milk fat quality, whereas FF goats showed a worsening in fat nutritional value.

Introduction

Goat polymorphism at α_{s1}-casein (CSN1S1) locus can affect casein, fat and milk fatty acid composition. The 17 alleles detected at this locus are commonly named null, weak, intermediate or strong, according to the level of α_{s1}-casein in milk ranging from 0 (CSN1S1 0_1, 0_2, N) to 3.6 g/L (CSN1S1 A, B_1, B_2, B_3, B_4, C, H, L) (Grosclaude et al., 1987). Strong genotypes are also associated to higher levels of fat, *de novo* synthesized fatty acids (Chilliard et al; 2006; Valenti et al., 2009) and milk yield (Avondo et al., 2008; Pagano et al., 2010), as compared to weak genotypes. Milk fatty acid composition can be modulated by the diet given to the animals. For example, it is well accepted that forage-to-concentrate ratio influences rumen microflora with remarkable effect on the proportions between milk fatty acids (Sanz Sampelayo et al.,

2007). The effects of interaction between genotype at CSN1S1 locus and dietary source have been investigated on milk yield and composition (Schmidely et al., 2002; de la Torre Adarve et al., 2009; Avondo et al., 2009; Pagano et al., 2010). However, few data are available on the effect of genotype × diet interaction on milk fatty acid profile of goat milk; the aim of this study is to evaluate if animals with different genotype at CSN1S1 locus respond in a different way when fed isoproteic diets at different energy levels.

Material and Methods

Eighteen Girgentana lactating goats, nine homozygous for strong (AA) alleles and nine homozygous for weak alleles (FF) at CSN1S1 locus, were used to evaluate the effect of genotype x diet interaction on goat milk fatty acid composition. To avoid genetic interference due to other caseins polymorphisms, the goats were selected also taking into account genotype at αs_2-casein (CSNS2) and β-casein (CSN2): in particular it was ascertained that, all the goats were uniformly characterized by strong alleles at the two loci. The genotypes of individuals at the CSN1S1, CSN2, and CSN1S2 were determined as reported by Pagano et al. (2010).

Average milk production, days of lactation and body weight was 1.3±0.3 Kg/d, 94±12 days and 36.9±4.7 Kg, respectively The selected animals were divided in two experimental groups and housed in individual pens where water and salt were always available. First group consisted of 5 AA and 4 FF animals, the second one consisted of 4 AA and 5 FF animals. Goats were used in a 2 x 2 factorial arrangement of treatments, with two genotypes (AA, FF) and two diets at different energy levels (100% and 65% of hay inclusion). The experiment consisted of two simultaneous 2 x 2 latin squares for the two genotypes (AA, FF), with one square for each level of energy in the diet. All the animals were managed according to the guidelines of the Animal Ethics Committee of the University of Catania. Goats were fed *ad libitum* with a mix (50:50) of the two diets for a 12 day pre-experimental period. The experiment lasted 46 days. Each experimental period lasted 23 d including 15 d for adaptation and 8 d for samples and data collection. During the experimental periods animals received the scheduled diet *ad libitum*. D100 diet consisted of 100% alfalfa pelleted hay, D65

diet consisted of a pelleted feed including 65% of alfalfa hay. All ingredients were ground and pelleted (6 mm diameter).

At the end of each experimental period, individual milk samples were collected from the morning (08.00 h.) and evening (17.00 h.) milkings and immediately refrigerated and stored at -18 °C.

Three samples for each diet were analysed for dry matter (DM), crude protein (CP), fat (AOAC, 1990), structural carbohydrates (Van Soest et al. 1991), water-soluble carbohydrates (Deriaz, 1961), starch by an enzymic procedure (Megazyme International Ireland Ltd., Bray, Co. Wicklow), protein fractions (Licitra et al., 1996) and fatty acid composition as reported by Avondo et al. (2008).

Milk samples, consisting of proportional volumes of morning and evening milk, were analysed for casein (FIL-IDF standard procedures,1964) and fatty acid composition (Avondo et al., 2008).

Statistical Analysis

Individual data for milk fatty acid composition were subjected to analysis of variance for two simultaneous 2 x 2 latin squares design, using the GLM procedure of statistical software Minitab (Release 14, 1995). The model included genotype, diet, block and genotype x diet (GxD). When interaction did not result statistically significant ($P>0.05$) this term was excluded from the model. Tukey's test was used to compare mean values.

Results

Table 1 reports ingredients and chemical composition of D100 and D65, designed in order to reach similar crude protein but different energy content, through a different inclusion of carbohydrates sources. Crude protein was identical (15.2% DM) between diets while fat was slightly higher in D65. The difference in net energy was principally due to the starch and crude fibre, respectively ten times higher and 25% lower in D65 than D100. Differences in diets fatty acid composition were: oleic (*cis*-9 C18:1) and linoleic acid (C18:2) higher in D65, while, as expected, linolenic acid (C18:3) was higher in D100.

Genotype affected milk yield and composition. AA animals were 353 g/d more productive than FF; moreover casein yield was significantly ($P<0.05$) greater for AA goats (respectively milk yield and casein yield: 1320 vs 967g/d, SEM: 88.60; 40 vs 24 g/d, SEM: 2.70). A significant genotype x diet interaction was recorded: shifting from D100 to D65, AA goats milk and casein yield increased, respectively by 53% and 43%, whereas FF goats milk yield remained unchanged and casein yield increased by 33% (equal to 7 g/d).

Table 1 - Ingredients and chemical composition of the diet

	D100	D65
Ingredients %		
Pelleted alfalfa hay	98.0	65.0
Maize	-	16.0
Barley	-	8.0
Soybean meal	-	3.0
Carob pulp	-	3.0
Corn gluten meal	-	3.0
Vitamin-mineral premix	2.0	2.0
Chemical Composition		
Dry matter %	87.7	85.4
Crude protein % DM	15.2	15.2
Crude fibre % DM	29.8	22.7
Neutral detergent fibre % DM	54.6	44.0
Acid detergent fibre % DM	36.5	24.4
Lignin % DM	13.3	6.4
Crude lipids % DM	2.0	2.4
Ash % DM	11.1	10.1
Water-soluble carbohydrates % DM	7.1	6.8
Starch % DM	1.9	19.5
NEl kcal/kg DM	1099.2	1386.3
Fatty acid Composition (g/kg DM)		
C12	0.504	0.312
C14	0.153	0.139
C14:1	0.00	0.00
C15	0.067	0.111
C15:1	0.048	0.028
C16	2.634	3.463
C16:1	0.077	0.084
C18	0.563	0.590
C18:1	0.824	3.358
C18:2	3.141	10.118
C18:3 γ	0.126	0.122
C:18:3 α	4.012	3.047

Milk fatty acid composition is shown in table 2. Energy level of diet affected percentage of 18 fatty acids: C8:0, C10:0, C11:0, C12:0, C14:0 and C18:2 were higher in D65 fed goats; C13:0, C14:1, C15 iso, C15:0, C17 iso, C16:1, C17 anteiso, C17:0, C17:1, *cis*-9 C18:1, *cis*-11 C18:1, C18:3 were higher in D100 fed goats. Genotype affected 9 different fatty acids: C12:0, C13:0, C14:0, C14:1, C15 iso, C15:0 and C18:0 were lower in FF milk, while C16:1 and *cis*-9 C18:1 were lower in AA milk. The genotype x diet interaction was significant for 11 different milk fatty acids: C8:0, C10:0, C12:0, C14:0 increased in FF goats, while the same fatty acids did not significantly changed in AA animals increasing the energy level of the diet; palmitic acid (C16:0) was

significantly lower in animals with strong genotype when the high energy diet was given. Shifting from D100 to D65, long chain fatty acids (> C18) significantly increased in AA and decrease in FF goat milk.

Tab. 2 Genotype x diet effect on milk fatty acid composition (g/100g total fatty acids)

	D 100		D 65		SEM	significance		
	AA	FF	AA	FF		G	D	G x D
C4	2.45	2.98	2.26	2.43	0.108	ns	ns	ns
C6	2.60	2.61	2.68	2.85	0.0525	ns	ns	ns
C8	2.63^{b}	$2.59b^{b}$	2.86^{ab}	2.96^{a}	0.0619	ns	**	*
C9	0.13	0.13	0.12	0.10	0.00538	ns	ns	ns
C10	10.71^{c}	9.98^{d}	11.49^{abc}	12.03^{b}	0.0242	ns	***	**
C11	0.23^{c}	0.22^{c}	0.22^{ac}	0.28^{b}	0.0104	ns	*	**
C12	6.22^{a}	4.78^{b}	6.28^{a}	6.05^{a}	0.199	*	*	*
C12:1	0.18	0.15	0.20	0.20	0.0122	ns	ns	ns
C13	0.14^{c}	0.11^{a}	0.11^{a}	0.08^{b}	0.00644	**	**	ns
C14	13.57^{a}	11.34^{b}	13.17^{a}	13.65^{a}	0.317	*	*	***
C14:1	0.15^{c}	0.12^{d}	0.09^{a}	0.06^{b}	0.0105	*	***	ns
C15 ISO	0.32^{b}	0.26^{ab}	0.24^{ab}	0.16^{a}	0.0189	**	**	ns
C15 ANTEISO	0.26^{a}	0.19^{b}	0.21^{ab}	0.22^{ab}	0.00953	ns	ns	*
C15	1.52^{b}	1.33^{cb}	0.93^{ac}	0.77^{a}	0.0795	*	***	ns
C15:1	0.07	0.03	0.04	0.07	0.0114	ns	ns	ns
C16	28.83^{b}	27.83^{b}	25.59^{a}	30.83^{c}	0.626	ns	ns	**
C17 ISO	0.34^{b}	0.40^{b}	0.31^{a}	0.23^{a}	0.0205	ns	*	ns
C16:1	0.72^{b}	0.75^{b}	0.55^{a}	0.70^{ab}	0.0255	*	**	ns
C17 ANTEISO	0.53^{b}	0.50^{b}	0.38^{a}	0.36^{a}	0.0283	ns	**	ns
C17	0.96^{b}	0.97^{b}	0.64^{a}	0.55^{a}	0.046	ns	**	ns
C17:1	0.37^{b}	0.42^{b}	0.21^{a}	0.21^{a}	0.0223	ns	***	ns
C18	5.78^{cb}	5.96^{ac}	6.96^{a}	4.68^{b}	0.254	*	ns	**
C18:1 9TR	0.30	0.24	0.20	0.23	0.04	ns	ns	ns
C18:1 11TR	0.10	0.13	0.10	0.11	0.00736	ns	ns	ns
C18:1 9 CIS	12.74^{b}	16.74^{c}	14.78^{a}	12.64^{b}	0.647	**	**	***
C18:1 11CIS	0.41^{ab}	0.46^{a}	0.42^{a}	0.31^{b}	0.0178	ns	*	**
C18:2 TR	0.37	0.38	0.36	0.35	0.0247	ns	ns	ns
C18:2 CIS	3.56^{b}	4.39^{ac}	5.88^{a}	4.26^{bc}	0.301	ns	**	***
C18:3 ALFA	2.23^{b}	2.10^{b}	1.33^{a}	0.92^{a}	0.154	ns	***	ns
9CIS-11TR CLA	1.11	1.29	0.95	1.22	0.0636	ns	ns	ns
10TR-12CIS CLA	0.10	0.09	0.09	0.08	0.0118	ns	ns	ns
C20:4	0.38	0.53	0.37	0.40	0.0334	ns	ns	ns
C6-C14	35.73^{b}	31.31^{a}	36.47^{b}	37.53^{b}	0.721	*	***	***
> C18	27.08^{a}	32.29^{b}	31.43^{c}	25.20^{a}	1.06	ns	*	***
C14:1/C14:0	0.011^{b}	0.011^{b}	0.007^{a}	0.005^{a}	0.000870	ns	***	ns
C16:1/C16:0	0.25^{a}	0.028^{b}	0.021^{a}	0.023^{a}	0.00108	ns	*	*
C17:1/C17:0	0.38^{a}	0.43^{b}	0.34^{a}	0.38^{a}	0.0113	*	*	*
C18:1/C18:0	2.24^{a}	2.75^{b}	2.19^{a}	2.75^{b}	0.0903	*	ns	ns

*Significance: ns P>0.05; * P<0.05; ** P<0.01; *** P<0.001*

[a.b] *Different letters within row indicate a significant difference between values (p<0.05)*

Discussion and conclusion

Polymorphism at CSN1S1 locus influenced milk fatty acid composition: the lower percentages in FF goats of fatty acids, entirely (C12:0, C14:0, C6-C14) or partially (C18:0, odd and branched chain fatty acid) *de novo* synthesized in the mammary tissues, are in line with literature (Lamberet et al., 1996; Chilliard et al., 2006, de la Torre Adarve et al., 2009; Valenti et al., 2009). Milk Δ^9 desaturated fatty acids (*cis*-9 C14:1, *cis*-9 C16:1, *cis*-9 C17:1 and *cis*-9 C18:1) were higher in FF milk; these fatty acids arise in part from diet and in part are synthesized in the mammary gland as a product of Δ^9 desaturase enzyme. Ratio between the saturated and the correspondent *cis*-9 unsaturated fatty acid can represent a good index for the activity of this enzyme; on the basis of these ratios, Chilliard et al. (2006) suggested that desaturase activity is higher in FF animals and, except for C14, our results are in line with that finding. On the contrary, de la Torre Adarve et al. (2009) did not report any genotype effect on Δ^9 desaturation ratios. This aspect has to be deeper investigated because, despite *in vivo* results, Ollier et al. (2008) found that the expression of the gene encoding for stearoyl-CoA desaturase is lower in FF animals.

As reviewed by Vlaeminck et al. (2006), diet composition has a considerable influence on the selection of rumen microflora population and consequently on milk fatty acid profile. The reduction of dietary fibre in favour of starch increases the rumen amylolytic bacteria and reduces cellulolytic bacteria. Our results for OBCFA are consistent with literature, showing that C15iso, C15:0, C17iso, C17anteiso and C17:0, mainly arising from cellulolytic microflora metabolism, were lower when D65 was given to the animals. Considering the different fatty acid composition of diets, the higher percentage of linolenic acid (C18:3) found in milk was an expected result when animals consumed D65. The sum of C6-C14 fatty acids (MCFA) was greater with D65; however, this mean result mainly depended on the genotype x diet effect. In fact, FF goats showed an increase of MCFA and a decrease of long chain fatty acids (LCA) when fed the higher energy diet; on the contrary, AA goats showed no differences in C8:0 to C14:0 fatty acids and a significant decrease in C16:0. To justify the increase in MCFA percentage in FF goats when fed D65 we could refer to Chanat et al. (1999) findings. These authors, observing the morphology of mammary epithelial cells of goats with different variants at CSN1S1 locus, revealed that the endoplasmatic

reticulum of FF animals was remarkably enlarged in comparison to AA genotype because of the accumulation of immature proteins and caseins. On the basis of these observations, Chilliard et al. (2006) suggested that this enlargement could also involve enzymes responsible for lipid biosynthesis. Moreover, Ollier et al. (2008) demonstrated that weak variants at CSN1S1 locus negatively affect gene expression of GPAM and FASN, which are two important genes implicated in the first step of triacylglycerols biosynthesis (Coleman et al., 2000) and in the endogenous biosynthesis of short and medium chain fatty acids (Smith, 1994), respectively. In our case, the increase of MCFA fatty acids in FF milk shifting from D100 to D65 could be related to the simultaneous increase in casein yield (+7 g/d) found when these animals were fed higher energy diet. It could be supposable that the higher availability of CSN1S1 alleviated the impediment of endoplasmatic reticulum in FF goats with positive effects on fat anabolism at mammary gland cells level. In AA goats, the analogue effect seems to be negligible, probably because in this genotype endoplasmatic reticulum does not show any impediment.

Taking in account these results, it would be desirable a differentiation of diet in function of CSN1S1 genotype. In fact, the improvement in productive performances can give a reason for the elevation of costs due to use of concentrates in the diet for AA animals, but not for FF animals whose unchanged productive level is coupled by milk fat quality detrimental.

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Effect of diet at different Energy level on milk casein composition of Girgentana goats differing in CSN1S1 locus

Valenti B., Pagano R.I., Avondo M. (2011). Effect of diet at different energy level on milk casein composition of Girgentana goats differing in CSN1S1 genotype. *Small Ruminant Research. (In press).*

Abstract

Eighteen Girgentana lactating goats, nine homozygous for strong alleles (AA) and nine homozygous for weak alleles (FF) at α_{s1}-casein (CSN1S1) locus, were used to evaluate the effect of genotype, diet and genotype × diet (G×D) interaction on the composition of goat milk caseins. Goats were used in a 2×2 factorial arrangement of treatments, with two genotypes (AA, FF) and two diets at different energy levels (high-energy diet (D65) and low-energy diet (D100)). The experiment consisted of two simultaneous 2×2 Latin squares for the two genotypes, with one square for each level of energy. Capillary electrophoresis was used for the determination of relative casein (CN) composition. α_{s1}-CN, κ-CN and β-CN yield were significantly higher with D65 than D100 (10.2 *vs* 7.2; 3.8 *vs* 2.6; 18.6 vs 13.6 g/d, respectively). Genotype significantly affected ($P<0.05$) α_{s2}-CN and α_{s1}-CN yield: α_{s1}-CN was higher in AA than FF goat milk (15.5 *vs* 2.4 g/d), while α_{s2}-CN was higher in FF than AA goat milk (4.7 *vs* 2.8 g/d); no genotype effect ($P>0.05$) was reported for κ-CN and β-CN yield. As concerning individual casein concentration, α_{s1}-CN was higher for AA than FF goat (12.4 *vs* 1.5 g/kg milk), whereas α_{s2}-CN and β-CN were higher in FF than AA milk (4.3 *vs* 1.4; 15.6 *vs* 12.9 g/kg, respectively); also κ-CN tended to be higher in FF goats. Diet did not significantly influence concentration of individual caseins. A significant G×D interaction was found only for α_{s1}-CN concentration, that decreased (-10%) when AA goats shifted from D100 to D65. In conclusion, high energy input consistently improved total casein yield beside genotype. The higher casein yield of AA goats mainly depends on α_{s1}-CN biosynthesis; moreover, the lower presence of α_{s1}-CN in FF goat milk may be partially counterbalanced by the other caseins.

Introduction

In the goat species, an extensive polymorphism at α_{s1}-casein (CSN1S1) locus has been reported. So far, 18 different alleles have been found at this locus (Meggiolaro et al., 2003). In the last decades, several studies have been done to investigate the effect of this polymorphism on goat milk composition and technological properties. The 18 allelic variants of CSN1S1 can be subdivided into four categories as a function of the quantity of α_{s1}-casein in goat milk (Moioli et al., 2007). The high-expressing, or strong, alleles (A, B_1, B_2, B_3, B_4, B', C, H, L and M) produce 3.6 g/L per allele,

intermediate alleles (E and I) produce 1.1 g/L each and weak alleles (D, F and G) 0.45 g/L per allele, while, null alleles (0_1, 0_2 and N) are responsible for the apparent absence of this fraction (Neveu et al. 2002). Milk from goat with strong alleles at CSN1S1 locus had been positively correlated with the amount of total protein, total solids and cheese-making properties as compared to milk lacking in α_{s1}-casein (Remeuf et al., 1993; Pirisi et al., 1994; Clark and Sherbon, 2000). Strong genotypes are also associated to higher level of fat, *de novo* synthesized fatty acids (Chilliard et al., 2006; Valenti et al., 2010) and milk yield (Avondo et al., 2009). Moreover, the relationship between feeding practices and CSN1S1 polymorphism have been studied in order to improve productive performances and milk composition. In particular, research focused on the utilization of dietary protein by either goats with low and high capacity to synthesize CSN1S1 in milk. Goats carrying strong alleles showed a greater efficiency in the exploitation of dietary nitrogen as compared to goat with weak alleles resulting in a higher milk protein yield. (Schmidely et al., 2002; de la Torre et al., 2007, de la Torre et al., 2008). Recently, our paper on the interaction between α_{s1}-casein polymorphism and dietary energy level demonstrated that a high energy input improves the efficiency of transformation of the diet into milk and casein yield in goats carrying strong alleles (Pagano et al., 2010).

Taking into account the importance of casein profile on milk nutritional and technological properties, it would be desirable to learn more about the effect of diet on relative composition of goat milk casein. Few data are available on this topic, so far. To our knowledge, the only data available on individual caseins report that low genotype goats increase α_{s1}-casein and α_{s2}-casein yield when fed higher protein diet, whereas their relative proportion in milk remains unchanged, but no information on the other caseins is given (de la Torre et al., 2009). The present study is part of a broader project aiming at studying the impact of different feeding practices on dairy goats performance. Specifically, here we tried to investigate the effect of diets at different energy level on casein composition in milk from goats with different genotype at CSN1S1 locus.

Materials and Methods

Animals and diet

Eighteen Girgentana lactating goats, nine homozygous for strong alleles (AA) and nine homozygous for weak alleles (FF) at CSN1S1 locus, were used to evaluate the effect of genotype, diet and genotype × diet interaction on the relative composition of goat milk caseins. Goats used in the experiment were also characterized by strong alleles at α_{s2}-casein (CSNS2) and β-casein (CSN2) locus. The genotypes of individuals at the CSN1S1, CSN2, and CSN1S2 were determined as reported by Pagano et al.(2010).

Average milk production, days of lactation and body weight was 1.3±0.3 kg/d, 94±12 days and 36.9±4.7 kg, respectively. The selected animals were assigned to two experimental groups and housed in individual pens where water and salt were always available. One group consisted of 5 AA and 4 FF animals, the other one consisted of 4 AA and 5 FF animals. Goats were used in a 2×2 factorial arrangement of treatments, with two genotypes (AA, FF) and two diets at different energy levels (100% and 65% of hay inclusion). The experiment consisted of two simultaneous 2×2 latin squares, one for each genotype, with one square for each level of energy in the diet. Goats were fed *ad libitum* with a mix (50:50) of the two diets for a 12 day pre-experimental period. Each 23-day experimental period included 15 days for adaptation and 8 days for sampling and data collection. During the experimental periods animals received the scheduled diet *ad libitum*. D100 diet consisted of 100% alfalfa pelleted hay, D65 diet consisted of a pelleted feed including 65% of alfalfa hay. All ingredients were ground and pelleted (6 mm diameter). All the animals were managed according to the guide lines of the Animal Ethics Committee of the University of Catania.

Chemical analyses

Individual intakes were daily measured, on the basis of residuals. Each diet was analyzed in triple for dry matter (DM), crude protein (CP), fat (AOAC, 1990), structural carbohydrates (Van Soest et al., 1991), water-soluble carbohydrates (Deriaz, 1961), starch by an enzymatic procedure (Megazyme International Ireland Ltd., Bray, Co. Wicklow), protein fractions (Licitra et al., 1996).

At the end of each experimental period, individual milk samples were collected from the morning (08.00 h.) and evening (17.00 h.) milkings and immediately refrigerated and stored at −18 ◦C until analyses. Milk total nitrogen (TN), non-protein nitrogen (NPN) and non-casein nitrogen (NCN) were determined by FIL-IDF standard procedures (1964). From these nitrogen fractions, total protein (TN*6.38) and casein [(TN–(NCN*0.994))*6.38] were calculated.

Capillary zone electrophoresis (CZE)

A Beckman P/ACE MDQ Capillary Electrophoresis system controlled by 32 Karat Software, version 8.0 (Beckman Instruments, Fullerton, CA, USA) equipped with a UV detector set at 214 nm was used in this study. Separations were carried out using an uncoated fused silica capillary (57 cm lenght, 50 μm i.d., 375 μm O.D.slit opening 100 x 800 μm; Beckman Instruments, Fullerton, CA, USA). Sample solutions were injected for 20s at 0.5 psi. Electrophoresis runs were carried out at 45 °C with a linear voltage gradient from 0 to 25 kV in 3 min, followed by a constant voltage at 25 kV. Buffers for CZE analyses were prepared according to Heck et al. (2008). Sample buffer (pH 8.6 ± 0.1) was 167 mM hydroxymethyl-aminomethane (TRIS - BIO-RAD), 42 mM 3-morpholinopropanesulphonic acid (MOPS - SIGMA), 67 mM ethylenediamine–tetraacetic acid disodium salt dihydrate (EDTA - SIGMA), 17 mM D,L-dithiothreitol (DTT - BIO-RAD), 6 M urea (BIO-RAD) and 0.05% (w/w) hydroxypropylmethylcellulose (MHPC - SIGMA). Run buffer (pH 3.0 ± 0.1) was 0.19 M citric acid (CARLO ERBA), 20 mM sodium citrate (CARLO ERBA), 6 M urea and 0.05% (w/w) PHCP. Individual samples were prepared by mixing individual milk and sample buffer (1:1.5), after 1 h at room temperature, samples were centrifuged at 5000 × *g* for 5 min and fat removed. Samples were analyzed without further preparation.

The caseins were identified by reference to literature (Feligini et al., 2005; Gomez-Ruiz et al., 2004; Recio et al., 1997a; Recio et al., 1997b). Since in CZE peak areas are inversely correlate to migration velocity, relative concentration of individual proteins was determined on the basis of the corrected area by Eq (1) as reported by Heck et al. (2008):

$$C_{x} = \frac{A_x / t_x}{\sum_{i=1}^{n} \left(A_i / t_i \right)} \times 100\%,$$

(1)

where C_x is the relative concentration, A_x the area in the electropherogram, tx the migration time of protein x and n the total number of peaks that together comprise 100% of the area. Quantities of individual caseins were calculated from total casein.

Statistical analysis

Individual data for relative casein composition were subjected to analysis of variance for two simultaneous 2×2 latin squares design, using the GLM procedure of statistical software Minitab (Release14,1995). The model included genotype, diet, block and genotype × diet (G×D). When interaction did not result statistically significant ($P > 0.05$) this term was excluded from the model. Tukey's test was used to compare mean values.

Results

Table 1 reports ingredients and chemical composition of D100 and D65, designed in order to reach similar crude protein, but different energy content, through a different inclusion of carbohydrates sources. Crude protein was identical (15.2% DM) between diets while fat was slightly higher in D65. The difference in net energy was principally due to the starch which was 10 times higher in D65 than D100.

Dry matter intake (DMI) was not affected ($P>0.05$) by genotype (respectively 2514 *vs* 2500 g/d for AA and FF) nor by the energy level (respectively 2525 *vs* 2490 g/d for D100 and D65), moreover no G×D interaction was recorded for intake levels. Milk yield was affected by genotype and diet. Milk was significantly ($P<0.05$) higher for AA than FF animals (1320 *vs* 967 g/d, SEM=88.6). High energy diet significantly ($P<0.05$) improved milk production as compared to diet at lower energy level (1338 *vs* 994 g/d). A significant G×D interaction was recorded: shifting from D100 to D65, milk yield increased ($P=0.039$) by 53% for AA goats whereas it was not affected in FF goats. Results on DMI and milk yield have been already presented by Valenti et al. (2010).

Table 1 - Ingredients and chemical composition of the diet .

	D100	D65
Ingredients, % of fresh weight		
Pelleted alfalfa hay	98.0	65.0
Maize	-	16.0
Barley	-	8.0
Soybean meal	-	3.0
Carob pulp	-	3.0
Corn gluten meal	-	3.0
Vitamin-mineral premix	2.0	2.0
Chemical composition		
Dry matter (DM) %	87.7	85.4
Crude protein % DM	15.2	15.2
Neutral detergent fibre % DM	54.6	44.0
Acid detergent fibre % DM	36.5	24.4
Lignin % DM	13.3	6.4
Crude lipids % DM	2.0	2.4
Ash % DM	11.1	10.1
Water-soluble carbohydrates % DM	7.1	6.8
Starch % DM	1.9	19.5
NEl† kcal/kg DM	1099.2	1386.3

† Net energy for lactation (Cornad et al., 1984)

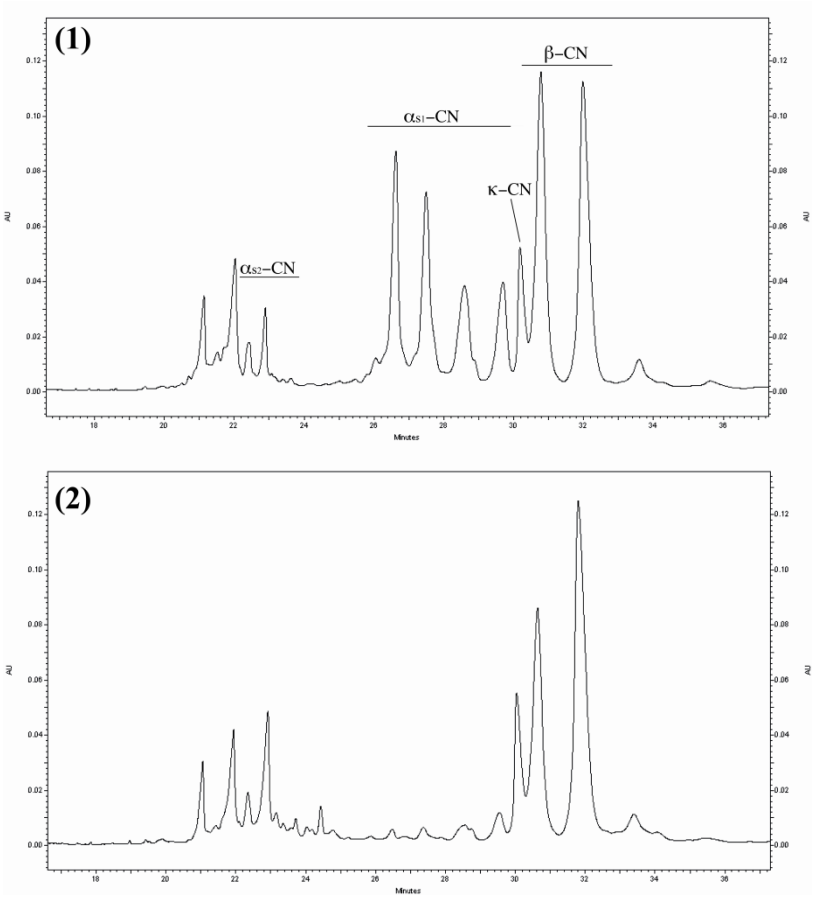

Figure 1 – Capillary electrophoresis of two individual caprine milk samples:
1. containing a high expression α_{s1}-CN variant, 2. containing a low expression α_{s1}-CN variant.
Peaks: α_{s2}-CN= α_{s2}-casein, α_{s1}-CN= α_{s1}-casein, κ-CN= κ-casein; β-CN=β–casein.

Fig. 1 shows the electropherograms of caprine milk. Most of the casein peaks migrated between 22 and 33 min. The order of electromigration of goat caseins was α_{s2}-CN, α_{s1}-CN, κ-CN and β-CN. The α_{s2}-CN was separated into three peaks migrating between 22 and 24 min; α_{s1}-CN was composed of at least four peaks migrating between 25.5 and 30 min; κ-CN peak migrated at 30.2 min before two peaks of β -CN, which appeared between 30.4 and 32.5 min.

Table 2 reports the effect of energy level of diet, genotype and their interaction on casein yield and composition. Casein yield (g/d) was significantly affected by energy level of diet and genotype. In particular, total casein, α_{s1}-CN , κ-CN and β-CN yield was higher with D65, while α_{s2}-CN did not differed between diets. As concerning the effect of genotype, total casein and α_{s1}-CN yield were higher in AA than FF goat milk, α_{s2}-CN was higher in FF than AA goat milk, while no genotype effect was reported for κ-CN and β-CN yield. Total and individual caseins concentration (g/kg) were affect only by genotype. Precisely, total and α_{s1}-CN concentration were higher for AA than FF goat milk, α_{s2}- and β-CN concentration was higher in FF than AA goat milk, while κ-CN tended (P=0.087) to be higher in FF goats. A significant interaction G×D was found only for the concentration of α_{s1}-CN, that decreased when AA goats shifted from D100 to D65.

Table 2 - Genotype × Diet effect on casein yield and composition of goat milk.

	D100		D65		SEM	Significance (P)		
	AA	FF	AA	FF		G	D	GxD
Casein g/d	29.4^{b}	23.7^{c}	43.3^{a}	30.4^{b}	2.83	**	**	*
Casein g/kg milk	31.7^{a}	21.8^{c}	28.8^{ab}	26.9^{b}	0.0921	***	ns	*
α_{s2}-CN g/d	2.1^{b}	4.2^{a}	3.5^{ab}	5.1^{a}	0.342	**	+	ns
α_{s1}-CN g/d	12.4^{b}	2.1^{c}	17.7^{a}	2.7^{c}	1.62	***	**	**
K-CN g/d	2.4^{b}	2.7^{ab}	3.8^{a}	3.8^{ab}	0.256	ns	**	ns
B-CN g/d	12.5^{b}	14.7^{b}	18.3^{a}	18.8^{a}	1.26	ns	*	ns
α_{s2}-CN g/kg milk	2.4^{b}	3.9^{a}	2.3^{b}	4.6^{a}	0.212	***	ns	ns
α_{s1}-CN g/kg milk	13.0^{a}	1.2^{b}	11.7^{a}	1.7^{b}	1.10	***	ns	*
K-CN g/kg milk	2.7	2.6	2.6	3.5	0.0997	+	ns	ns
B-CN g/kg milk	13.6^{ab}	14.1^{ab}	12.2^{b}	17.1^{a}	0.487	**	ns	ns

Significance: ns P>0.1; +P<0.1 *P<0.05; **P<0.01; ***P<0.001
^{a,b} Different letters within row indicate a significant difference between values (P<0.05)

Discussion

The presence of multiple peaks of the same casein along the electropherogram is due to the phosphorilation state of the casein. Phosphorilation is one of the most important post-translational modification responsible for the addiction of a variable number of phosphate groups to the caseins. The order of electromigration of caseins (α_{s2}-CN, α_{s1}-

CN, κ-CN and β-CN) was identical to those reported by Feligini et al. (2005), Gomez-Ruiz et al. (2004), Recio et al. (1997a; 1997b), but the migration times were slightly higher. This could be due to the polymeric additive and the capillary used in our experiment to improve the separation. Indeed, it is reported that the use of MHPC instead of MHEC increases migration times (De Jong et al., 1993); moreover, Rodriguez-Nogales (2006) found similar migration times using an uncoated fused silica capillary.

High energy diet increased total casein yield as compared to low energy diet. This finding is in accordance with similar works carried out to evaluated the effect of diet on milk composition from goat differing by CSN1S1 genotype (Schmidely et al., 2002; de la Torre et al., 2009). In these papers, opposite to our conditions, diets differed in crude protein level, but it is supposable that the higher energy availability arising from D65 improved protein synthesis at rumen level (Koenig et al., 2003) and had an effect similar to that produced by an increase of dietary protein level. Moreover, taking into account that milk yield increased when D65 was given to the animal, a decrease of casein concentration (g/kg milk) would have been expected; nevertheless, the concentration of total caseins was not affected by the diet, confirming that D65 had a positive effect on the casein biosynthesis rate (Morand-Fehr et al., 2000).

Casein yield was higher in AA goats than FF goats; this expected results depends on the positive relationship between strong alleles at α_{s1}-CN locus and casein yield (Grosclaude et al., 1987; Clark et al., 2000; Schmidely et al., 2002; Avondo et al., 2009). Moreover, our results suggest that the higher total casein level in AA goat than FF goats is due only to the higher biosynthesis of α_{s1}-CN by goat carrying strong alleles at CSN1S1 locus and not on the other casein fractions. In fact, we found that total casein yield was higher in AA goat milk despite α_{s2}-CN was higher in FF goats (de la Torre et al., 2009), and κ- and β-CN did not differ according to the genotype. Moreover, except for α_{s1}-CN, concentration of α_{s2}-CN, κ-CN and β-CN was higher in FF goat. These data, consistent with previous papers reporting the concentration of α_{s2}-CN in milk from goats homozygous for weak (Tziboula, 1997) and null (Criscione et al., 2011) alleles at CSN1S1 locus, suggest that in FF goat milk the lower content of α_{s1}-CN may be partially compensated by other caseins. Indeed, values of β-CN

concentration in AA goat milk were similar to mean values reported in literature for non-null genetic variant of β-CN, whereas FF goats showed values 13-15% higher (Moatsou et al., 2006; Tziboula, 1997).

A significant G×D effect on casein composition was reported. Daily production of α_{s1}-CN significantly increased for AA goat shifting from D100 to D65. At the same time its concentration lowered as a consequence of a greater increase of milk yield.

Conclusion

In conclusion we found that dietary high energy input consistently improved casein yield and concentration beside genotype at α_{s1}-CN locus. Genotype at CSN1S1 locus influences milk protein yield and composition. In particular, the here-presented results suggest that the higher casein yield of goats carrying strong alleles mainly depends on biosynthesis of α_{s1}-CN. Moreover, the lower content of α_{s1}-CN may be partially compensated by the other caseins in FF goat milk.

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General conclusion

This research indicates that an interaction between genetic polymorphism of α_{s1}-casein and dietary factors occurs, thus affecting the efficiency of nutrient transfer into milk.
In particular, our results suggest that in a free choice feeding system goats are able to select a diet according to their genetic aptitude to produce casein. In particular, besides the genotype, the energy requirements were over-satisfied, but goats carrying strong alleles voluntary selected a diet with a higher percentage of energy-rich feeds thus increasing their milk and casein production as compared to goats with weak alleles. For these reason a second trial has been carried out to investigate how goats selected according to different α_{s1}-casein genotype could respond to diets with similar protein content and different energy levels. This second trial confirmed that high energy input improves the efficiency of transformation of the diet into milk and casein yield in goats carrying strong alleles, whereas it does not exert noticeable effects in goats carrying weak alleles.
As regard fine milk composition, our results suggest that polymorphism at α_{s1}-casein locus affect milk fatty acid composition. In particular, in similar feeding conditions, *de novo* synthesized fatty acids have been found to be higher in the fat of milk of goat with strong alleles. However, this difference tends to be lost when weak alleles goats receive a high energy diet because of an increase of these fatty acids also in the fat of FF goats. Lastly, the study on relative milk casein composition indicates that the higher casein yield and content of goats carrying strong alleles exclusively depends on the biosynthesis rate of α_{s1}-casein. Moreover, the lower content of α_{s1}-casein in goat with weak alleles seems to be partially compensated by the other caseins in FF goat milk.

Taking in account these results, it would be desirable a differentiation of the diet in function of α_{s1}-casein genotype. It is known that milk from goats with strong alleles has more protein and casein than animal with weak alleles; in our conditions for the first time a positive relation between strong genotype and milk yield was found. This relation was further improved by increased dietary energy; in addiction the higher production level was associated with an enhanced protein, casein and fat content as well. Even if no cheese-making trials have been carried out, it is seems obvious that milk obtained by AA goat fed high energy diet has higher cheese-making properties

than FF goat milk, also taking into account that the same improvements in terms of milk yield and composition were not achieved by FF. Therefore, the improvement in productive performances of AA animals can give a reason for the elevation of costs due to use of concentrates in the diet to give means.

Differently from AA goats, the main effect of high dietary energy on milk from goats with weak alleles, was to modify milk fatty acid composition with no significant effect on milk and protein. Unfortunately, the changing in fat composition consists in an increased level of fatty acids with an high atherogenic effect (lauric, myristic, and palmitic acids). These findings seems to support the choice of the farmers which preferentially select dairy goats with strong genotype.

However, the presence in the farm of dairy goats with weak genotype at α_{s1}-casein locus could be justified by a different productive destination of their milk (drinking milk). Indeed, under hay-based feeding conditions, the quantity of atherogenic fatty acids is lower in FF milk than AA milk. Moreover, a study conducted on pigs to evaluate the allergenic power of goat milk, demonstrated that milk lacking of α_{s1}-casein fraction is less allergenic than other goat milk especially when it is replaced by α_{s2}-casein. According to our results, the smaller quantity of as1-casein is compensated also by an higher quantity of α_{s2}-casein.

Thus, the differentiation of feeding strategies according to the productive potential of dairy goats coupled with a different destination of use for milk produce by goat with weak alleles could result in important economic advantages for the farmers.

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