Influence of fresh forage-based diets and α_{S1} -casein (*CSN1S1*) genotype on nutrient intake and productive, metabolic, and hormonal responses in milking goats

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ABSTRACT

Polymorphism at the α_{S1} -casein locus (*CSN1S1*) in goats influences several milk production traits. Milk from goats carrying strong alleles, which are associated with high α_{S1} -casein (α_{S1} -CN) synthesis, has higher fat and casein contents, longer coagulation time and higher curd firmness than milk from goats with weak alleles linked to low α_{S1} -CN content. Nutrition also affects these milk properties; therefore, it is important to better understand the interaction between dietary characteristics and the *CSN1S1* genotype in goats. This study aimed to investigate the effect of fresh forage based diet or energy supplement on feeding behavior, milk production, and metabolic and hormonal parameters of Girgentana goats with different genotypes at *CSN1S1* loci. From a group of goats genotyped by PCR at the DNA level, 12 were selected because they had the same genotype for α_{S2} -CN, β -CN, and κ -CN but a different genotype for α_{S1} -CN: 6 were homozygous for strong alleles at the *CSN1S1* loci (AA) and 6 were heterozygous for a weak allele (AF). Goats of each genotype were allocated to 3 subgroups and fed 3 diets ad libitum in a 3 × 3 Latin square design. The diets were sulla (*Hedysarum coronarium* L.) fresh forage, sulla fresh forage plus 800 g/d of barley meal (SFB), and mixed hay plus 800 g/d of barley meal (MHB). Diet had a stronger effect than *CSN1S1* genotype. The SFB diet led to the highest energy intake, dry matter (DM) digestibility, and milk yield. The fresh forage diets (SFF and SFB) increased DM and crude protein (CP) intake, CP digestibility, and milk CN compared with the MHB diet. The diets supplemented with energy (SFB, MHB) reduced milk fat and urea, improved CP utilization for casein synthesis, and limited body fat mobilization, in

accordance with a lower level of nonesterified fatty acids and higher levels of glucose and IGF-1. With regard to *CSN1S1* genotype, AA goats showed higher CP digestibility and lower free thyroxine hormone and cholesterol levels than AF goats. Significant diet × genotype interactions indicated how AA goats, compared with AF goats, showed higher DM digestibility and milk yield when fed the SFB diet, which had more energy. A reduction in free triiodothyronine hormone occurred in AF goats fed the MHB diet, whereas no differences were observed in AA goats. These results demonstrate how goats with a higher capacity for α_{S1} -CN synthesis exhibit more efficient energy and protein utilization, evident at the digestive level, and better productive responses to high-nutrition diets.

Key words: goat, milk, α_{S1} -casein, diet by genotype interaction

INTRODUCTION

Both genetic and nutritional factors contribute to determine the production, composition, and technological properties of milk. The characteristics of goat milk depend strongly on the genetic variants of key proteins; in particular, polymorphisms in α_{S1} -CN have a marked effect on milk composition, especially casein content and clotting ability (Clark and Sherbon, 2000a,b).

Studies on goat milk protein polymorphisms have shown that the *CSN1S1* loci are highly polymorphic. In fact, 18 alleles have been detected and classified into 4 groups associated with different rates of synthesis of milk CN: strong (A, B1, B2, B3, B4, B', C, H, L, M), intermediate (E and I), and weak (D, F, G) alleles. Those groups synthesize a high (about 3.5 g/L), medium (about 1.1 g/L), and low (0.45 g/L) quantity of α_{S1} -CN, respectively, in addition to null alleles (01, 02, N), which do not synthesize α_{S1} -CN (Sacchi et al., 2005; Moiola et al., 2007).

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The milk of goats carrying strong alleles, which are associated with a high α_{S1} -CN content, has higher percentages of milk casein, fat, calcium, and phosphorus, and smaller CN micelles than the milk of goats with weak alleles; these characteristics increase coagulation time and curd firmness (Grosclaude et al., 1987; Clark and Sherbon, 2000a). Moreover, the milk of homozygous AA goats is characterized by a less intense taste and smell than that of FF goats (Remeuf, 1993). Recently, it was also found that the *CSN1S1* genotype influences milk fatty acid composition, in particular the short and medium chain fatty acid content and the activity of Δ^9 -desaturase (Chilliard et al., 2006; Valenti et al., 2010).

Because the nutritional characteristics of an animal's diet also exert a strong influence on milk production and properties, interest exists in studying the relationships between genetic polymorphisms in milk CN proteins and goat nutrition to verify the expression of genes coding for α_{S1} -CN as a function of feeding regimen.

Recent studies on goats fed dry pellet diets with different amounts of hay examined the possible relationships between *CSN1S1* genotype and goat feeding. They found that, compared with FF individuals, AA individuals use protein more efficiently (Schmidely et al., 2002; De la Torre Adarve et al., 2009) and respond to higher diet energy, therefore increasing milk production (Pagano et al., 2010a) and α_{S1} -CN concentration (Valenti et al., 2011). These results seem to depend more on a different hormonal status than on a dissimilar metabolic efficiency (Pagano et al., 2010a,b).

The aim of the present study was to contribute to the knowledge base on relationships between *CSN1S1* genotype and nutrition in goats. The purpose was to determine whether differences in the milk production of goats with different genetic aptitudes for α_{S1} -CN synthesis depend on the goats' different levels of efficiency of nutrient utilization—as supported by a different metabolic or hormonal status—or whether the satisfaction of energy and protein requirements by diet could mitigate the productive gap among genotypes. The aim was to obtain data that could usefully direct the work of genetically selecting goats bred for high milk production and more efficient feed utilization, which would contribute to a beneficial reduction in the environmental impact of livestock production systems.

Therefore, the nutritional, metabolic, hormonal, and productive responses of Girgentana goats to fresh forage diets with different levels of energy and protein were investigated according to different *CSN1S1* genotypes in goats. A comparison was made between goats homozygous for strong alleles at *CSN1S1* loci (AA) and those heterozygous for a weak allele (AF), which are associated with high and intermediate levels of α_{S1} -CN

synthesis, respectively. The choice of the AF genotype was dictated primarily by the high frequency of goats heterozygous at *CSN1S1* loci in local farms, but also by the limited number of studies of the heterozygous *CSN1S1* genotype.

Specifically, this study evaluated the effect of a diet based on sulla fresh forage (*Hedysarum coronarium* L.), supplemented or not with an energy concentrate such as barley, on feed intake, digestibility, metabolic and hormonal responses, and milk production of goats according to their *CSN1S1* genotype. The choice of sulla was based on the widespread availability of this legume forage in Mediterranean areas, where it is greatly appreciated for the positive effects of its nutrient and condensed tannins contents on milk yield and composition, as demonstrated in both sheep (Bonanno et al., 2007a; Molle et al., 2009) and goats (Bonanno et al., 2007b).

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was conducted at a private farm in Sicily (Santa Margherita Belice, Agrigento, Sicily, Italy) for a period lasting 11 wk, from March to May. At the farm, 40 milking goats were genotyped at the *CSN1S1*, *CSN1S2*, *CSN2*, and *CSN3* loci, coding for α_{S1} -CN, α_{S2} -CN, β -CN (Ramunno et al., 2000, 2001; Cosenza et al., 2003), and κ -CN (Caroli et al., 2006; Gigli et al., 2008), respectively, using specific PCR protocols at the DNA level.

Twelve milking goats, averaging 37.2 ± 3.5 kg in live weight, were selected for having the same genotype at *CSN1S2* (AA), *CSN2* (AA), and *CSN3* (AA) loci. They were split into 2 groups by *CSN1S1* genotype: "strong," composed of 6 goats homozygous for strong alleles (AA), and "intermediate," composed of 6 goats heterozygous for a weak allele (AF).

For the entire experimental period, the goats were housed in individual large pens placed inside a shed. After a 2-wk period of adaptation to changed housing conditions, the 6 goats of each *CSN1S1* genotype (AA and AF) were allocated homogeneously, based on DIM (86 ± 41), to 3 subgroups. The subgroups received 3 different diets in succession, according to a 3×3 Latin square design with 3 phases composed of 14 d for adaptation to the diets and 7 d for data and sample collection.

The 3 experimental diets, with different levels of energy and protein, contained sulla fresh forage ad libitum (SFF), sulla fresh forage ad libitum and 800 g/d barley meal (SFB), and mixed hay ad libitum and 800 g/d barley meal (MHB). The sulla fresh forage was

mowed daily in the morning, cut roughly, and supplied to goats in the feeding trough 2×/d, at 1000 and 1700 h, whereas the barley meal was divided into 2 meals.

Sampling and Analysis

At the beginning and the end of each phase, all goats were weighed and monitored for their BCS, and individual feces samples were taken directly from the rectum. During each phase, the offered and refused feed of each goat was weighed daily and sampled twice. The individual milk yield was recorded daily at morning (0700 h) and evening (1600 h) milking and sampled 3 times on d 3, 5, and 7 of the sampling week in each phase. The samples of offered and refused *sulla* fresh forage were disaggregated into their different botanical parts (leaves, flowers, stalks, and stems) to estimate, based on their incidence and their chemical composition, the quality of the ingested forage.

The samples of barley and botanical parts of *sulla* were analyzed for the determination of DM, CP, ether extract, ash (AOAC, 1995), and structural carbohydrates (NDF, ADF, ADL; Van Soest et al., 1991). The energy value of the samples (expressed in Mcal of NE_L) was estimated using NRC (2001) equations based on digestibility and ADF content. In addition, freeze-dried samples of *sulla* botanical parts were analyzed by spectrophotometer for condensed tannins using the butanol-HCl method (Porter et al., 1986) and delphinidin as the reference standard (Tava et al., 2005).

Nitrogen and lignin (ADL) contents in the feces were determined and used as a marker to estimate the digestibility of DM and CP (Sundvold and Cochran, 1991). Fecal recovery data for the same ADL were taken from previous *in vivo* digestibility trials (A. Bonanno, unpublished data).

Individual milk samples were analyzed for lactose, fat, and SCC by infrared method (Combi-foss 6000, Foss Electric, Hillerød, Denmark); total bacterial count by BactoScan instrument (Foss Electric); pH by HI 9025 pH meter (Hanna Instruments, Ann Arbor, MI); titratable acidity by Soxhlet-Henkel method (°SH/50 mL); and urea by the enzymatic method using the difference in pH (CL-10 Plus, Eurochem, Italy). Fat-corrected milk at 3.5% fat was calculated as follows (Pulina et al., 1991): FCM, g/d = milk, g/d × (0.634 + 0.1046 × fat %).

The protein fractions (CN, whey protein, and NPN) were determined by analyzing individual milk samples for total nitrogen (TN), noncasein nitrogen (NCN), and NPN by FIL-IDF (1964, 1993) standard procedures. From these nitrogenous components, total protein (TN × 6.38), whey proteins [(NCN − NPN) × 6.38], and CN [TN − (NCN × 0.994) × 6.38] were calculated.

At the end of the preexperimental and experimental phases, blood samples from each fasted goat were collected in the morning, via jugular venipuncture, into 2 Vacutainer tubes—one containing lithium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ) and the other containing no anticoagulant. The tube containing lithium heparin was immediately placed on ice. The plasma was centrifuged at $1892 \times g$ for 10 min at 4°C in a refrigerated centrifuge. Serum samples were kept at room temperature until a clot formed and then centrifuged. Plasma and serum samples were stored at −80°C until analysis.

The metabolic and hormonal profiles of the goats were measured. An automated analyzer (TARGA model 2000, Technology Advanced Random Generation Analyzer, Biotechnica Instruments, Rome, Italy) was used to determine glucose, cholesterol, urea, total protein, albumin, and calcium (Mercury, Riardo, Italy) in plasma samples. Globulin content was calculated as the difference between total protein and albumin. Nonesterified fatty acids and BHBA were analyzed using FA 115 and Ranbut commercial kits, respectively (Randox Laboratories, Crumlin, UK). Insulin (Merco-dia 10-1202-01, Uppsala, Sweden), IGF-1 (600; DRG Diagnostics, Marburg, Germany), free triiodothyronine (fT3; DiaMetra Srl, Milan, Italy), and free thyroxine (fT4; DiaMetra Srl) were determined using commercially available ELISA kits.

Statistical Analyses

Statistical analyses were performed using the MIXED procedure in SAS 9.1.2 (SAS Institute, 2004). Experimental phase (1, 2, 3), diet (D: SFF, SFB, MHB), genotype (G: AA, AF), and D × G interaction were fixed factors, and the goat was considered a random factor. Preexperimental data on milk production and blood parameters were used as covariates only when significant. Before the analyses, the normal distribution of variables was assessed and SCC, total bacteria count, NEFA, insulin, fT3, and fT4 data were transformed in log₁₀, log₁₀, ln (y+1), ln (y+10), ln (y+1) and ln (y+10), respectively. Least squares means were compared using Tukey's test ($P < 0.05$). Pearson correlation coefficients were calculated between some variables measured in this study.

RESULTS AND DISCUSSION

Feed Intake and Digestibility

The live weight and BCS of goats did not change as a function of *CSN1S1* genotype or diet (Table 1). Regarding the feeding behavior of goats (Table 1), the intake

of DM and major nutrients was strongly influenced by diet and did not reveal a significant effect of *CSN1S1* genotype and $D \times G$ interaction. Similar results were found by other researchers (Bonanno et al., 2007c; Pagano et al., 2010a); in particular, with regard to DM intake, Bonanno et al. (2007c) found no difference between goats with strong (*AA*) and intermediate (*AF*) genotypes, in line with the present findings, although they observed a lower feed intake in goats with weak (*FF*) genotype.

Regarding the effect of diet (Table 1), compared with hay, sulla fresh forage was the favored DM intake regardless of barley energy supplementation. At the same time, protein intake, as well as condensed tannins intake, increased with the increasing level of fresh forage ingested. The SFF diet resulted in the maximum fiber intake, followed by the MHB diet; whereas the SFB diet, due to the lower fiber intake, corresponded to the highest energy intake and a more balanced protein to energy ratio. These results confirm the positive effect of sulla forage on voluntary feed intake (Bonanno et al., 2007a; Molle et al., 2009), attributed to its high protein percentage and low NDF content but also to the high ratio of nonstructural to structural carbohydrates (Burke et al., 2004).

Interesting results not found in the literature emerged for diet digestibility (Table 1). The digestibility coefficients of both DM and protein were strongly influenced by diet, with the highest values recorded with the SFB diet and the lowest with the MHB diet. Protein digestibility was also significantly influenced by genotype, as goats with strong alleles showed a greater digestive capacity. In contrast to these results, Schmidely et al. (2002) and De la Torre et al. (2008) found no effect of *CSN1S1* genotype on nitrogen digestibility; however, in goats with a high genetic capability for α_{S1} -CN synthesis, those authors observed a higher utilization of digested nitrogen for milk production. The $D \times G$ interaction was significant for the digestibility of DM, and tended to be significant for protein. Table 2 shows how the best DM digestive capacity of SA goats emerged only with the SFB diet, that diet being higher in energy and, presumably, the most nutritionally balanced. The digestibility of protein in goats with strong alleles was higher with the SFF diet, which was richer in protein, but no difference was found for the MHB diet characterized by lower protein to energy and protein to fiber ratios (Table 1).

These results confirm that goats with strong alleles at *CSN1S1* loci respond better to diets higher in energy (Pagano et al., 2010a). However, they also indicate that the higher efficiency of energy and protein utilization is already revealed at the digestive level. A hypothesis could be advanced about a positive influence of strong

genotype on the expression of genes coding for the synthesis of digestive enzymes, rather than implications for the rumen or mammary gland.

Milk Yield and Composition

As in feeding behavior, milk production showed a more pronounced effect of diet rather than genotype (Table 2). In fact, daily milk yield increased from the SFF diet to the MHB diet and reached its maximum with the SFB diet, in which sulla forage was supplemented with barley. Moreover, daily FCM yield (adjusted to 3.5% fat) was higher with the SFB diet, whereas no difference was found between SFF and MHB goats.

This is not the first time the lack of an effect of *CSN1S1* genotype on the milk yield of goats has been reported in the literature. In fact, several researchers have found no significant difference between goats with strong genotype *AA* and weak genotype at *CSN1S1* loci (Schmidely et al., 2002; Chilliard et al., 2006; Bonanno et al., 2009; De la Torre Adarve et al., 2009); only Avondo et al. (2009) reported increased milk production of *AA* goats compared with *FF* goats.

When the intermediate genotype *AF* was considered, the milk yields of *AA* and *AF* goats did not differ significantly, and both yields were higher than that of *FF* goats (Bonanno et al., 2007c). However, Pagano et al. (2010a) found a higher milk yield in goats with the strong genotype compared with those with the weak and intermediate genotypes, which did not differ between themselves. One explanation for these discrepancies might be that the milk production of goats with different *CSN1S1* genotypes depends greatly on dietary intake. In fact, in this study, although the *CSN1S1* genotype did not influence the milk yield, a significant $D \times G$ interaction was noted, according to other authors (De la Torre Adarve et al., 2009; Pagano et al., 2010a). In this regard, Table 2 shows that the productive superiority of the *AA* genotype over the *AF* genotype occurred only when goats were fed the higher energy SFB diet (1,720 vs. 1,606 g/d; $P < 0.05$). In fact, the daily milk yield produced by the *AA* goats fed the SFB diet was more than 350 g/d higher than that of goats fed the other diets, and this difference among diets was markedly less in *AF* goats. These results confirm the better milking response of goats with the strong genotype when fed diets balanced for energy and protein content (De la Torre Adarve et al., 2009; Pagano et al., 2010a).

Regarding milk composition (Table 2), only SCC and total bacteria count remained unaffected by the considered factors. With regard to the effect of diet, energy supplementation with barley reduced milk fat and urea content. The reduction in milk fat is attribut-

Table 1. Effects of diet and *CSN1S1* genotype of goats on live weight, BCS, nutrient intake, and digestibility (LSM)

Item	Diet (D) ¹			Genotype (G) ²		AA			AF			SEM	Significance		
	SFF	SFB	MHB	AA	AF	SFF	SFB	MHB	SFF	SFB	MHB		D	G	D × G
Final live weight, kg	38.8	38.9	38.6	39.5	38.1	39.7	39.5	39.2	37.9	38.2	38.0	1.51	NS	NS	NS
Final BCS	2.79	2.79	2.75	2.75	2.81	2.79	2.71	2.75	2.79	2.87	2.75	0.092	NS	NS	NS
DMI, g/d	1,820 ^a	1,807 ^a	1,655 ^b	1,746	1,776	1,769	1,776	1,692	1,872	1,837	1,618	86.8	**	NS	NS
CP intake, g/d	321 ^a	290 ^b	203 ^c	272	270	322	286	209	320	293	197	15.3	***	NS	NS
NDF intake, g/d	632 ^a	483 ^c	539 ^b	535	568	592	463	550	673	503	527	37.4	***	NS	NS
Condensed tannins intake, g/d	47.2 ^a	35.6 ^b	3.50 ^c	29.1	28.5	47.7	35.7	3.70	46.6	35.4	3.31	1.64	***	NS	NS
NE _L intake, Mcal/d	2.40 ^b	3.03 ^a	2.34 ^b	2.60	2.58	2.36	3.05	2.37	2.44	3.01	2.31	0.088	***	NS	NS
CP/NE _L , g/Mcal	133.5 ^a	94.9 ^b	85.6 ^c	105.5	103.9	135.9	93.6	87.1	131.1	96.2	84.2	3.30	***	NS	NS
CP/NDF	0.54 ^b	0.63 ^a	0.39 ^c	0.54	0.50	0.58	0.65	0.39	0.50	0.62	0.39	0.020	***	NS	NS
DM digestibility, %	68.1 ^b	74.4 ^a	58.9 ^c	67.6	66.6	69.1 ^c	76.4 ^a	57.4 ^d	67.1 ^c	72.3 ^b	60.5 ^d	1.15	***	NS	**
CP digestibility, %	66.8 ^a	66.9 ^a	47.6 ^b	62.1 ^a	58.7 ^b	68.7 ^{ef}	69.9 ^e	47.7 ^b	64.9 ^{fg}	63.8 ^g	47.5 ^h	1.61	***	*	†

^{a-d}Means within a row with different superscripts differ ($P \leq 0.05$).^{e-h}Means within a row with different superscripts differ ($P \leq 0.10$).¹Diets: SFF = sulla (*Hedysarum coronarium* L.) fresh forage; SFB = sulla fresh forage plus 800 g/d barley meal; MHB = mixed hay plus 800 g/d barley meal.²Genotypes: AA = homozygous for strong alleles; AF = heterozygous for a weak allele.† $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.**Table 2.** Effects of diet and *CSN1S1* genotype of goats on milk yield and composition (LSM)

Item	Diet (D) ¹			Genotype (G) ²		AA			AF			SEM	Significance		
	SFF	SFB	MHB	AA	AF	SFF	SFB	MHB	SFF	SFB	MHB		D	G	D × G
Milk, g/d	1,353 ^c	1,664 ^a	1,423 ^b	1,487	1,473	1,356 ^{cd}	1,720 ^a	1,384 ^{cd}	1,348 ^d	1,606 ^b	1,465 ^c	44.3	***	NS	**
FCM, ³ g/d	1,359 ^b	1,599 ^a	1,344 ^b	1,439	1,430	1,362 ^c	1,640 ^a	1,315 ^c	1,351 ^c	1,557 ^b	1,382 ^c	102.1	***	NS	*
Lactose, %	4.50	4.46	4.45	4.45	4.50	4.48	4.44	4.43	4.53	4.48	4.47	0.068	NS	NS	NS
Fat, %	3.59 ^a	3.17 ^b	2.95 ^c	3.17	3.31	3.52	3.06	2.92	3.66	3.28	2.99	0.21	***	NS	NS
Protein (total N × 6.38), %	3.54 ^a	3.52 ^a	3.38 ^b	3.49	3.47	3.55 ^d	3.49 ^d	3.43 ^d	3.52 ^d	3.56 ^d	3.33 ^e	0.10	***	NS	†
CN, %	2.69 ^a	2.73 ^a	2.61 ^b	2.74	2.61	2.76 ^d	2.76 ^d	2.70 ^d	2.62 ^d	2.70 ^d	2.51 ^e	0.092	***	NS	†
CN yield, g/d	35.6 ^b	45.3 ^a	36.1 ^b	40.2	37.8	37.2	46.8	36.6	33.9	43.8	35.6	3.00	***	NS	NS
CN/CP intake, g/kg	115 ^c	164 ^b	191 ^a	156	157	117	168	184	113	161	198	12.7	***	NS	NS
CN/digestible CP intake, g/kg	172 ^c	242 ^b	409 ^a	267	281	172 ^c	237 ^b	393 ^a	173 ^c	247 ^b	424 ^a	20.6	***	NS	NS
Whey protein, %	0.60 ^a	0.55 ^b	0.53 ^b	0.54	0.59	0.58	0.52	0.51	0.62	0.59	0.55	0.024	**	NS	NS
NP _N , %	0.039	0.038	0.039	0.038	0.040	0.039	0.038	0.039	0.040	0.039	0.039	0.001	NS	NS	NS
Urea, mg/dL	35.4 ^a	32.1 ^b	30.9 ^b	33.8	31.8	35.8	33.4	32.3	35.0	30.9	29.5	2.19	***	NS	NS
SCC, log ₁₀ cells/mL	5.27	5.28	5.27	5.13	5.42	5.15	5.09	5.14	5.39	5.46	5.41	0.15	NS	NS	NS
Total bacteria count, log ₁₀ , cfu/mL	5.71	5.90	5.76	5.73	5.84	5.71	5.78	5.71	5.70	6.01	5.81	0.098	NS	NS	NS

^{a-d}Means within a row with different superscripts differ ($P \leq 0.05$).¹Diets: SFF = sulla (*Hedysarum coronarium* L.) fresh forage; SFB = sulla fresh forage plus 800 g/d barley meal; MHB = mixed hay plus 800 g/d barley meal.²Genotypes: AA = homozygous for strong alleles; AF = heterozygous for a weak allele.³Milk corrected at 3.5% fat: milk g/d × (0.634 + 0.1046 × fat %); Pulina et al., 1991.† $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

able to the lower forage to concentrate ratio of the diets supplemented with barley, and therefore to the lower cellulose intake. The reduction in milk urea is likely a consequence of the more balanced protein to energy ratio in diets with barley supplementation (Table 2), which results in higher rumen microbial growth (Harmeyer and Martens, 1980).

In addition, the sulla fresh forage, independent of the barley supplementation, resulted in an increase in milk protein and CN percentages, probably favored by the higher intake of condensed tannins (Table 2). These secondary metabolites are present in the sulla forage in a moderate amount (<6% DM) that, in Mediterranean areas, can vary between 0.8 and 5% of the whole-plant DM in relation to environment, genetic type, and stage of development (Amato et al., 2005). Condensed tannins, together with their anthelmintic action (Hoste et al., 2006), reduce protein degradability in the rumen and consequently result in a greater amount of amino acids being absorbed in the intestinal tract (Niezen et al., 2002; Min et al., 2003; Waghorn and McNabb, 2003), thus improving the efficiency of dietary protein utilization for the synthesis of milk casein.

However, a higher daily CN yield (45.3 g/d; $P < 0.01$; Table 2) was obtained with the SFB diet, likely because it is associated with a higher daily milk yield. Furthermore, efficiency in the use of dietary protein for milk CN synthesis, expressed in grams of CN per kilogram of crude or digestible protein intake, was influenced by diet but not by *CSN1S1* genotype. In fact, it tended to increase as a function of reduced protein intake, but at the same time it was positively influenced by the energy supplementation with barley that, by increasing the energy availability in the rumen, favored the conversion of nitrogen into microbial protein, resulting in a reduction in milk urea and especially in the increased availability of amino acids used in the udder for milk CN synthesis. A strong negative correlation between the efficiency of milk CN synthesis (CN/CP intake, g/kg) and milk urea ($r = -0.70$; $P < 0.01$) was found in the SFB group.

The *CSN1S1* genotype did not significantly influence milk composition, even in terms of CN percentage. Many reports in the literature (Avondo et al., 2009; Bonanno et al., 2009; De la Torre Adarve et al., 2009; Valenti et al., 2011) indicate that the milk produced by goats with the strong *CSN1S1* genotype AA contained a higher percentage of CN than that produced by goats with the weak *CSN1S1* genotype, whereas goats with the heterozygous genotype AF had an intermediate level of CN (Bonanno et al., 2007c; Pagano et al., 2010a) that was statistically different from that of both strong and weak goats. However, in the current study, the CN showed a tendency to significance of the D $\times$

G interaction (Table 2): in fact, in goats with the AA (strong) genotype, diet did not affect the level of milk casein, whereas goats with the AF heterozygous genotype showed a lower CN content if fed the MHB diet. This trend suggests a major effect of condensed tannins of the sulla fresh forage in AF goats; in fact, when fed SFF and SFB diets, they improved slightly the dietary protein utilization for the synthesis of milk CN reaching a level near to that of AA goats. Moreover, in AA goats, a strong negative correlation between milk production and milk urea was found ($r = -0.61$; $P < 0.007$).

Metabolic and Hormonal Variables

The metabolic and hormonal variables are shown in Table 3. The plasma concentrations of the energy metabolism indicators, such as glucose, NEFA, BHBA, and cholesterol, were within the normal range for caprines (Kaneko et al., 1997); all of these variables except cholesterol were affected by diet. We observed no effect of genetic variants at the *CSN1S1* locus (AA vs. AF) on glucose, NEFA, or BHBA, in line with Chilliard et al. (2006); however, a significant effect of *CSN1S1* genotype was noted for cholesterol ($P < 0.05$) and the D $\times$ G interaction for BHBA ($P \leq 0.10$).

Overall, the expression of energy metabolism measures indicated an energy deficiency in goats receiving the SFF diet compared with the SFB and MHB diets. Consequently, the mobilization of body reserves was lower in the SFB and MHB groups, and this was reflected in their lower concentrations of NEFA and greater concentrations of glucose and IGF-1 compared with goats fed the SFF diet. Because of the negative correlation between energy balance and concentrations of circulating IGF-1 (McGuire et al., 1992), a lower level of IGF-1 was found in the SFF group compared with the SFB and MHB groups.

Plasma BHBA increased ($P < 0.05$) in the SFB and MHB groups compared with the SFF group. This result was in line with Fernández et al. (2007), who observed a higher level of BHBA in goats supplemented with cereals than in goats that received no supplement. Overall, relationships were found between plasma BHBA and milk production ($r = 0.30$; $P < 0.05$), CP intake ($r = -0.33$; $P < 0.04$), and milk protein ($r = -0.41$; $P < 0.01$).

A slight D $\times$ G interaction was noted for BHBA ($P \leq 0.10$), with an increase in AFWA goats of 47% for the SFF group and decreases of 22 and 14% for the SFB and MHB groups, respectively. Because of this interaction, a negative correlation between BHBA and milk protein was found only in AF goats ($r = -0.64$; $P < 0.004$). In a previous study, Schmidely et al. (1997) suggest a positive relationship between milk protein content and the

Table 3. Effects of diet and CSN1S1 genotype of goats on metabolic and hormonal profiles (LSM)

Item	Diet (D) ¹			Genotype (G) ²		AA			AF			SEM	Significance		
	SFF	SFB	MHB	AA	AF	SFF	SFB	MHB	SFF	SFB	MHB		D	G	D × G
Metabolic profile															
Glucose, mg/dL	40.60 ^b	47.97 ^a	45.70 ^{ab}	44.16	45.36	38.53	47.25	46.70	42.68	48.68	44.70	2.59	†	NS	NS
NEFA, ln+1 mmol/L	0.318 ^a	0.186 ^b	0.201 ^b	0.234	0.236	0.310	0.210	0.170	0.320	0.160	0.230	0.034	**	NS	NS
BHBA, mmol/L	0.276 ^b	0.486 ^a	0.446 ^{ab}	0.416	0.389	0.224 ^g	0.545 ^d	0.480 ^e	0.329 ^f	0.426 ^e	0.412 ^e	0.047	**	NS	†
Cholesterol, mg/dL	66.19	68.73	64.57	59.26 ^b	73.73 ^a	59.10	62.82	55.87	73.28	74.63	73.27	3.94	NS	*	NS
Urea, mg/100 mL	25.68	22.82	22.56	25.18 ^d	22.18 ^e	26.47	25.53	23.55	24.88	20.10	21.57	1.86	NS	†	NS
Total protein, g/L	82.18	80.33	80.44	80.46	81.50	80.97	81.43	78.99	83.40	79.22	81.88	1.67	NS	NS	NS
Albumin, g/L	47.08	46.82	47.27	46.98	47.13	46.65	47.13	47.17	47.52	46.50	47.37	0.49	NS	NS	NS
Globulin, g/L	35.10	33.51	33.17	33.48	34.37	34.32	34.30	31.83	35.88	32.72	34.52	1.69	NS	NS	NS
Calcium, mg/100 mL	5.56	5.57	5.56	5.55	5.58	5.63	5.57	5.46	5.50	5.58	5.65	0.30	NS	NS	NS
Hormonal profile															
Insulin, ln + 10 µg/L	2.29	2.34	2.26	2.27	2.32	2.25	2.32	2.23	2.32	2.36	2.30	0.04	NS	NS	NS
IGF-1, ng/mL	117.12	129.75	121.94	114.89 ^e	130.98 ^d	106.72	128.91	109.04	127.51	130.59	134.83	10.15	NS	†	NS
fT3, ³ ln + 1 pg/mL	1.43	1.52	1.48	1.51 ^d	1.45 ^e	1.42 ^{ab}	1.52 ^{ab}	1.58 ^a	1.45 ^{ab}	1.52 ^{ab}	1.37 ^b	0.039	NS	†	*
fT4, ⁴ ln + 10 ng/dL	2.29	2.40	2.40	2.39 ^b	2.40 ^a	2.39	2.39	2.39	2.39	2.41	2.40	0.004	NS	*	NS

^{a-c}Means within a row with different superscripts differ ($P \leq 0.05$).^{d-g}Means within a row with different superscripts differ ($P \leq 0.10$).¹Diets: SFF = sulla (*Hedysarum coronarium* L.) fresh forage, SFB = sulla fresh forage plus 800 g/d barley meal, MHB = mixed hay plus 800 g/d barley meal.²Genotypes: AA = homozygous for strong alleles; AF = heterozygous for a weak allele.³Free triiodothyronine.⁴Free thyroxine.† $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

plasma clearance rate of BHBA in goats with different α_{SI} -CN genotypes ($AA > EE > FF$).

Pagano et al. (2010a) reported a significant effect of feeding regimen on plasma BHBA in goats with 3 genotypes at the *CSN1S1* locus but, in contrast to the present study, they did not find a significant interaction between feeding regimen and *CSN1S1* genotype. This discrepancy might be because, in the study of Pagano et al. (2010a), the goats received 3 isoprotein content diets with different amounts of energy.

Serum cholesterol values were higher in *AF* than in *AA* *CSN1S1* goats ($P < 0.05$). Contrary to findings from Pagano et al. (2010a) about a positive correlation between cholesterol and energy intake, in the present study the absence of a relationship between energy intake and cholesterol level in the 2 genotypes makes it difficult to explain the difference between the *AA* and *AF* genotypes.

No significant effect of diet, *CSN1S1* genotype, or $D \times G$ interaction was observed on plasma content of total protein, albumin, globulin, or calcium. Unlike for milk urea, plasma concentration of urea was not affected by diet, even though a positive correlation between blood urea and milk urea was found in the SFB ($r = 0.73$; $P < 0.01$) and MHB ($r = 0.69$; $P < 0.01$) groups. However, blood urea was slightly influenced by *CSN1S1* genotype ($P \leq 0.10$), and higher values were obtained in the *AA* goats compared with the *AF* goats. Schmidely et al. (1997) reported different values, even though not statistically significant, for different α_{SI} -CN genotypes ($EE > FF > AA$) and different diets (12% > 16% > 20% CP/DM) on plasma urea levels. Conversely, Pagano et al. (2010a) did not find an effect of *CSN1S1* genotype on blood urea in goats fed iso-protein content diets.

The *CSN1S1* genotype and diet did not influence the amount of insulin in the blood, in agreement with results found by Schmidely et al. (1997) comparing the *AA* and *FF* genotypes. However, other authors (Pagano et al., 2010b) have reported an effect of *CSN1S1* genotype, with higher levels of insulin in *AA* than in *FF* goats.

Both fT3 and fT4 thyroid hormones were affected by *CSN1S1* genotype: *AF* goats tended to have a lower level of fT3 ($P \leq 0.10$); for that variable a significant $D \times G$ interaction was found (Table 3) because of a reduction in fT3 in *AF* goats fed the MHB diet, which did not occur in *AA* goats. The fT4 concentration was statistically lower ($P < 0.05$) in *AA* than in *AF* goats. Concerning the relationship between thyroid hormones and milk production, in *AA* goats the correlation between fT4 and milk yield was strong ($r = -0.63$; $P < 0.01$), and in *AF* goats it was slightly negative ($r = -0.28$; $P \leq 0.10$) in spite of the slight increase in milk

production in *AA* goats compared with *AF* goats. Also, in the SFB group, characterized by the highest milk production, a correlation was found between fT4 and milk yield ($r = -0.64$; $P < 0.01$). These results might be explained by an increased conversion of T4 to T3 in the goats characterized by a higher milk yield.

Some studies have reported a negative correlation between milk yield and serum concentrations of fT4 in dairy cattle (Blum et al., 1983; Nixon et al., 1988). The thyroid gland secretes mostly T4, which is mono-deiodinated to T3 before interacting with target cells to exert its biologic effect. Only the free fractions of serum thyroid hormones are available for utilization by peripheral tissues, and only the free fractions control the rates of secretion of thyroid-releasing hormone from the hypothalamus and thyroid-stimulating hormone from the adenohypophysis (Nixon et al., 1988). The thyroid hormones are indicators of the nutritional and metabolic status of animals and in ruminant species are positively correlated with feed intake and energy and nitrogen balance; in sheep, energy deficiency reduces concentrations of T3 and fT3, whereas subsequent overfeeding increases them (Todini, 2007). Therefore, the highest plasma concentration of fT3 and the lowest fT4 level in goats with the strong genotype, characterized by higher milk production responses, could be explained by higher energy and protein requirements that, in this trial, seem warranted mostly by better digestibility than by the higher feed intake.

Only Pagano et al. (2010b) investigated the effect of *CSN1S1* genotype on thyroid hormones. They found, in line with the findings of the present study, a higher value of fT3 in *AA* than in *FF* goats and a significant $D \times G$ interaction ($P \leq 0.10$), due to the higher concentration of the hormone in *AA* goats that received a diet with higher energy content. However, the authors found no effect on the fT4 hormone.

A slight influence of *CSN1S1* genotype on IGF-1 ($P \leq 0.10$) was found. The plasma level of IGF-1 was lower in *AA* than in *AF* goats ($P \leq 0.10$), although the 2 genotypes exhibited the same CN yield. Insulin-like growth factor 1 secretion is a very complex mechanism dependent on several factors, such as protein intake, energy intake, environmental temperature, environmental stress, and micronutrient intake. These factors involve certain hormones, including growth hormone, insulin, thyroid hormone, and glucocorticoids (McCusker, 1998). The effects of IGF-1 on cell proliferation appear to be consistent across species. Various results have been reported for the effects of IGF-1 on the regulation of differentiated secretory functions (Cohick, 1998). In *in vitro* studies, Prosser et al. (1987) observed that CN gene expression and synthesis, α -LA activity, and glucose transport were all stimulated by the addition

of IGF-I to murine mammary gland explants. Those results do not agree with the current study, where a negative correlation between IGF-1 and CN ($r = -0.36$; $P < 0.012$) was observed. Because CN was affected by the $D \times G$ interaction in the present study, the relationship between IGF-1 and CN cannot be explained by the findings of the cited studies.

These results seem to indicate, as Pagano et al. (2010b) suggested, that the role played by hormones in determining differences between *CSN1S1* genotypes of goats is prevalent than that of metabolic components. The involvement of some metabolic (cholesterol and urea) and hormonal (IGF-1, fT3, and fT4) variables in determining differences between *CSN1S1* genotypes has to be explored, in light of recent research that relates polymorphisms in some genes to blood and productive measures. Clempson et al. (2012) revealed associations between polymorphisms at insulin-like growth factor binding protein (IGFBP)2 with fertility, milk production, and metabolic traits (glucose, insulin, and BHBA) in dairy cows. The effect of polymorphisms in somatotrophic axis genes (GH-*AluI* and IGF-I-*SnabI* polymorphisms) on metabolic and endocrine profiles (NEFA, BHB, insulin, and IGF-1), and on measures of production and reproduction in dairy cows was also found (Rupprechter et al., 2011). Finally, a relationship between variants of the leptin gene and biomarkers of energy metabolism has been reported in goats (P. Di Gregorio, unpublished data) and in women (Hinuy et al., 2010).

Goats with genotypes associated with the synthesis of either a high AA or intermediate AF level of α_{S1} -CN were compared based on their feeding behavior, metabolic and hormonal responses, and milk production resulting from different nutrient intake. The results indicate that the effect of diet was more pronounced than the effect of *CSN1S1* genotype. Regardless of *CSN1S1* genotype and barley supplementation, diets based on sulla fresh forage, compared with that based on hay, resulted in a higher DM and protein intake, improved DM and protein digestibility, and increased milk protein and CN content. Barley supplementation appeared to prevent energy deficiency and, therefore, limited the body fat mobilization of the goats; however, it also allowed for a more efficient utilization of dietary protein for the synthesis of milk CN and a reduction in milk fat and urea content. With the SFB diet, which had more energy and was more balanced, it was possible to maximize energy intake, DM digestibility, and milk production.

With regard to *CSN1S1* genotype, the goats with the AA genotype at *CSN1S1* showed higher protein digestibility and plasma fT3 levels than the AF goats, which indicates their greater energy and protein intake,

required to support the greater nutritional exigencies of milk production. Therefore, *CSN1S1* genotype interacted significantly with diet to affect DM digestibility, the level of the fT3 hormone, milk production, and, to a lesser extent for protein digestibility, BHBA, and milk CN content. In fact, goats with the AA genotype showed higher digestibility and daily milk yield when fed sulla and barley, and also higher protein digestibility when fed both diets based on sulla forage, whereas those effects were less evident in goats with heterozygous AF genotype. The reduction in milk CN and the fT3 hormone in AF goats fed hay and barley was not observed in AA goats.

These results demonstrate that goats with the AA genotype at *CSN1S1*, compared with AF goats, showed more efficient feed utilization and, consequently, higher milk production when they received diets high in energy with a balanced protein to energy ratio. They also demonstrate that these effects were already evident at the digestive level. Therefore, these results confirm that milk production potential of AA goats, carrying the strong genotype at *CSN1S1*, is superior to that of AF heterozygous goats.

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