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Effect of the αs_1-casein genotype and its interaction with diet degradability on milk production, milk quality, metabolic and endocrinal response of Girgentana goats

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ABSTRACT

A study was carried out to evaluate if diets at different starch and protein degradability could influence productive, metabolic and hormonal response of goats at different αs_1-casein genotype. Nineteen Girgentana goats at mid lactation were selected on the basis of their genotype at αs_1-casein locus: nine goats homozygous for strong (AA) alleles and ten goats homozygous for weak alleles (FF). The goats were used in a 2×2 factorial arrangement of treatments, with two genotypes (AA, FF) and two diets at different degradability (H diet: RDP, 60.5% of crude protein; starch degradability, 78,4%; L diet: RDP, 50.9% of crude protein; starch degradability, 66.8%). The dry matter intake was not affected by αs_1-genotype (respectively AA and FF goats: 2107 and 2086 g/d; $P = 0.160$), whereas it resulted significantly higher when goats were fed with the L diet (2130 g/d vs 2063 g/d; $P < 0.001$). Milk yield was much higher in AA compared to FF goats (1401 g/d vs 1076 g/d; $P = 0.03$). As expected, milk protein and casein resulted significantly higher and urea resulted significantly lower in AA goats (protein, 3.72% vs 3.20%; $P < 0.001$; casein, 3.11% vs 2.56%, $P < 0.001$; urea 56.6 mg/dl vs 69.2 mg/dl, $P < 0.001$) whereas no differences were found, between genotypes, in milk fat and lactose. No diet effect was detected on milk production and composition. The αs_1 genotype has strongly influenced free triiodothyronine (fT3) levels, which were higher in AA goats (1.64 vs 1.37 ln + 1 pg/ml; $P = 0.009$). No other differences were evident between genotypes or diets. In conclusion, the hypothesis that diets at different degradability could modify the productive response of goats at different CSN1S1 genotype has not been demonstrated. It has not been clarified the metabolic and endocrinal mechanisms involved in the manifestation of the differences found in relation to the studied polymorphism.

1. Introduction

The milk nitrogen content in goats is strongly affected by the genotype at αs_1-casein locus (CSN1S1). This highly polymorphic gene (Marletta et al., 2005, 2007) also appears to exert effects on milk fat concentration (Schmidely et al., 2002) and composition (Chilliard et al., 2006; Valenti et al.,

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2010), milk yield (Avondo et al., 2009; Pagano et al., 2010a; Bonanno et al., 2013a) and milk clotting properties (Bonanno et al., 2013b, 2009; Delacroix-Buchet et al., 1996). In addition, significant effects of CSN1S1 genotype have been found on thyroid hormones concentration in blood (Pagano et al., 2010b; Bonanno et al., 2013a).

Recently some interactions have been highlighted between the characteristics of diet and the different dairy performance of goats, associated to this polymorphism. Significant interactions have been evidentiate with the crude protein content of diet (De la Torre et al., 2009) and with the energy level associated to different levels of hay inclusion in the diet (Pagano et al., 2010a). In particular it has been found that goats homozygous for strong alleles (AA goats) showed higher milk production only when fed at high energy level, compared to goats homozygous for weak alleles (FF goats) (Pagano et al., 2010a) or to heterozygous goats (Bonanno et al., 2013a).

The aim of the study was to deepen the knowledges on the interaction between the diet characteristics and the genotype at CSN1S1 locus, in order to optimize the performance of goats. It has been reported that a higher rate of starch and protein fermentation was associated to a higher microbial growth in the rumen (Herrera-Saldana and Huber, 1989; Herrera-Saldana et al., 1990) and, as a consequence, to higher levels of microbial protein for digestion and a higher aminoacids availability for milk protein synthesis. In a previous study it has been found that, between 100%, 65% and 30% of hay in a complete diet, the intermediate level resulted in a better productive response of goats with strong alleles at CSN1S1 locus. Maintaining the same 65% level of hay, a trial was conducted to evaluate if diets at different rumen degradability could influence productive, metabolic and hormonal response of goats at different αs_1-casein genotype.

2. Materials and methods

2.1. Experimental design

Nineteen Girgentana goats in their 2nd to 3rd lactation, homogeneous for days of lactation (75 ± 10 d), milk production (1.2 ± 0.4 kg/d) and body weight (35.7 ± 6.5 kg), were selected on the basis of their genotype at αs_1-casein locus, as follows: nine goats homozygous for strong (AA) alleles and ten goats homozygous for weak alleles (FF). Moreover all the goats were selected taking into account CSN2 and CSN1S2 genotype. In particular all the goats were characterized by strong alleles at the two loci. Goat DNA samples were obtained from hair bulbs. The genotypes of individuals at the CSN1S1, CSN2, and CSN1S2 were determined by means of PCR analyses, following the protocol previously detailed by Avondo et al. (2009). Goats in each genetic group were derived from two different farms. The goats were used according to a change over design in a 2 × 2 factorial arrangement of treatments, with two genotypes (AA, FF) and two diets at different degradability (higher degradability, H; lower degradability, L) (Table 1).

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. The preexperimental period consisted of a 7-d period during which the animals received hay ad libitum and 400 g of a mix of the two experimental diets; during this period preexperimental data for milk production, body weight and dry matter intake were detected and milk and blood samples were collected. The experiment lasted 40 d, from 11 March to 20 April. Each experimental period lasted 20 d and consisted of 15 d for adaptation and 5 d for data and samples collection, during which the goats received the scheduled diet ad libitum. All ingredients were ground and pelleted (6 mm diameter).

Table 1
Diets composition and chemical analyses.

	High degradability (H)	Low degradability (L)
Ingredients % fresh matter		
Maize	–	16
Barley	18	8
Faba bean meal	13	–
Soybean meal 44% CP	–	3
Carrob pulp	2	3
Maize gluten meal	–	3
Vitamin-mineral mix	2	2
Alfalfa pelleted hay	65	65
Chemical composition		
Dry matter %	89.4	88.0
Crude protein % DM[a]	16.4	16.6
NDF % DM	38.1	36.1
Lignin % DM	5.2	5.1
RDP[b] % CP	60.5	50.9
RUP[c] % CP	39.5	49.1
Water soluble carbohydrates % DM	6.2	6.3
Starch % DM	18.7	18.7
Crude lipid % DM	2.3	2.4
Ash % DM	9.2	9.1
NEl[d] MJ/kg DM	5.55	5.62
Starch degradability[e] %	78.4	66.2

[a] Dry matter.
[b] Rumen degradable protein.
[c] Rumen undegradable protein.
[d] Net energy for lactation (Conrad et al., 1984).
[e] Calculated on the basis of literature data (Nocek and Tamminga, 1991; Offner et al., 2003; Gencoglu et al., 2011).

2.2. Sample collection and analysis

Individual intakes were measured daily, on the basis of refusals. Individual milk production and milk samples were collected from the morning and evening milking two times for each 5-d collection period. Three samples for each pelleted diet were analyzed for dry matter (DM), crude protein (CP), fat (Association of Official Analytical Chemists, 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, Ireland). RDP and RUP were calculated from NRC (2001).

Milk samples, consisting of proportional volumes of morning and evening milk yield, were analyzed for lactose, fat, protein, casein, urea and SCC, by an automated Fourier transform infrared absorption spectrophotometric analyzer (Combi-foss 6000, Foss Electric, Hillerød, Denmark). Body weight was measured at the start and at the end of the trial.

Blood samples (8 ml) were taken from all goats at the end of the preexperimental period and at the end of each experimental period 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* at 4 °C for 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. Nonesterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) were analyzed by using respectively FA 115 and Ranbut commercial kits (Randox Laboratories, Crumlin, Antrim, UK). Insulin (Mercodia 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, Milan, Italy) were measured in duplicate by ELISA kits.

2.3. Statistical analysis

Pre-experimental data for milk production were analyzed for genotype effect, using a one-way ANOVA. Individual data for intake, milk production and composition were analyzed using the GLM procedure

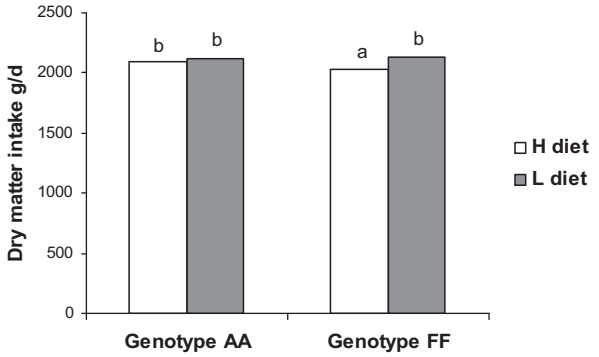

Fig. 1. Effect of the interaction between αs_1-casein and diet degradability on dry matter intake of goats. H, high degradable diet; L, low degradable diet. $^{a,b}P < 0.05$.

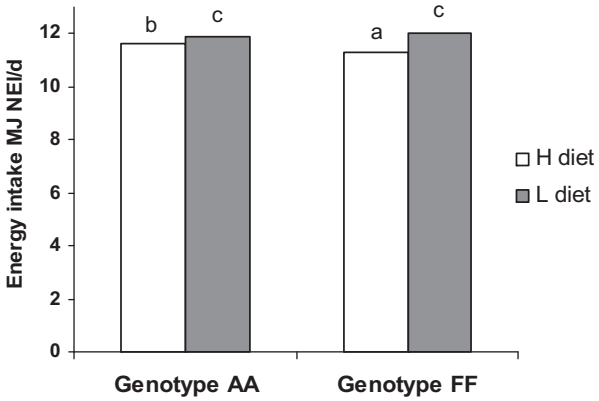

Fig. 2. Effect of the interaction between αs_1-casein and diet degradability on energy intake of goats. H, high degradable diet; L, low degradable diet. NEl, net energy for lactation. $^{a-c}P < 0.05$.

for repeated measures of SPSS (SPSS for Windows, SPSS Inc., Chicago IL, USA). Body weight and plasma concentrations of metabolites and hormones were analyzed by means of GLM procedure and analysis included main effect of αs_1-casein genotype (FF, AA), diet (H, L) and interaction genotype × diet.

3. Results

Diets ingredients and chemical analysis are reported in Table 1. The inclusion of protein-rich feeds at different protein degradability (faba bean meal in H diet, soyabean meal and corn gluten meal in L diet) and grains at different starch degradability (barley in H diet and maize in L diet) determined the formulation of two complete diets that resulted similar for the main dietary components, except for the protein and starch degradability (Table 2).

Intake of dry matter and net energy was not affected by αs_1-genotype, whereas it resulted significantly higher when goats were fed with L diet. A significant interaction for dry matter and energy intakes was also found between genotype and diet (Figs. 1 and 2). Milk yield was much higher in AA, compared to FF goats. As expected, milk protein and casein resulted significantly higher and urea significantly lower in AA goats, whereas no differences were found between genotypes in milk fat and lactose percentages. No diet effect was detected for milk production

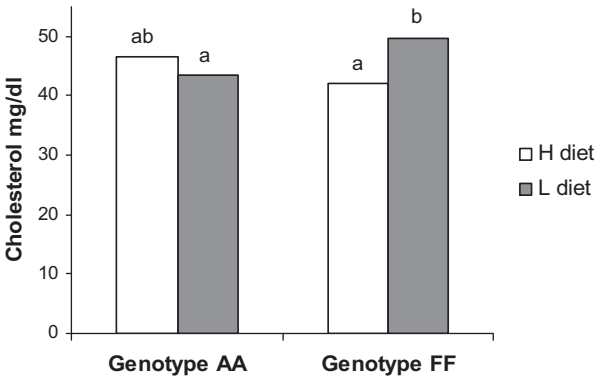

Fig. 3. Effect of the interaction between αs_1-casein and diet degradability on plasma cholesterol concentration in goats. H, high degradable diet; L, low degradable diet. $^{a,b}P < 0.05$.

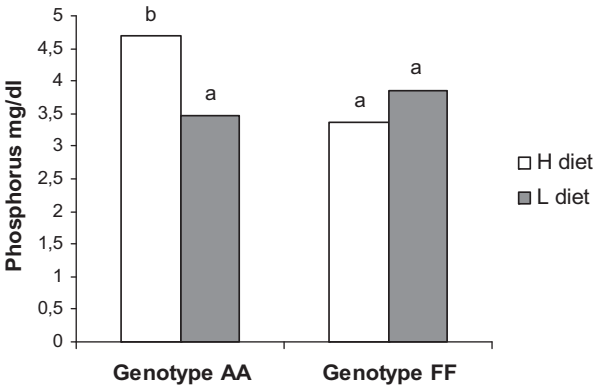

Fig. 4. Effect of the interaction between αs_1-casein and diet degradability on plasma phosphorus concentration in goats. H, high degradable diet; L, low degradable diet. $^{a,b}P < 0.05$.

and composition. Final body weight was not significantly affected by treatments.

In Table 3 data on plasma metabolites concentrations and hormonal profile are reported. Data for each parameter resulted within the normal range. The only parameter affected by genotype was blood urea, that was significantly higher in FF goats. The H diet administration caused only an increase in BHBA and a decrease in NEFA blood concentrations, compared to L diet. A significant interaction genotype × diet was found for cholesterol and phosphorus concentrations (Figs. 3 and 4): H diet, compared to L diet, determined an increase in cholesterol and phosphorus, when supplied to AA goats (respectively H and L: cholesterol, 46.7 and 43.6 mg/100 ml; P, 4.69 and 3.48 mg/100 ml), whereas the same diet determined a decrease of these parameters when administered to FF goats (respectively H and L: cholesterol, 42.1 and 49.6 mg/100 ml; P, 3.38 and 3.85 mg/100 ml).

The αs_1-genotype strongly influenced the thyroid hormones: the fT3 and fT4 levels resulted higher in AA goats. No other difference was evident between genotypes or diets.

Table 2
Effects of CSN1S1 genotype and diet on dry matter intake, milk yield and composition.

	αs_1 genotype (G)		Diet degradability (D)		SEM	Significance		
	AA	FF	High (H)	Low (L)		G	D	GxD
Dry matter intake (g/d)	2107	2086	2062	2130	15	0.16	<0.01	0.02
Energy intake (MJ NEl[a]/d)	11.8	11.6	11.4	12.0	0.2	0.16	<0.01	0.02
Milk yield (g/d)	1400	1075	1225	1251	68	0.03	0.86	0.89
Milk composition								
Fat %	3.3	3.2	3.3	3.2	0.7	0.26	0.45	0.74
Protein %	3.7	3.2	3.4	3.5	0.1	<0.01	0.42	0.68
Lactose %	4.4	4.4	4.4	4.4	0.1	0.34	0.50	0.65
Casein %	3.1	2.6	2.8	2.9	0.6	<0.01	0.52	0.80
Urea mg/dl	56.6	69.2	62.8	63.0	1.7	<0.01	0.93	0.51

[a] Net energy for lactation.

Table 3
Effects of CSN1S1 genotype and diet on metabolic and hormonal profile of goats.

	αs_1 genotype (G)		Diet degradability (D)			Significance		
	AA	FF	High (H)	Low (L)	SEM	G	D	G × D
Metabolites								
Glucose mg/dl	43.7	44.7	44.4	44.0	0.6	0.31	0.76	0.05
BHBA[a] mol/l	0.40	0.42	0.51	0.32	0.03	0.65	<0.01	0.61
NEFA[b] mmol/l	0.16	0.16	0.15	0.18	0.01	0.54	<0.01	0.87
Cholesterol mg/dl	45.1	45.8	44.4	46.6	1.3	0.77	0.35	0.03
Triglicerids mg/dl	2.39	2.84	2.57	2.66	0.24	0.32	0.85	0.75
Urea mg/dl	35.5	40.4	37.5	38.5	1.0	<0.01	0.51	0.29
Total protein g/l	61.9	63.9	62.3	63.4	0.7	0.19	0.50	0.89
Albumin g/l	45.3	45.0	45.1	45.3	0.2	0.44	0.68	0.07
Globulin g/l	16.5	18.8	17.3	18.1	0.7	0.14	0.58	0.71
Calcium mg/dl	5.88	5.37	5.79	5.46	0.20	0.21	0.42	0.34
Phosphorus mg/dl	4.09	3.62	4.04	3.66	0.31	0.23	0.34	0.03
Hormonal profile								
Insulin μg/l	0.29	0.25	0.26	0.28	0.02	0.47	0.75	0.46
IGF[c]-1 μg/l	106.8	96.8	105.7	97.9	6.0	0.40	0.51	0.70
fT3[d] ln +1 pg/ml	1.64	1.37	1.51	1.50	0.29	<0.01	0.96	0.47
fT4[e] ln + 10 ng/dl	2.33	2.32	2.33	2.33	0.01	<0.01	0.98	0.52

[a] Betahydroxybutyrate.
[b] Nonesterified fatty acids.
[c] Insulin-like growth factor 1.
[d] Triiodothyronine.
[e] Thyroxine.

4. Discussion

The dry matter intake resulted high, although not reaching the level already found for goats fed with a similar complete pelleted diet supplied ad libitum, with the same level (65%) of hay inclusion (Pagano et al., 2010a). Intake was not influenced by genotype but resulted slightly higher when goats were fed with the diet at lower degradability in contrast with Biricik et al. (2006) that found identical DMI in sheep fed with diets at different protein and starch degradability.

Milk yield was about 30% higher in AA goats, compared to FF genotype. This result is in line with previous experiments with goats with strong or weak alleles at αs_1 locus, fed ad libitum with pelleted hay and whole grains (Avondo et al., 2009) or with a complete pelleted diet with at least 35% of concentrate inclusion (Pagano et al., 2010a). Bonanno et al. (2013a) found an increase in milk production in AA goats even compared to heterozygous goats (AF), when the animals were fed with pasture supplemented with a highly energetic concentrate. On the contrary, goats with different αs_1 genotype, fed with a 100% pelleted hay (Pagano et al., 2010a) or extensively grazing (Balia et al., 2013) did not show any difference in milk production. Furthermore, in our conditions, during the pre-experimental period, when goats were fed with hay and 400 g of a mix of the H and L diets, milk production level was similar in the two genetic groups (respectively in AA and FF goats, 1257 and 1263 g/d; $P = 0.975$; SEM = 104.7). All these results seem to confirm that AA goats show their higher aptitude to produce milk only when fed at high feeding levels.

The hypothesis that a higher diet degradability could improve milk performance in AA goats, as an effect of a possible higher efficiency of microbial protein synthesis (Nocek and Russell, 1988), was not verified in our conditions, in contrast with results reported by Herrera-Saldana and Huber (1989). The lack of differences between diets could be justified by the synchronization of the degradability of starch and protein probably obtained by modulating the ingredients in the two diets, that did not allow to

evidentiate significant differences in milk production and quality among them. In fact, synchronizing the ruminal availability of energy and nitrogen, a high microbial protein synthesis is expected (Herrera-Saldana et al., 1990; Chumpawadee et al., 2006; Yang et al., 2010) probably independently from speed of degradation. As a demonstration of a similar ruminal efficiency in the protein utilization with the two diets, the levels of urea in milk and serum were not significantly affected by diets.

As expected from the different genetic aptitude to synthetize αs_1 casein, the N components of milk were strongly affected by the genotype: AA goats had 16% more protein, 21.5% more casein and 22.3% less urea, compared to FF goats, confirming previous results (Avondo et al., 2009; Pagano et al., 2010a). Coherently with these results, blood urea resulted significantly lower in AA goats, thus demonstrating a better utilization of N feeding sources.

No other differences in milk composition were found between genotype and between diets. The lack of differences between genotype in milk fat content is in contrast with Schmidely et al. (2002), De la Torre et al. (2009) and Pagano et al. (2010a) that reported a greater fat level in goats with strong alleles at CSN1S1 locus. A diluition effect of the higher milk production in AA goats may have reduced fat percentage in this genetic group, thus nullifying differences.

Milk fat, on average, resulted lower than protein. The inversion of fat and protein percentages, has been already highlighted in Girgentana goats (Avondo et al., 2009; Pagano et al., 2010a; Bonanno et al., 2013a) and in Saanen goats (Pulina et al., 2008). In general this anomaly in milk composition could be attributable to the low fiber content of the diet; however it has been demonstrated that, in goats, fat percentage is less sensitive to low fiber levels (Rapetti and Bava, 2008). Other factors, such as the spring season, a mid stage of lactation, a long day lenght could contribute to decrease milk fat content (Pulina et al., 2008). These conditions were all present during the experimental period.

The blood parameters resulted within the normal range of values reported for goats (Kaneko et al., 1997), thus indicating a good health status of the experimental animals, confirmed by the absence of any clinical signs of disease. The blood energy indicators, such as glucose, BHBA, NEFA and cholesterol, were consistent with values reported in other experiments (Bonanno et al., 2013a; Pagano et al., 2010a), and suggest a positive energy balance, as confirmed by body weight increases reached at the end of the trial, even in AA goats which produced much more milk, compared to FF group. The higher BHBA level found in goats fed with H diet compared to L, that could be indicative of a certain utilization of body reserves, does not seem to be justified by the little decrease in DMI found in H goats. Goats fed on H diet also showed lower NEFA plasma levels compared to goats fed on L diet; however it is difficult to associate this result to the different diet characteristics. Cholesterol showed a significant interaction between genotype and diet. Pagano et al. (2010a) found a strong positive correlation ($r = 0.94$; $P = 0.04$) between serum cholesterol and energy intake. In our conditions the same correlation was not significant ($r = 0.28$; $P = 0.09$) even though the trend was similar in FF goats (respectively with H and L diet, cholesterol and net energy intake: 42.1 mg/100 ml and 2696.8 kcal NEl/d; 49.6 mg/100 ml and 2871.7 kcal NEl/d).

No differences were found in blood insulin concentration as effect of genotype or diet, in line with Bonanno et al. (2013a) and Schmidely et al. (2002), but in contrast with the higher values reported by Pagano et al. (2010b) in AA goats. Coherently with insulin results, also IGF-1 blood levels were not influenced by treatments; in fact Magistrelli et al. (2005) found a positive correlation between these two substances in plasma of goats. As the insulin and IGF-1 blood concentration depends on energy and protein inputs, the lack of differences in intake levels in the two genotypes and the little difference found between diets could explain results.

The higher levels of the free thyroid hormones found in AA goats, independently from feeding treatment, are in line with findings of Pagano et al. (2010b) and Bonanno et al. (2013a). Taking into account that AA goats produced more milk, the higher levels of T3 and T4 in these goats could be associated to their temporary galactopoietic role; in fact, it has been demonstrated that administration of these hormones stimulates lactation in many species (Todini, 2007). In contrast, Blum et al. (1983) and Nixon et al. (1988) found negative correlations between T3 and T4 levels and milk yield. It has been also well established that T3 and T4 are positively correlated to energy balance (Capuco et al., 2001; Cassar-Malek et al., 2001); on the contrary, in the present study, the energy balance in AA goats appeared to be lower than in FF goats, because they produced more milk associated with lower feed intake and similar changes in body weight; however, the well known effect of thyroid hormones in promoting the intestinal absorption of glucose and in stimulating the metabolism of nutrients may have improved the feed utilization efficiency, compared to FF goats. Another explanation of the higher levels of T3 and T4 found in goats with strong alleles could be directly related to their higher aptitude to produce milk protein, taking into account the anabolic effects of these hormones in terms of protein synthesis (Kaneko et al., 1997).

The study reasserts the well known effects of the polymorphism at the αs_1-casein locus (CSN1S1) on nitrogen content in milk: goats homozygous for strong alleles contained more casein and less urea. The hypothesis that a different degradability of protein and energy sources in the diet could improve the productive response of goats at different genotype has not been demonstrated probably because the two diets were well balanced in terms of speed of degradability of carbohydrates and proteins. This has probably resulted in an optimal metabolic response in both the genetic groups, as demonstrated by the hematological values of the main metabolic parameters within the normal range. The hormonal status was influenced by genotype: the blood concentration of fT3 and fT4 was higher in goats with strong alleles. However, it has not been clarified whether thyroid hormones results represent the cause or the effect of the different performance associated to the two genotypes. Further researches should be aimed at understanding the metabolic and endocrinal mechanisms involved in the manifestation of these differences in relation to polymorphism at CSN1S1 locus.

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