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Milk quality as affected by grazing time of day in Mediterranean goats

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We evaluated the effect of grazing time of day on goat milk chemical composition, renneting properties and milk fatty acid profile in a Mediterranean grazing system. Sixteen lactating Girgentana goats were divided into two experimental groups and housed in individual pens, where they received 500 g/d of barley grain. For 5 weeks the two groups were left to graze in two fenced plots on a ryegrass sward as follows: morning group (AM), from 9·00 to 13·00; afternoon group (PM), from 12·00 to 16·00. In selected herbage, water-soluble carbohydrates (WSC) increased in the afternoon (204 *v.* 174 g/kg dry matter, DM; $P=0·01$), whereas crude protein (CP) and linolenic acid decreased (respectively, 16·7 *v.* 19·8% DM; $P<0·01$ and 26·8 *v.* 30·4 g/kg DM; $P<0·01$). Pasture dry matter intake (DMI) was significantly higher in the afternoon (0·82 *v.* 0·75 kg/d; $P=0·026$). Fat corrected milk production (FCM), milk fat and lactose content were not affected by treatment, whereas protein and titrable acidity (°SH) increased in the PM group (respectively 3·56 *v.* 3·42%; $P=0·01$; 3·55 *v.* 3·22 °SH/50 ml; $P=0·01$). In contrast, milk urea content was significantly higher in the AM group (381 *v.* 358 mg/l; $P=0·037$). The results seem to indicate that an improvement in ruminal efficiency might be obtained by shifting grazing time from morning to afternoon, as a consequence of a more balanced ratio between nitrogenous compounds and sugars. Indeed, the higher linolenic acid and the lower conjugated linoleic acid (CLA) (respectively 1·02 *v.* 0·90, $P=0·037$; 0·71 *v.* 0·81% of total fatty acids, $P=0·022$) in the milk of goats grazing in the afternoon seem to indicate a reduced biohydrogenation activity in the PM group.

Keywords: Grazing time of day, goat, milk quality, fatty acid composition.

Fresh herbage is generally characterized by a high content of soluble protein often associated with an inadequate level of water-soluble carbohydrates (WSC). The unbalanced availability of N and energy sources for bacterial growth in the rumen might lead to a consequent reduction in microbial protein synthesis and protein concentration in milk. For the same reason, a low level of WSC in the diet can cause an increase in milk and blood levels of urea and in urinary N excretion (Miller et al. 2001). Consequently, a greater availability of sugars should result in an improvement of pasture utilization.

Short grazing is a widely adopted system for dairy small ruminants in Mediterranean countries. This system consists of taking the animals out to pasture for 4–8 h a day between the two daily milkings, often supplying a feed supplement during milkings or during the night (Avondo et al. 2007).

Taking into account that the WSC concentration tends to increase over the day (Smith, 1973; Avondo et al. 1996) through the accumulation of the products of photosynthesis, the present trial evaluated the effect of varying the grazing time of day, in a short grazing system, on chemical composition, renneting properties and fatty acid profile of goat milk.

Materials and Methods

Animals and their management

Sixteen Girgentana goats at 135±4·4 days in milk were divided into two groups of eight, which were balanced for milk yield (1108±320 g/d), body weight (37·1±5·2 kg)

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and body condition (2·8±0·2) scored as reported by Santucci & Maestrini (1985). The goats, housed in individual wooden pens equipped with trough and bucket, received 500 g/d of barley grain (10·9% crude protein in DM) supplied into two meals, before and after 4-h grazing on a ryegrass sward (*Lolium multiflorum* Lam. subsp. *Westerwoldicum, var.* Elunaria); water was freely available. Goats were hand-milked twice daily.

The experiment began on 15 April after a 10-d pre-experimental period and lasted 5 weeks (mean daily temperature averaged 16 °C; mean maximum values 24 °C with peaks close to 30 °C; mean humidity was 71%). During the pre-experimental period all the goats grazed together from 10·00 to 15·00. During the experimental period the groups were left to graze separately, at a stocking rate of 64 goats/ha, on two contiguous fenced areas of the sward as follows: morning group (**AM**), from 9·00 to 13·00; afternoon group (**PM**), from 12·00 to 16·00.

Data collection

Each week, the a.m. and p.m. milk production was recorded and individual milk samples from each milking were collected; individual herbage dry matter intake (DMI) was estimated by the *n*-alkane method (Mayes et al. 1986); three herbage samples per week for each group were collected as follows: during grazing, each sample was collected by hand-plucking herbage every hour, as previously reported (D'Urso et al. 1998) from 9·00 to 13·00 in the morning plot and from 12·00 to 16·00 in the afternoon plot.

Herbage chemical analysis

Analysis for DM, crude protein (CP) (AOAC, 1990) and structural carbohydrates (Van Soest et al. 1991) were carried out on forage and barley. Herbage and faeces were processed for *n*-alkane analysis, as described by Mayes et al. (1986). WSC was determined by a modified anthrone method (Deriaz, 1961). Fatty acids were determined on freeze-dried samples ground to pass a 1-mm screen. Lipid extraction and esterification were performed as reported by Palmquist & Jenkins (2003). Nonadecanoic acid (19 : 0) was added as internal standard. Fatty acid methyl esters were analysed on a Trace Thermo Finnigan GC equipped with a flame ionization detector, on a 60 m × 0·25-mm i.d. fused-silica capillary column (SP-2340, Supelco Inc., Bellefonte PA, USA). The temperatures of injector and detector were maintained at 220 °C and 250 °C, respectively. The oven temperature was initially set at 160 °C for 1 min, then increased at a rate of 3 deg C/min to 210 °C for 3 min. Helium was the carrier gas. Split flow was 20 ml/min. Injected volume was 1 µl, split ratio, 17.

Milk analysis and coagulation properties

Individual milk samples, consisting of proportional volumes of morning and evening milk, were divided into two samples. One was stored at 4 °C without any preservative and analysed on the same day for pH, titrable acidity, lactose, fat and protein by an infrared method (Combi-foss 6000, Foss Electric, Hillerød, Denmark). The other sample, without preservative, was immediately stored at −15 °C until analysis of fatty acid profile. Total nitrogen (TN), non-protein nitrogen (NPN) and non-casein nitrogen (NCN) were determined by standard procedures (IDF, 1964). From these N fractions, total protein (TN * 6·38) and casein ((TN−(NCN * 0·994)) * 6·38) were calculated. Milk urea content was determined by an enzymic method using difference in pH (CL10 instrument, Eurochem, Italy). Milk rennet coagulation properties were measured, according to Zannoni & Annibaldi (1981), using a Formagraph instrument (Foss Italia, Padova, Italy). Three clotting parameters were considered: coagulation time (r), as the time from rennet addition to the beginning of coagulation; rate of clot formation (k_{20}) as the time from coagulation until reaching the curd firmness corresponding to an amplitude of 20 mm on the Formagraph trace; curd firmness (a_{30}), as the amplitude of the trace 30 min. after the rennet addition.

Fat-corrected milk at 3·5% fat (FCM) was calculated according to the following equation: FCM=Milk production (g/d) * (0·6340+0·1046% fat) (Pulina et al. 1991).

Milk lipids were extracted as described by Alonso et al. (1999). Fatty acid methyl esters were prepared according to Kramer et al. (1997). Nonadecanoic acid (19 : 0) was added as internal standard. Separation of methyl esters was achieved through a 60 m × 0·25-mm i.d. fused-silica capillary column (SP-2340, Supelco Inc., Bellefonte PA, USA). The temperatures of injector and detector were maintained at 220 °C and 250 °C, respectively. The oven temperature was initially set at 70 °C for 1 min, increased at a rate of 8 deg C/min to 170 °C for 18 min, then increased at 9 deg C/min to 230 °C for 4 min. Helium was the carrier gas. Split flow was 26 ml/min. Injected volume was 1 µl, split ratio, 33.

Statistical analysis

Individual data of DMI, milk production and composition, renneting parameters and fatty acid profile were analysed using the GLM procedure for repeated measures of SPSS (SPSS for Windows, SPSS Inc., Chicago IL, USA). The pre-treatment mean of each parameter was used as a covariate. When the covariate was not significant ($P>0·05$) it was removed from the model. Herbage chemical composition variables were analysed by a two-way analysis of variance (time of day × week).

Results

Chemical composition of herbage and dry matter intake

Tables 1 and 2 report the chemical composition and the fatty acid content of plucked herbage. The grazing time of

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Table 1. Chemical composition of herbage plucked samples and DM intake from pasture grazed in the morning (AM) or in the afternoon (PM)

	DM, %		Crude protein, % DM		NDF†, % DM		WSC, % DM		WSC/CP	
Week	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	15·2	16·0	17·4	15·2	44·1	39·7	18·7	22·6	1·07	1·49
2	14·2	15·3	17·7	13·1	42·6	43·4	19·2	23·9	1·08	1·82
3	15·1	15·6	19·7	17·2	44·5	42·4	17·0	21·9	0·86	1·27
4	16·3	17·4	22·3	18·6	42·5	43·0	17·9	18·5	0·80	0·99
5	18·6	19·3	21·9	19·5	42·5	44·4	14·4	15·3	0·66	0·78
Mean	15·9	16·7	19·8	16·7	43·2	42·6	17·4	20·4	0·89	1·27
Significance:										
Time of day (T)	0·08		<0·01		0·38		0·01		<0·01	
Week (W)	<0·01		<0·01		0·70		<0·01		<0·01	
T × W	0·991		0·31		0·10		<0·01		<0·01	
Error MS	2·17		1·25		4·45		1·33		<0·01	

† Neutral detergent fibre

Table 2. Fatty acid content (g/kg DM) of plucked herbage samples taken during the morning (AM) or the afternoon (PM)

	12 : 0		14 : 0		16 : 0		18 : 0		18 : 1		18 : 2		18 : 3	
Week	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	1·28	1·01	0·13	0·15	4·03	3·53	0·62	0·49	0·35	0·39	3·71	3·45	30·0	24·1
2	1·42	1·44	0·16	0·15	4·46	4·61	0·61	0·63	0·48	0·50	3·95	3·99	28·6	29·2
3	1·36	1·22	0·17	0·24	3·89	3·90	0·52	0·55	0·40	0·46	3·83	3·79	27·3	26·2
4	1·81	1·45	0·29	0·23	5·15	4·76	0·67	0·66	0·50	0·61	4·82	4·24	37·0	28·4
5	1·59	1·56	0·21	0·33	5·19	6·54	0·66	0·90	0·74	1·23	4·93	5·36	29·2	26·1
Mean	1·49	1·33	0·19	0·22	4·55	4·67	0·61	0·64	0·49	0·64	4·25	4·17	30·4	26·8
Significance:														
Time of day	0·02		0·01		0·55		0·32		0·02		0·57		<0·01	
Week	<0·01		<0·01		<0·01		<0·01		<0·01		<0·01		0·01	
T × W	0·05		<0·01		0·06		0·01		0·08		0·14		0·06	
Error MS	0·01		0·01		0·30		0·01		0·02		0·10		7·23	

day caused significant variations in chemical composition of plucked herbage. On average, the PM herbage was significantly richer in WSC, myristic and oleic acids and poorer in CP, lauric and linolenic acids. The WSC/CP ratio, due to the opposite trend of the two variables in AM and PM herbage, was much higher in PM samples.

Some variations in herbage quality were detected during the experiment, as demonstrated by the significant increase in DM and the decrease in WSC from week 1 to week 5. On the contrary, CP and all the fatty acids increased during the experiment, except for linolenic acid, which showed an irregular trend from week 1 to week 5.

Intake, body weight, milk production and composition and coagulation properties

Barley was entirely consumed by each goat. Table 3 reports the mean data on herbage intake, milk production and composition. On average, herbage DMI increased significantly ($P<0·026$) in the PM group. As a consequence, WSC intake was significantly higher in the PM group ($P=0·002$), the highest differences occurring during the first 3 weeks (Fig. 1). On the contrary, CP intake, compensated by the higher percentages found in AM herbage, was not significantly affected by treatment ($P=0·498$).

Final body weight was not affected by treatment (37·8 and 36·7 kg, respectively, in AM and PM groups, $P=0·702$).

FCM, fat, lactose and casein content were not affected by treatment. The protein levels were significantly increased, whereas urea was significantly decreased in the PM group. Grazing time of day also affected milk titrable acidity (°SH) and coagulation properties: in the PM group a significant increase of °SH value and an improvement in renneting properties were observed, in terms of curd firmness (A30).

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Table 3. Herbage dry matter intake (DMI), fat corrected milk (FCM), milk chemical composition, titrable acidity and rennet coagulation properties in goats grazing in the morning (AM) or in the afternoon (PM)

			Significance			
	AM	PM	Time of day (T)	Week (W)	T × W	Error MS
Herbage DMI, kg/d	0·75	0·82	0·03	0·07	0·47	0·02
FCM, g/d	927·4	879·7	0·69	<0·01	0·35	4864·0
Fat, %	4·06	3·94	0·47	0·29	0·03	0·12
Protein, %	3·42	3·56	0·01	<0·01	0·10	0·01
Lactose, %	4·62	4·57	0·41	<0·01	0·83	0·01
Casein, %	2·65	2·78	0·79	<0·01	0·03	0·02
Urea, mg/l milk	381	358	0·04	<0·01	<0·01	65·6
Titrable acidity °SH/50 ml	3·22	3·55	0·01	<0·01	0·07	0·04
Coagulation time, min	11·2	10·8	0·35	0·03	0·02	1·72
K20	1·99	1·69	0·28	0·07	0·16	0·18
A30	46·0	52·3	0·03	0·06	0·14	22·17

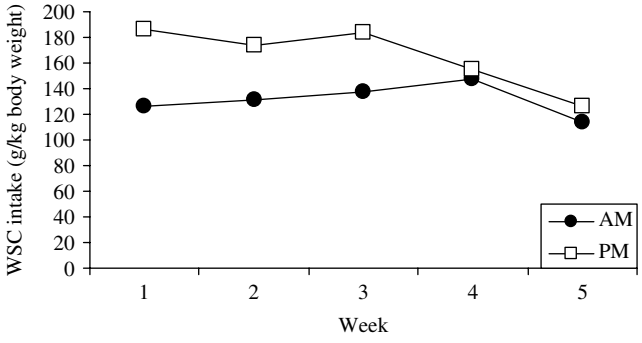

Fig. 1. Intake of water soluble carbohydrate (WSC, g/kg body weight) throughout the 5 weeks of the experiment for groups grazing in the morning (AM) and in the afternoon (PM). See text for details of treatments.

Milk fatty acid composition

Milk fatty acid composition (Table 4) was affected in part by grazing time of day. No significant differences were found between groups in the classes of short, medium and long chain, saturated, unsaturated and polyunsaturated, odd and branched chain fatty acids. As regards single acids, we found a significant mean increase of 13·3% in 18:3 in the PM group, which amounted to an increase of 50% during week 3 (respectively in AM and PM groups: week 1: 0·89 *v.* 1·10; week 2: 0·87 *v.* 1·04; week 3: 0·81 *v.* 1·24; week 4: 1·06 *v.* 0·75; week 5: 0·86 *v.* 0·98%). In contrast, CLA concentrations were significantly decreased in the PM group (respectively in AM and PM groups, week 1: 0·72 *v.* 0·63; week 2: 0·68 *v.* 0·60; week 3: 0·95 *v.* 0·81; week 4: 0·75 *v.* 0·73; week 5: 0·92 *v.* 0·76).

Discussion

The main variation in herbage chemical composition linked to grazing time of day, as expected, was the increased WSC in PM herbage. Similar results are reported for hays of different species cut in the morning and in the afternoon (Fisher et al. 2002, 2005; Burns et al. 2005), in grazed herbage (Orr et al. 1997) and even in silages (Avondo et al. 1996; Huntington & Burns, 2007) and are clearly caused by the accumulation of photosynthesized simple sugars throughout the plant during daylight (Smith, 1973). In our experimental conditions, collecting samples by plucking the herbage every hour from 9·00 to 13·00 and from 12·00 to 16·00, respectively in AM and PM plots, we found a mean WSC increase from AM to PM of about 17%. Lower differences are reported by Orr et al. (1997); they collected ryegrass samples at 7·30, 11·30, 15·30 and 19·30, finding an increase of about 10% in WSC content from 7·30 to 11·30, and lower increases in the following cuts. Taweel et al. (2005), comparing ryegrass varieties at different sugar contents, concluded that the high sugar levels occurred at the expense of CP and neutral detergent fibre. Other authors report that WSC in forages are inversely related to CP (Elgersma et al. 2005; Tas et al. 2005). In our conditions we found a significant reduction in CP linked to the higher WSC percentages of PM herbage. This opposite trend led to a WSC/CP ratio 43% higher on average than in the AM group, with a peak of 68% in week 2. Fatty acid content of herbage was significantly affected by time of day. A significant decrease of linolenic acid content during the afternoon was observed and this is particularly important because linolenic acid represents the major component of herbage fatty acids. To our knowledge, no references are available on herbage fatty acids variations during the day. Hawke (1973) discussed the role of different light conditions on fatty acid composition of leaf lipids, concluding that linolenic acid increases with increasing light intensity. Similar results are reported by Dewhurst & King (1998). However, it is not justified to draw comparisons with these results under artificial shading treatments, to the differences that occurred in our experimental conditions, where a natural photosynthetic pattern was applied.

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Table 4. Milk fatty acid composition (% of total fatty acids) of milk from goats grazing in the morning (AM) or in the afternoon (PM)

			Significance			
	AM	PM	Time of day (T)	Week (W)	T × W	Error MS
4 : 0	4·86	4·87	0·88	< 0·01	0·30	1·85
6 : 0	5·82	5·95	0·47	0·02	0·27	1·53
8 : 0	5·12	5·34	0·23	< 0·01	0·30	0·87
9 : 0	0·23	0·20	0·17	0·15	0·30	0·04
10 : 0	15·60	16·00	0·42	< 0·01	0·24	4·42
11 : 0	0·24	0·19	0·06	< 0·01	0·24	0·01
12 : 0	5·53	5·88	0·24	< 0·01	0·03	0·27
14 : 0	9·9	9·8	0·84	0·53	0·11	1·48
14 : 1	0·11	0·12	0·38	0·02	0·07	< 0·01
Iso 15 : 0	0·24	0·25	0·21	0·01	0·41	0·01
Anteiso 15 : 0	0·15	0·15	0·94	0·02	0·01	0·01
15 : 0	1·06	1·04	0·47	< 0·01	0·51	0·01
16 : 0	20·20	18·90	0·10	< 0·01	0·14	3·78
16 : 1	0·61	0·61	0·97	0·02	0·03	0·01
Iso 17 : 0	0·48	0·45	0·43	< 0·01	0·86	0·01
Anteiso 17 : 0	0·40	0·41	0·78	0·05	0·74	0·01
17 : 0	0·53	0·55	0·58	0·01	0·11	0·01
17 : 1	0·16	0·17	0·60	< 0·01	0·03	0·01
18 : 0	7·89	8·00	0·82	0·01	0·10	1·75
18 : 1 *t*†	1·17	1·08	0·27	0·01	0·09	0·11
18 : 1 *c*-9	14·90	14·90	0·96	< 0·01	0·27	5·67
18 : 2	1·28	1·29	0·89	0·01	0·67	0·05
CLA 9-11	0·81	0·71	0·02	0·07	0·29	0·04
18 : 3	0·90	1·02	0·04	0·82	0·10	0·02

† Identification based on *trans*-11 and *trans*-9 isomers. Procedure does not preclude the presence of the *trans* isomers

More simply, the decrease of linolenic acid in PM herbage could be linked to the corresponding lower CP level of herbage. In fact, as Elgersma et al. (2006) report, a strong linear relationship exists between linolenic acid and the N concentration of the herbage even though the cause of this correlation is not clear.

On average, goats ate more herbage in the PM group, as a function of increased WSC level; intake increase linked to higher level of WSC as a consequence of PM cutting, compared with AM, have been found with alfalfa hay (Fisher et al. 2002; Burns et al. 2005) and on forages stored as baleage (Huntington & Burns, 2007). A greater palatability due to forage sweetness can explain these results. Higher intake in the PM group further increased the difference in WSC inputs between groups.

Milk protein and urea were significantly affected by treatment. Taweel et al. (2005), on testing perennial ryegrass varieties at different WSC content on dairy cows, did not find significant differences in milk production and quality. The authors ascribe the lack of significance to the small difference in WSC content between the two grass varieties. However, they found higher milk urea levels in the group fed with the variety characterized by lower WSC content, associated with a slightly higher CP content. In our experimental conditions, with a difference in WSC content of the herbage on average equal to 30 g/kg, FCM was not affected but protein was higher and urea lower in the PM group. As supported by previous findings (Godden et al. 2001; Broderick, 2003; Moharrery, 2004) we should hypothesize that in goats grazing during PM hours an improvement of the efficiency of microbial protein synthesis had occurred, as a consequence of the increased WSC/CP ratio in the diet.

Curd firmness increased in the PM group; a firm curd, encouraging the retention of milk components, should be considered the most important attribute determining quality and yield of cheese and thus economic returns (Clark & Sherbon, 2000). According to Bencini et al (2002), for sheep and cow milk, the increase found in PM group is probably a function of the favourable combination of higher protein content and higher acidity of milk.

No important differences were found in the main classes of fatty acids, as an effect of the grazing time of day. Cabrita et al. (2003) report increases in the fatty acids synthesized de novo (8 : 0, 12 : 0 and 14 : 0) when a diet rich in sugars and low in fat was given to dairy cows. In our conditions the variations in WSC of herbage between AM and PM were not sufficient to expect similar results; however, a significant reduction of 8 : 0 (from 6·0 to 4·6% of total fatty acids), 10 : 0 (from 17·5 to 14·1% of total fatty acids) and 12 : 0 (from 6·1 to 5·2% of total fatty acids) was found from week 1 to week 5, which can be explained by the corresponding marked decrease in WSC in herbage. The odd- and branched-chain fatty acids (OBCFA) are mainly derived from bacteria leaving the rumen, whereas they are generally absent from feeds (Vlaeminck et al. 2006a). For this reason, these acids have been used as markers of different aspects of bacterial activity in the rumen (Cabrita et al. 2003; Kim et al. 2005). Vlaeminck et al. (2006b) developed a model to predict volatile fatty acid (VFA) concentration in the rumen as a function of OBCFA concentration in milk. Results in the literature highlight the positive correlation between forage : concentrate ratio and OBCFA in milk (Vlaeminck et al. 2006c), but a positive correlation between sugar supply and milk *anteiso* 15 : 0 (Cabrita et al. 2003). In our experimental conditions the forage:concentrate ratio was higher in the PM group, as a consequence of the higher herbage intake level and, in the same group, the fermentable carbohydrate intake was also higher than for the AM group. For these reasons and taking into account the effects on milk urea and protein levels already discussed, which seem to indicate a better microbial efficiency, we would have expected an increase of OBCFA in the PM group. In this regard, Dewhurst et al. (2007) found little change in the yield of OBCFA in milk, in response to increasing the level of concentrate in the diet of dairy cows and no significant relationships with

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microbial protein yield; the synthesis of some of these acids in animal tissues might explain these results.

Milk CLA content was higher in the AM group. Considering the higher linolenic acid in AM herbage, our results seem to be in line with those of Elgersma et al. (2003). Those authors, feeding cows with *Lolium perenne* at different linolenic acid concentrations, found a positive correlation between milk CLA and forage linolenic acid. However, taking into account that in our conditions the PM group ate more herbage, the linolenic acid input in the two groups were similar (22·8 and 22·0 g/d, respectively, in AM and PM group).

It is well known that linolenic acid is subjected to extensive microbial biohydrogenation, up to 92% according to Chilliard & Ferlay (2004). The most important intermediates of this process are vaccenic acid (18 : 1 *trans* 11) and CLA (18 : 2 *cis* 9 *trans* 11). Even though the differences between groups in 18 : 1 *trans* isomers did not reach significance, our results seem to suggest that a lower degree of saturation occurred in the PM group, perhaps as a consequence of a lower N availability (Cabrita et al. 2003) and/or a lower ruminal pH linked to the higher soluble sugar input. On this matter, Peyraud et al. (1997) observed higher propionate and butyrate and lower acetate proportions in the rumen when a Lolium variety with high WSC content was given to dairy cows, compared with a low WSC variety. As reported by Elgersma et al. (2006), results of in-vitro experiments indicate that the amount of readily fermentable carbohydrates negatively affects the rate of biohydrogenation. In our conditions the hypothesis that a lower biohydrogenation activity occurred in the PM group seems to be supported by the higher milk linolenic acid concentration in this group, which could be linked to a probable higher duodenal flow of this acid, having escaped bacterial saturation activity.

In conclusion, shifting grazing time of day from morning to afternoon could represent a good strategy in Mediterranean dairy goat system, where short grazing is a common practice. An increase in herbage sugar content, by means of the accumulation of the product of photosynthesis throughout the day, seems to improve milk quality by increasing protein level, curd firmness and linolenic acid concentration.

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