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Fatty acid profile of milk from goats fed diets with different levels of conserved and fresh forages

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UNIVERSITÀ DEGLI STUDI DI TORINO

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ABSTRACT

Aim of the study was to evaluate the effect of different proportions of hay and fresh grass in goats' diet on milk fatty acid profile. Nine Camosciata goats were fed a fixed amount of concentrate (30% of total diet) and different percentages (40% vs 30%, 50% vs 20%, and 60% vs 10%) of hay and fresh grass, respectively. Diminishing amounts of fresh grass percentages in the diet led to significant increases of lauric, myristic, and palmitic acids ($P \leq 0.001$) and to significant decreases of C18:1 ω -6-11, rumenic and α -linolenic acids ($P \leq 0.001$) in milk, thus determining a worsening of the health value of milk fat.

Keywords Goat milk, Fatty acids, Conjugated linoleic acid, Hay, Fresh grass, Human health.

INTRODUCTION

In the last decades some claimed negative health effects have been attributed to dairy fat, mainly due to its high content of saturated fatty acids. Consequently, limitations to dairy fat intakes have been recommended by international public health policies (World Health Organization 2008). These factors have led to a general negative perception of dairy products by consumers, who are nowadays more and more aware of the potential health-related benefits and damages linked to food consumption (Smed and Jensen 2005). However, the intense research activity carried out in the last few years has led to a reappraisal of milk and dairy products from ruminants. The latter have been recently recognized as "functional foods", that means natural sources of biologically-active compounds able to exert an important positive

1 role in human nutrition by providing health benefits beyond basic nutrition
2 (Prates and Mateus 2002).

3 Specific unsaturated fatty acids, such as conjugated linoleic acids (CLA)
4 and n3 (omega-3) fatty acids, have been shown to exert potential human
5 health benefits including protection against carcinogenesis, atherosclerosis,
6 diabetes, inflammation, cardiovascular, and autoimmune diseases (Parodi
7 2009). The amount of these relevant biologically active molecules in milk fat
8 from ruminants is greatly affected by the dietary regimen applied at farm level
9 (Morand-Fehr *et al.* 2007).

10 Goat milk and dairy products are acquiring great importance in human
11 nutrition (Haenlein 2004). Notwithstanding, the number of studies aimed at
12 assessing the effects of different diet components on the fatty acid
13 composition of goat milk is relatively limited (Sanz Sampelayo *et al.* 2007) if
14 compared to the great amount of research carried out with dairy ewes, and
15 even more with dairy cows. Moreover, the available research studies have
16 essentially been conducted with the purpose to evaluate the effects of
17 different dietary forage:concentrate ratios, showing that decreasing the fibre
18 and increasing the grain contents in the diet lead to higher contents of
19 undesirable saturated and *trans* fatty acids and contemporarily to lower
20 contents of CLA and other beneficial unsaturated fatty acids in goat milk
21 (Morand-Fehr *et al.* 2007).

22 The method of forage preservation has been reported to affect the content
23 of fatty acids in plants (Morel *et al.* 2006; Doreau *et al.* 2005; Morand-Fehr
24 and Tran 2001). Since dietary unsaturated fatty acids are important
25 precursors for the biosynthesis of fatty acids with functional properties in milk
26 (Antongiovanni *et al.* 2003), some differences in goat milk fatty acid

1 composition could be expected in relation to the type of forage (fresh or
2 conserved) fed to animals. Pajor *et al.* (2009) reported that milk from goats fed
3 pasture had higher amounts of nutritionally peculiar fatty acids than milk from
4 goats fed with hay. No studies are currently available on the effects of
5 different proportions of conserved and fresh forages in goats' diet on the fatty
6 acid profile of milk fat.

7 The aim of this study was therefore to evaluate the changes in the fatty
8 acid profile of milk from goats fed diets characterized by a fixed amount of
9 concentrate and different proportions of hay and fresh cut grass.

10 **MATERIALS AND METHODS**

11 **Animals, feeding and management**

12 The experiment lasted five months and was carried out in a dairy goat farm
13 located in North-Western Italy (latitude: 45°37'16"; longitude: 08°02'03";
14 altitude: 750 m a.s.l.). Nine multiparous Camosciata goats were selected from
15 a flock of 50 heads on the basis of their stage of lactation, milk yield, and milk
16 gross composition. The main changes in milk fatty acids are known to occur in
17 early lactation, while a relative stable fatty acid pattern is generally observed
18 in mid and late lactation (Ataşoğlu *et al.* 2009; De La Fuente *et al.* 2009). In
19 order to avoid the presence of confounding factors (e.g., stage of lactation), all
20 selected goats were in mid lactation at the beginning of the experimental
21 period (107±9 days in milk post partum). Means and standard deviations of
22 milk yield and milk fat, protein and lactose contents were equal to 3.30±0.51
23 kg head⁻¹ day⁻¹, 2.72±0.40 g 100g⁻¹, 3.18±0.32 g 100g⁻¹ and 4.08±0.29 g
24 100g⁻¹, respectively.

1 During a 16 days pre-experimental period (May 16th to May 31st) the
2 selected goats were fed a diet consisting of 0.8 kg concentrate, 1.2 kg mixed
3 meadow hay, and fresh cut grass offered *ad libitum*.

4 The experimental period (June 1st to October 15th) was divided into three
5 phases (P1, P2, and P3) during which the goats were fed three different diets
6 containing a fixed percentage (30% of the total diet on a dry matter -DM-
7 basis) of concentrate and variable proportions of mixed meadow hay and
8 fresh cut grass: 40% vs 30% (diet G30, from June 1st to July 15th – P1), 50%
9 vs 20% (diet G20, from July 16th to August 31st – P2), and 60% vs 10% (diet
10 G10, from September 1st to October 15th – P3), respectively. Both hay and
11 concentrate were the same ones used during the pre-experimental period. In
12 all phases, the fresh grass was cut from the same meadow, sown as a
13 combination of perennial ryegrass (*Lolium perenne*) and white clover
14 (*Trifolium repens*). At the beginning of the trial the meadow was divided into
15 two plots. The first plot was used in P1; the second one was used in P2, while
16 in P3 fresh grass was cut again from the first plot, being consequently in a
17 regrowth stage.

18 The chemical compositions of feedstuffs were used to verify that all diets
19 fulfilled the nutrients requirements of the goats according to National
20 Research Council (NRC 1981). The diet G30 consisted of 0.9 kg concentrate,
21 1.2 kg hay, and 3.1 kg fresh cut grass. In the G20 diet goats received 0.8 kg
22 concentrate, 1.3 kg hay, and 1.4 kg of fresh grass. Finally in the G10 diet, 0.8
23 kg concentrate, 1.5 kg hay, and 1.4 kg fresh cut grass were offered to the
24 goats.

25 Feeds offered and refused were measured individually.

26

1

Feed sampling and analysis

2 Representative fresh grass samples were hand-plucked at random transects
3 once at the beginning of each dietary phase and stored at -20°C until analysis
4 for chemical and fatty acid compositions. Hay and concentrate samples were
5 instead taken at the beginning of the trial for chemical analysis.

6 All feed samples (concentrate, hay, and fresh grass) were analysed for dry
7 matter (DM), ash, crude protein (CP), ether extract (EE), and neutral
8 detergent fibre (NDF) according to AOAC procedures (2000). For fatty acids
9 analysis, total lipids were extracted according to Folch *et al.* (1957). Fatty acid
10 methyl esters (FAMES) were prepared by methylation procedure (AOAC
11 2000) and were separated and quantified by gas chromatography (Shimadzu
12 GC17A, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan)
13 using a DB-Wax capillary column (60 m x 0.53 mm ID, 1.0 mm film thickness;
14 J&W Scientific). The column temperature was held at 180°C for one min, then
15 raised $5^{\circ}\text{C min}^{-1}$ up to 225°C , and maintained for 30 min. The temperatures of
16 the injector and flame-ionization detector were maintained at 250 and 270°C ,
17 respectively; the injection volume was $0.1 \mu\text{L}$; nitrogen constant linear flow
18 rate was set at 24 mL min^{-1} . Peaks were identified by comparison of retention
19 times with FAME standards (Restek Corporation, Bellefonte, PA, USA).
20 Results were expressed as $\text{g } 100\text{g}^{-1}$ methyl esters.

21

Milk sampling and analysis

22 The goats were manually milked twice a day (at 06.00 and 18.00 h). Milk
23 yield recording and samples collection started after two weeks of adaptation to
24 the new diet conditions in each phase. Individual daily milk yields were
25 recorded during the morning and afternoon milkings every three weeks (twice

1 for each phase). For laboratory analysis, individual composite samples (1:1
2 ratio of morning and afternoon milkings) were collected following the same
3 time schedule as for milk yield recording. One aliquot of each milk sample was
4 stored at 4°C in a portable refrigerator, and then immediately transported to
5 the laboratory for the analysis of fat, protein, lactose, and somatic cell count
6 (Combi-Foss™ 6000 FC; Foss, Hillerød, Denmark). A second aliquot was
7 frozen at -20°C, until analysed for the fatty acid composition. Fatty acids were
8 determined as previously reported by Collomb and Bühler (2000). Milk fat
9 extraction was obtained by centrifugation at 7,300 rpm for 30 min at -4°C.
10 After the resulting molten butter had been filtered through a hydrophobic filter
11 (Whatman 1, Whatman International Ltd, Maidstone England), the pure milk
12 fat was dissolved in heptane and FAMES were obtained by *trans*-esterification
13 of glycerides by using a solution of KOH in methanol (IOfS 2002). FAMES
14 were then separated and quantified by a gas chromatograph (Shimadzu
15 GC17A, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan)
16 equipped with a CP-Sil 88 capillary column (100 m x 0.25 mm ID, 0.20 mm
17 film thickness; Varian Inc., Lake Forest, CA). The column temperature was
18 held at 45°C for 5 min, then raised 20°C min⁻¹ up to 195°C and maintained for
19 65 min. The temperatures of the injector and the flame-ionization detector
20 were maintained at 250 and 280°C, respectively; the injection volume was 0.1
21 µL; nitrogen constant linear flow rate was set at 40 mL min⁻¹. Peaks were
22 identified by comparing the retention times with pure FAME standards
23 (Matreya Inc., Pleasant Gap, PA, USA and Restek Corporation, Bellefonte,
24 PA, USA). Results were expressed as g 100 g⁻¹ methyl esters.

Statistical analysis

The Kolmogorov-Smirnov test was used to check dependent variables for normality. Somatic cell count was not normally distributed; this variable was consequently log-transformed prior to further statistical analysis, but the presented results are shown as non-transformed data.

The changes in milk yield, main constituents and fatty acids were analysed as a repeated measures design using the Proc MIXED procedure of SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). The following mixed linear model was used:

$$Y_{ijkl} = \mu + D_i + bX_j + G_k + P_l + (D \times P)_{il} + \epsilon_{ijkl},$$

where Y_{ijkl} = mean of response variable, μ = overall mean, D_i = fixed effect of the diet, bX_j = covariable represented by the DIM at which the first record occurred, G_k = random effect of goat, P_l = fixed effect of parity, $(D \times P)_{il}$ = effect of interaction between diet and parity, and ϵ_{ijkl} = random residual error.

Parity and the interaction between diet and parity were not statistically significant for any of the detected parameters. Both effects were consequently removed from the statistical model and least square means have been presented for diets only. When significant ($P \leq 0.05$) effects due to dietary treatments were detected, mean separation was conducted by the PDIFF option in SAS.

RESULTS AND DISCUSSION

Characteristics of feedstuffs and diets

The chemical compositions of feedstuffs (concentrate, hay, and fresh grass) and of the three experimental diets are presented in Table 1. Fresh

1 grass was particularly rich in α -linolenic acid (C18:3 $n-3$, ALA), which
2 comprised alone about 45-50% of total fatty acids. The α -linolenic acid
3 content of plants was especially high in P1, in coincidence of the plants initial
4 growth. As expected (Clapham *et al.* 2005), the second and third most
5 abundant fatty acids in fresh grass were palmitic (C16:0) and linoleic (C18:2
6 $n-6$, LA) acids, which were set at approximately 16-20% of total fatty acids.
7 These three fatty acids were the most abundant ones in hay as well, but
8 notable lower amounts of ALA were detected in hay if compared to those
9 observed in fresh grass. As a method of forage preservation, drying is known
10 to affect the concentrations of fatty acids in plants, also by decreasing the
11 content of ALA (Morel *et al.* 2006; Morand-Fehr and Tran 2001). Differently
12 from the other feedstuffs, the predominant fatty acid in the concentrate was
13 linoleic acid (about 55% of total fatty acids), followed in order of abundance by
14 oleic (C18:1 $n-7$) and palmitic (C16:0) acids.

15 The three diets were similar if considering major components (protein, fat,
16 and fibre contents). However, their fatty acid composition showed some
17 differences, mainly in the proportions of palmitic and α -linolenic acids. The
18 former acid increased whereas the latter decreased while increasing the ratio
19 between hay and fresh grass in the diet.

20

Animal performance

21 Only negligible feed refusals were recorded in the three phases showing
22 that the diets were correctly formulated.

23 Milk yield and gross composition during the three phases are reported in
24 Table 2. Milk yield significantly and progressively declined during the trial
25 ($P \leq 0.001$). No statistically significant variations were observed in the fat

1 percentage of milk. Protein percentages were higher in P3 if compared to P1
2 and P2 ($P \leq 0.001$). The somatic cell count significantly and progressively
3 increased during the experiment ($P \leq 0.05$). No differences were observed in
4 the lactose percentage of milk. The stage of lactation is one of the main
5 parameters able to influence milk production performance in dairy goats
6 (Ciappesoni *et al.* 2004). The observed variations are most likely to be
7 attributed to the effect of lactation progression rather than to the changes in
8 the dietary regimen.

9 **Milk fatty acid composition**

10 Results on the fatty acid composition of goat milk fat obtained in the three
11 experimental phases are presented in Table 3. Among detected fatty acids,
12 only caproic (C6:0), caprylic (C8:0), and dodecenoic (C12:1) acids were not
13 significantly affected by diet.

14 Significantly higher levels of total saturated fatty acids were observed in P2
15 and P3 if compared to P1 ($P \leq 0.001$). Considering individual saturated fatty
16 acids, those that underwent significant increases during the trial were lauric
17 (C12:0), myristic (C14:0), palmitic (C16:0), and heptadecanoic (C17:0) acids
18 ($P \leq 0.001$). Lauric, myristic and palmitic acids have been shown to raise
19 cholesterol levels, being consequently considered detrimental for human
20 health (Parodi 2009). Their sum, referred in Table 3 as HSFA
21 (Hypercholesterolemic Saturated Fatty Acids), was found to be significantly
22 lower ($P \leq 0.001$) when the goats were fed the G30 diet, which comprised the
23 higher percentage of fresh grass in the diet. Differently from other detected
24 saturated fatty acids, stearic acid (C18:0) showed significantly higher levels in
25 P1 relative to both P2 and P3 ($P \leq 0.001$). Stearic acid is the final product of

1 rumen bacterial biohydrogenation of dietary unsaturated fatty acids. The
2 higher amount of C18:0 found in P1 seems to be mainly related to the higher
3 levels of ALA in the G30 diet. Dietary ALA, in fact, is usually almost
4 completely biohydrogenated within the rumen (Lock and Garnsworthy 2002),
5 leading to high amounts of both intermediate and final biohydrogenation
6 products in milk fat from ruminants.

7 Unsaturated fatty acids (both total mono- and polyunsaturated ones)
8 showed an opposite trend as that observed for the majority of saturated fatty
9 acids. Their content in milk fat was significantly lower in P2 and P3 if
10 compared to values observed in P1. In particular, among monounsaturated
11 fatty acids such a decreasing trend was shown to occur for myristoleic (C14:1
12 ω 9; $P \leq 0.01$), palmitoleic (C16:1 ω 9; $P \leq 0.01$), heptadecenoic (C17:1 ω 9;
13 $P \leq 0.001$), and the sum of ω 6 to ω 11 octadecenoic isomers ($P \leq 0.001$). Among
14 polyunsaturated fatty acids, a significant decrease from the G30 diet to the
15 G10 diet was found in the content of rumenic (C18:2 ω 9 ω 11, CLA; $P \leq 0.001$)
16 and α -linolenic (C18:3 ω 9 ω 12 ω 15; $P \leq 0.01$) acids.

17 Vaccenic acid (VA) is by far the most abundant among *trans* octadecenoic
18 isomers in milk fat from ruminants, being one of the main intermediate
19 products of the biohydrogenation process occurring within the rumen.
20 Similarly to what previously discussed for stearic acid, since ALA is one of the
21 dietary precursors for VA synthesis (Collomb *et al.* 2006), the explanation for
22 the higher C18:1 ω 6-11 content in P1 have to be related to higher ALA level in
23 the G30 treatment.

24 The majority of rumenic acid (the most abundant among CLA isomers in
25 ruminant-derived food products) originates endogenously in the mammary
26 gland from VA thanks to the activity of the Δ 9-desaturase enzyme (Mosley *et*

1 *al.* 2006). Δ 9-desaturase is able to add a *cis* double bond between carbons 9
2 and 10 of saturated and unsaturated fatty acids with a chain length of 10 to 18
3 carbons (Soyeurt *et al.* 2008). In order to assess the influence of experimental
4 diets on the activity of this enzyme within the mammary gland, a desaturase
5 index was calculated as the ratio between myristoleic and myristic acids
6 (C14:1 *c9*/C14:0, DI₁₄). This index is considered the best indicator for the Δ 9-
7 desaturase activity because all myristoleic acid is formed from myristic acid
8 thanks to the activity of this enzyme (Griinari *et al.* 2000). Increasing levels of
9 DI₁₄ indicate increasing activity of the enzyme within the mammary gland. The
10 diet significantly affected DI₁₄, which was found to decrease from G30 to G10.
11 Such result confirms previous findings by Lock and Garnsworthy (2003) and
12 Impemba *et al.* (2007) who both found that the feeding regimen can
13 significantly influence the desaturase index in dairy cows and goats, with fresh
14 grass being able to enhance the activity of the enzyme. The decreasing
15 contents of myristoleic, palmitoleic, heptadecenoic and rumenic acids found
16 from the G30 diet to the G10 diet can be essentially related to the lower
17 estimated Δ 9-desaturase activity within the mammary gland. In addition, the
18 significant variations observed in the rumenic acid content are also the
19 consequence of the lower availability of VA as substrate for Δ 9-desaturase
20 activity.

21 It is worth mentioning that Couvreur *et al.* (2006) previously found linear
22 relationships existing between the proportion of fresh grass in the diet of dairy
23 cows and the content of the majority of fatty acids in milk fat. In our trial, the
24 lack of significant differences between P2 and P3 in the levels of some
25 detected fatty acids in milk (e.g., C18:1 ω -11, CLA, ALA), could be ascribed to
26 the variation in the phenological phase of fresh cut grass that occurred in the

1 three experimental phases. In fact, it is known that increasing maturity and
2 flowering determine a reduction of FAs concentrations in plants (Clapham *et*
3 *al.* 2005). Consequently, it is reasonable to hypothesize that in P3 (regrowth
4 stage of fresh grass) high FAs intake from grass occurred despite the low
5 percentage of this feedstuff in the diet, thus explaining the lack of significant
6 differences in milk fatty acid profiles between P2 and P3.

7 Fatty acids are able to strongly affect human health. The Atherogenicity
8 and Trombogenicity Indexes (Ulbricht and Southgate 1991), widely used as
9 markers of cardiovascular disease risk, showed lower levels when the goats
10 were fed the G30 diet if compared to both G20 and G10 treatments. The
11 same was also observed if considering rumenic acid, vaccenic acid and
12 omega-3 fatty acids, which are able to exert many beneficial biological effects
13 including protection against carcinogenesis, arteriosclerosis, and some other
14 widespread diseases (Collomb *et al.* 2006; Tyburczy *et al.* 2009; Field *et al.*
15 2009; Anderson and Ma 2009). The obtained results showed that milk fat had
16 an overall superior health value when the goats were fed the higher amount of
17 fresh forages in the diet (G30).

18 **CONCLUSIONS**

19 Increasing the amount of hay at the expense of fresh grass in the diet of dairy
20 goats can significantly worsen the fatty acid composition of milk fat. Such
21 worsening is mainly associated to an increase in the percentages of
22 hypercholesterolemic saturated fatty acids and to a decrease of the
23 percentages of both mono- and polyunsaturated fatty acids. Of particular
24 remark is the decrease in the percentages of vaccenic, rumenic, and α -
25 linolenic acids that are known to be able to exert many beneficial effects on

1 human health. Keeping the animal management more natural as possible (by
2 using fresh cut grass or, even better, allowing ruminants to graze) allows the
3 optimisation of the balance between detrimental and valuable fatty acids in
4 dairy products, thus obtaining putative beneficial effects for the consumer's
5 health. The cheese fatty acid profile is known to reflect the improvement
6 obtained in milk as affected by dietary regime (Lucas *et al.* 2006). This is
7 particularly important in goat milk, since it is mainly processed into cheeses
8 and other typical dairy products.

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1 **Table 1** Chemical composition (% DM, unless otherwise stated) and fatty acid profile (g 100g⁻¹ methyl esters) of
 2 feedstuffs (concentrate, hay, and fresh grass) and experimental diets
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	Feedstuffs					Diets [‡]		
	Concentrate [†]	Hay	Fresh grass			G30	G20	G10
			P1	P2	P3			
Main nutrients								
Dry matter (%)	90.8	89.1	26.1	34.7	16.0	68.8	78.7	83.0
Ash	9.6	9.3	8.4	9.2	8.7	9.1	9.4	9.3
Crude protein	18.7	14.5	16.5	14.4	17.1	16.3	15.6	15.9
Ether extract	2.4	1.5	3.0	2.7	2.2	2.2	2.0	1.8
Neutral detergent fibre	28.0	57.5	57.9	65.0	53.2	49.7	51.0	49.1
UFL kg ⁻¹ DM	0.98	0.73	0.75	0.65	0.79	0.86	0.85	0.85
Fatty acids								
C10	nd	0.68	0.30	0.60	2.63	0.37	0.48	0.67
C12	nd	0.35	0.19	0.39	0.59	0.20	0.27	0.28
C14	0.45	3.24	3.02	3.37	1.65	2.41	2.51	2.34
C14:1	0.12	2.22	0.72	1.37	1.17	1.16	1.48	1.56
C15	0.05	0.81	0.33	0.77	1.26	0.45	0.60	0.65
C15:1	0.05	1.65	2.00	2.56	1.17	1.33	1.40	1.17
C16	13.51	27.72	17.84	19.59	19.45	20.62	22.26	23.14
C16:1	0.12	1.24	2.08	1.53	1.78	1.21	0.99	0.98
C18	2.15	5.29	1.55	2.80	3.53	3.21	3.94	4.28
C18:1 c9	26.06	7.23	3.38	5.18	5.73	11.04	11.90	12.18

C18:2 <i>c9c12</i> (LA)	55.06	21.97	16.72	16.32	16.19	29.17	29.78	30.39
C18:3 <i>c9c12c15</i> (ALA)	2.43	27.61	51.88	45.53	44.85	28.82	24.39	22.36
SFA	16.16	38.09	23.23	27.52	29.11	27.27	30.06	31.36
MUFA	26.35	12.34	8.18	10.64	9.85	14.75	15.78	15.89
PUFA	57.49	49.58	68.60	61.85	61.04	57.99	54.17	52.75

Abbreviations: P1, phase 1; P2, phase 2; P3, phase 3; DM, dry matter; *c*, *cis*; LA, linoleic acid; ALA, α -linolenic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

† Commercial concentrate based on: corn meal, sunflower meal, fine wheat bran, wheat middlings, genetically modified soybean meal, barley meal, genetically modified soybean seeds, sugarcane molasses, calcium carbonate, dicalcium phosphate, sodium chloride, inactivated yeast culture of *Saccharomyces cerevisiae*, magnesium oxide, vitamin-mineral premix.

‡ Diet G30 consisting of: 30% concentrate, 40% hay, and 30% fresh grass; Diet G20 consisting of: 30% concentrate, 50% hay, and 20% fresh grass; Diet G10 consisting of: 30% concentrate, 60% hay, and 10% fresh grass.

1 **Table 2** Relationship between the proportion of hay and fresh
 2 grass in the diet and milk yield, milk main constituents and somatic
 3 cell count
 4

	Diets [†]			Significance [‡]
	G30 – P1 n = 18	G20 – P2 n = 18	G10 – P3 n = 18	
Milk yield (kg head ⁻¹ day ⁻¹)	3.24 ^a	2.40 ^b	2.04 ^c	***
Fat (g 100g ⁻¹)	3.12	2.81	2.87	ns
Protein (g 100g ⁻¹)	3.31 ^b	3.21 ^b	3.86 ^a	***
Lactose (g 100g ⁻¹)	4.24	4.02	4.10	ns
SCC [§] (n*10 ³ mL ⁻¹)	446.00 ^b	552.00 ^{ab}	690.00 ^a	*

5
 6 Abbreviations: P1, phase 1; P2, phase 2; P3, phase 3; SCC, somatic cell count.
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8 † Diet G30 consisting of: 30% concentrate, 40% hay, and 30% fresh grass; Diet G20
 9 consisting of: 30% concentrate, 50% hay, and 20% fresh grass; Diet G10 consisting of: 30%
 10 concentrate, 60% hay, and 10% fresh grass. Commercial concentrate based on: corn meal,
 11 sunflower meal, fine wheat bran, wheat middlings, genetically modified soybean meal, barley
 12 meal, genetically modified soybean seeds, sugarcane molasses, calcium carbonate,
 13 dicalcium phosphate, sodium chloride, inactivated yeast culture of *Saccharomyces cerevisiae*,
 14 magnesium oxide, vitamin-mineral premix.
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16 ‡ Probability: * P≤0.05; ** P≤0.01; *** P≤0.001; ns, not significant (P>0.05). Different letters
 17 within rows indicate statistically significant differences between diets.
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1 **Table 3** Relationship between the proportion of hay and fresh
 2 grass in the diet and fatty acid profile (g 100g⁻¹ methyl esters) of
 3 goat milk fat
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	Diets [†]			Significance [‡]
	G30 – P1 n = 18	G20 – P2 n = 18	G10 – P3 n = 18	
C6	1.15	1.43	1.13	ns
C8	1.95	2.01	1.94	ns
C10	7.85 ^c	9.42 ^a	8.65 ^b	***
C10:1 <i>c</i> 9	0.13 ^b	0.19 ^a	0.17 ^{ab}	**
C12	3.87 ^c	5.27 ^b	6.73 ^a	***
C12:1 <i>c</i> 9	0.18	0.22	0.17	ns
C14	10.03 ^c	11.04 ^b	13.11 ^a	***
C14:1 <i>c</i> 9	0.30 ^a	0.32 ^a	0.25 ^b	**
C15	0.47 ^a	0.49 ^a	0.32 ^b	***
C15:1	1.03 ^b	1.19 ^a	0.86 ^c	***
C16	26.34 ^b	31.28 ^a	30.95 ^a	***
C16:1 <i>c</i> 9	0.44 ^a	0.40 ^a	0.33 ^b	**
C17	0.85 ^b	1.07 ^a	1.08 ^a	***
C17:1 <i>c</i> 9	0.77 ^a	0.77 ^a	0.45 ^b	***
C18	14.77 ^a	9.31 ^b	8.12 ^b	***
C18:1 <i>c</i> 9	21.19 ^a	18.83 ^b	19.86 ^{ab}	*
C18:1 <i>t</i> 6-11	3.62 ^a	1.91 ^b	1.85 ^b	***
C18:2 <i>c</i> 9 <i>c</i> 12 (LA)	2.63 ^b	2.93 ^a	2.23 ^c	***
C20	0.30 ^a	0.29 ^a	0.17 ^b	***
CLA <i>c</i> 9 <i>t</i> 11 (CLA)	0.95 ^a	0.72 ^b	0.67 ^b	***
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15 (ALA)	1.16 ^a	0.91 ^b	0.89 ^b	**
SFA	67.60 ^b	71.61 ^a	72.26 ^a	***
MUFA	27.63 ^a	23.83 ^b	23.93 ^b	***
PUFA	4.73 ^a	4.56 ^a	3.78 ^b	***
SFA / UFA	2.11 ^b	2.60 ^a	2.72 ^a	***
AI [§]	2.28 ^b	3.01 ^a	3.52 ^a	***
TI [§]	2.72 ^b	3.22 ^a	3.38 ^a	**
DI ₁₄ [#]	0.03 ^a	0.03 ^a	0.02 ^b	***
HSFA [^]	40.27 ^b	47.59 ^a	50.90 ^a	***

5
 6 Abbreviations: P1, phase 1; P2, phase 2; P3, phase 3; *c*, *cis*; *t*, *trans*; LA, linoleic acid; CLA,
 7 conjugated linoleic acid; ALA, α -linolenic acid; SFA, saturated fatty acids; MUFA,
 8 monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty
 9 acids; AI, atherogenicity index; TI, trombogenicity index; DI, desaturase index; HSFA,
 10 hypercholesterolemic saturated fatty acids.

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 12 [†] Diet G30 consisting of: 30% concentrate, 40% hay, and 30% fresh grass; Diet G20
 13 consisting of: 30% concentrate, 50% hay, and 20% fresh grass; Diet G10 consisting of: 30%
 14 concentrate, 60% hay, and 10% fresh grass. Commercial concentrate based on: corn meal,
 15 sunflower meal, fine wheat bran, wheat middlings, genetically modified soybean meal, barley

1 meal, genetically modified soybean seeds, sugarcane molasses, calcium carbonate,
2 dicalcium phosphate, sodium chloride, inactivated yeast culture of *Saccharomyces cerevisiae*,
3 magnesium oxide, vitamin-mineral premix.

4
5 ‡ Probability: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, not significant ($P > 0.05$). Different letters
6 within rows indicate statistically significant differences between diets.

7
8 § Calculated as (Ulbricht and Southgate, 1991): $AI = (C12:0 + 4 * C14:0 + C16:0) / (n3 + n6 + MUFA)$;
9 $TI = (C14:0 + C16:0 + C18:0) / (0.5 * MUFA + 0.5 * n6 + 3 * n3 + n3/n6)$.

10
11 # Calculated as $C14:1\ c9 / C14:0$.

12
13 ^ Calculated as $C12:0 + 4 * C14:0 + C16:0$.

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