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FATTY ACID PROFILE OF MILK FROM GOATS
FED DIETS WITH DIFFERENT LEVELS
OF CONSERVED AND FRESH FORAGES

Fresh grass:hay ratio and goat milk fatty acids

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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≤0.001) and to significant decreases of C18:1 t6-11, rumenic and α-linolenic acids (P≤0.001) in milk, thus determining a worsening of the health value of milk fat.

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

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

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

The method of forage preservation has been reported to affect the content of fatty acids in plants (Morel et al. 2006; Doreau et al. 2005; Morand-Fehr and Tran 2001). Since dietary unsaturated fatty acids are important precursors for the biosynthesis of fatty acids with functional properties in milk (Antongiovanni et al. 2003), some differences in goat milk fatty acid
composition could be expected in relation to the type of forage (fresh or
conserved) fed to animals. Pajor et al. (2009) reported that milk from goats fed
pasture had higher amounts of nutritionally peculiar fatty acids than milk from
goats fed with hay. No studies are currently available on the effects of
different proportions of conserved and fresh forages in goats’ diet on the fatty
acid profile of milk fat.

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

MATERIALS AND METHODS

Animals, feeding and management

The experiment lasted five months and was carried out in a dairy goat farm
located in North-Western Italy (latitude: 45°37’16’; longitude: 08°02’03’;
alitude: 750 m a.s.l.). Nine multiparous Camosciata goats were selected from
a flock of 50 heads on the basis of their stage of lactation, milk yield, and milk
gross composition. The main changes in milk fatty acids are known to occur in
early lactation, while a relative stable fatty acid pattern is generally observed
in mid and late lactation (Ataşoğlu et al. 2009; De La Fuente et al. 2009). In
order to avoid the presence of confounding factors (e.g., stage of lactation), all
selected goats were in mid lactation at the beginning of the experimental
period (107±9 days in milk post partum). Means and standard deviations of
milk yield and milk fat, protein and lactose contents were equal to 3.30±0.51
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
100g⁻¹, respectively.
During a 16 days pre-experimental period (May 16th to May 31st) the selected goats were fed a diet consisting of 0.8 kg concentrate, 1.2 kg mixed meadow hay, and fresh cut grass offered *ad libitum*.

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

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

Feeds offered and refused were measured individually.
Feed sampling and analysis

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

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

Milk sampling and analysis

The goats were manually milked twice a day (at 06.00 and 18.00 h). Milk yield recording and samples collection started after two weeks of adaptation to the new diet conditions in each phase. Individual daily milk yields were recorded during the morning and afternoon milkings every three weeks (twice
for each phase). For laboratory analysis, individual composite samples (1:1 ratio of morning and afternoon milkings) were collected following the same time schedule as for milk yield recording. One aliquot of each milk sample was stored at 4°C in a portable refrigerator, and then immediately transported to the laboratory for the analysis of fat, protein, lactose, and somatic cell count (Combi-Foss™ 6000 FC; Foss, Hillerød, Denmark). A second aliquot was frozen at –20°C, until analysed for the fatty acid composition. Fatty acids were determined as previously reported by Collomb and Bühler (2000). Milk fat extraction was obtained by centrifugation at 7,300 rpm for 30 min at –4°C. After the resulting molten butter had been filtered through a hydrophobic filter (Whatman 1, Whatman International Ltd, Maidstone England), the pure milk fat was dissolved in heptane and FAMEs were obtained by trans-esterification of glycerides by using a solution of KOH in methanol (IOfS 2002). FAMEs were then separated and quantified by a gas chromatograph (Shimadzu GC17A, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan) equipped with a CP-Sil 88 capillary column (100 m x 0.25 mm ID, 0.20 mm film thickness; Varian Inc., Lake Forest, CA). The column temperature was held at 45°C for 5 min, then raised 20°C min⁻¹ up to 195°C and maintained for 65 min. The temperatures of the injector and the flame-ionization detector were maintained at 250 and 280°C, respectively; the injection volume was 0.1 μL; nitrogen constant linear flow rate was set at 40 mL min⁻¹. Peaks were identified by comparing the retention times with pure FAME standards (Matreya Inc., Pleasant Gap, PA, USA and Restek Corporation, Bellefonte, 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:

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

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

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
grass was particularly rich in $\alpha$-linolenic acid (C18:3 c9c12c15, ALA), which comprised alone about 45-50% of total fatty acids. The $\alpha$-linolenic acid content of plants was especially high in P1, in coincidence of the plants initial growth. As expected (Clapham et al. 2005), the second and third most abundant fatty acids in fresh grass were palmitic (C16:0) and linoleic (C18:2 c9c12, LA) acids, which were set at approximately 16-20% of total fatty acids. These three fatty acids were the most abundant ones in hay as well, but notable lower amounts of ALA were detected in hay if compared to those observed in fresh grass. As a method of forage preservation, drying is known to affect the concentrations of fatty acids in plants, also by decreasing the content of ALA (Morel et al. 2006; Morand-Fehr and Tran 2001). Differently from the other feedstuffs, the predominant fatty acid in the concentrate was linoleic acid (about 55% of total fatty acids), followed in order of abundance by oleic (C18:1 c9) and palmitic (C16:0) acids.

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

Animal performance

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

Milk yield and gross composition during the three phases are reported in Table 2. Milk yield significantly and progressively declined during the trial (P ≤ 0.001). No statistically significant variations were observed in the fat
percentage of milk. Protein percentages were higher in P3 if compared to P1 and P2 ($P \leq 0.001$). The somatic cell count significantly and progressively increased during the experiment ($P \leq 0.05$). No differences were observed in the lactose percentage of milk. The stage of lactation is one of the main parameters able to influence milk production performance in dairy goats (Ciappesoni et al. 2004). The observed variations are most likely to be attributed to the effect of lactation progression rather than to the changes in the dietary regimen.

**Milk fatty acid composition**

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

Significantly higher levels of total saturated fatty acids were observed in P2 and P3 if compared to P1 ($P \leq 0.001$). Considering individual saturated fatty acids, those that underwent significant increases during the trial were lauric (C12:0), myristic (C14:0), palmitic (C16:0), and heptadecanoic (C17:0) acids ($P \leq 0.001$). Lauric, myristic and palmitic acids have been shown to raise cholesterol levels, being consequently considered detrimental for human health (Parodi 2009). Their sum, referred in Table 3 as HSFA (Hypercholesterolemic Saturated Fatty Acids), was found to be significantly lower ($P \leq 0.001$) when the goats were fed the G30 diet, which comprised the higher percentage of fresh grass in the diet. Differently from other detected saturated fatty acids, stearic acid (C18:0) showed significantly higher levels in P1 relative to both P2 and P3 ($P \leq 0.001$). Stearic acid is the final product of
rumen bacterial biohydrogenation of dietary unsaturated fatty acids. The higher amount of C18:0 found in P1 seems to be mainly related to the higher levels of ALA in the G30 diet. Dietary ALA, in fact, is usually almost completely biohydrogenated within the rumen (Lock and Garnsworthy 2002), leading to high amounts of both intermediate and final biohydrogenation products in milk fat from ruminants.

Unsaturated fatty acids (both total mono- and polyunsaturated ones) showed an opposite trend as that observed for the majority of saturated fatty acids. Their content in milk fat was significantly lower in P2 and P3 if compared to values observed in P1. In particular, among monounsaturated fatty acids such a decreasing trend was shown to occur for myristoleic (C14:1 c9; P≤0.01), palmitoleic (C16:1 c9; P≤0.01), heptadecenoic (C17:1 c9; P≤0.001), and the sum of t6 to t11 octadecenoic isomers (P≤0.001). Among polyunsaturated fatty acids, a significant decrease from the G30 diet to the G10 diet was found in the content of rumenic (C18:2 c9t11, CLA; P≤0.001) and α-linolenic (C18:3 c9c12c15; P≤0.01) acids.

Vaccenic acid (VA) is by far the most abundant among trans octadecenoic isomers in milk fat from ruminants, being one of the main intermediate products of the biohydrogenation process occurring within the rumen. Similarly to what previously discussed for stearic acid, since ALA is one of the dietary precursors for VA synthesis (Collomb et al. 2006), the explanation for the higher C18:1 t6-11 content in P1 have to be related to higher ALA level in the G30 treatment.

The majority of rumenic acid (the most abundant among CLA isomers in ruminant-derived food products) originates endogenously in the mammary gland from VA thanks to the activity of the Δ9-desaturase enzyme (Mosley et
al. 2006). \(\Delta 9\)-desaturase is able to add a cis double bond between carbons 9 and 10 of saturated and unsaturated fatty acids with a chain length of 10 to 18 carbons (Soyeurt et al. 2008). In order to assess the influence of experimental diets on the activity of this enzyme within the mammary gland, a desaturase index was calculated as the ratio between myristoleic and myristic acids (C14:1 \(c9\)/C14:0, DI\(_{14}\)). This index is considered the best indicator for the \(\Delta 9\)-desaturase activity because all myristoleic acid is formed from myristic acid thanks to the activity of this enzyme (Griinari et al. 2000). Increasing levels of DI\(_{14}\) indicate increasing activity of the enzyme within the mammary gland. The diet significantly affected DI\(_{14}\), which was found to decrease from G30 to G10. Such result confirms previous findings by Lock and Garnsworthy (2003) and Impemba et al. (2007) who both found that the feeding regimen can significantly influence the desaturase index in dairy cows and goats, with fresh grass being able to enhance the activity of the enzyme. The decreasing contents of myristoleic, palmitoleic, heptadecenoic and rumenic acids found from the G30 diet to the G10 diet can be essentially related to the lower estimated \(\Delta 9\)-desaturase activity within the mammary gland. In addition, the significant variations observed in the rumenic acid content are also the consequence of the lower availability of VA as substrate for \(\Delta 9\)-desaturase activity.

It is worth mentioning that Couvreur et al. (2006) previously found linear relationships existing between the proportion of fresh grass in the diet of dairy cows and the content of the majority of fatty acids in milk fat. In our trial, the lack of significant differences between P2 and P3 in the levels of some detected fatty acids in milk (e.g., C18:1 \(t6\)-11, CLA, ALA), could be ascribed to the variation in the phenological phase of fresh cut grass that occurred in the
three experimental phases. In fact, it is known that increasing maturity and flowering determine a reduction of FAs concentrations in plants (Clapham et al. 2005). Consequently, it is reasonable to hypothesize that in P3 (regrowth stage of fresh grass) high FAs intake from grass occurred despite the low percentage of this feedstuff in the diet, thus explaining the lack of significant differences in milk fatty acid profiles between P2 and P3.

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

**CONCLUSIONS**

Increasing the amount of hay at the expense of fresh grass in the diet of dairy goats can significantly worsen the fatty acid composition of milk fat. Such worsening is mainly associated to an increase in the percentages of hypercholesterolemic saturated fatty acids and to a decrease of the percentages of both mono- and polyunsaturated fatty acids. Of particular remark is the decrease in the percentages of vaccenic, rumenic, and \( \alpha \)-linolenic acids that are known to be able to exert many beneficial effects on
human health. Keeping the animal management more natural as possible (by
using fresh cut grass or, even better, allowing ruminants to graze) allows the
optimisation of the balance between detrimental and valuable fatty acids in
dairy products, thus obtaining putative beneficial effects for the consumer's
health. The cheese fatty acid profile is known to reflect the improvement
obtained in milk as affected by dietary regime (Lucas et al. 2006). This is
particularly important in goat milk, since it is mainly processed into cheeses
and other typical dairy products.

REFERENCES
Anderson B M and Ma D W L (2009) Are all polyunsaturated fatty acids
created equal? Lipids in Health and Disease 8 33, doi: 10.1186/1476-
511X-8-33.
Antongiovanni M, Buccioni A, Petacchi F, Secchiari P, Mele M and Serra A.
(2003) Upgrading the lipid fraction of foods of animal origin by dietary
means: rumen activity and presence of trans fatty acids and CLA in milk
Analytical Chemists, Gaithersburg, MD, USA, 2178 pp.
acid composition of goats during lactation in a semi-intensive production
465–473.


Table 1 Chemical composition (% DM, unless otherwise stated) and fatty acid profile (g 100g⁻¹ methyl esters) of feedstuffs (concentrate, hay, and fresh grass) and experimental diets

<table>
<thead>
<tr>
<th>Feedstuffs</th>
<th>Concentrate†</th>
<th>Hay</th>
<th>Fresh grass</th>
<th>Diets‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P1/P2/P3</td>
<td>G30/G20/G10</td>
</tr>
<tr>
<td><strong>Main nutrients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>90.8</td>
<td>89.1</td>
<td>26.1/34.7/16.0</td>
<td>68.8/78.7/83.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>18.7</td>
<td>14.5</td>
<td>16.5/14.4/17.1</td>
<td>16.3/15.6/15.9</td>
</tr>
<tr>
<td>Ether extract</td>
<td>2.4</td>
<td>1.5</td>
<td>3.0/2.7/2.2</td>
<td>2.2/2.0/1.8</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>28.0</td>
<td>57.5</td>
<td>57.9/65.0/53.2</td>
<td>49.7/51.0/49.1</td>
</tr>
<tr>
<td>UFL kg⁻¹ DM</td>
<td>0.98</td>
<td>0.73</td>
<td>0.75/0.65/0.79</td>
<td>0.86/0.85/0.85</td>
</tr>
<tr>
<td><strong>Fatty acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>nd</td>
<td>0.68</td>
<td>0.30/0.60/2.63</td>
<td>0.37/0.48/0.67</td>
</tr>
<tr>
<td>C12</td>
<td>nd</td>
<td>0.35</td>
<td>0.19/0.39/0.59</td>
<td>0.20/0.27/0.28</td>
</tr>
<tr>
<td>C14</td>
<td>0.45</td>
<td>3.24</td>
<td>3.02/3.37/1.65</td>
<td>2.41/2.51/2.34</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.12</td>
<td>2.22</td>
<td>0.72/1.37/1.17</td>
<td>1.16/1.48/1.56</td>
</tr>
<tr>
<td>C15</td>
<td>0.05</td>
<td>0.81</td>
<td>0.33/0.77/1.26</td>
<td>0.45/0.60/0.65</td>
</tr>
<tr>
<td>C15:1</td>
<td>0.05</td>
<td>1.65</td>
<td>2.00/2.56/1.17</td>
<td>1.33/1.40/1.17</td>
</tr>
<tr>
<td>C16</td>
<td>13.51</td>
<td>27.72</td>
<td>17.84/19.59/19.45</td>
<td>20.62/22.26/23.14</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.12</td>
<td>1.24</td>
<td>2.08/1.53/1.78</td>
<td>1.21/0.99/0.98</td>
</tr>
<tr>
<td>C18</td>
<td>2.15</td>
<td>5.29</td>
<td>1.55/2.80/3.53</td>
<td>3.21/3.94/4.28</td>
</tr>
<tr>
<td>C18:1 c9</td>
<td>26.06</td>
<td>7.23</td>
<td>3.38/5.18/5.73</td>
<td>11.04/11.90/12.18</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>----------------</td>
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<td>------</td>
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<td>-------</td>
</tr>
<tr>
<td><strong>C18:2 c9c12 (LA)</strong></td>
<td>55.06</td>
<td>21.97</td>
<td>16.72</td>
<td>16.32</td>
</tr>
<tr>
<td><strong>C18:3 c9c12c15 (ALA)</strong></td>
<td>2.43</td>
<td>27.61</td>
<td>51.88</td>
<td>45.53</td>
</tr>
<tr>
<td><strong>SFA</strong></td>
<td>16.16</td>
<td>38.09</td>
<td>23.23</td>
<td>27.52</td>
</tr>
<tr>
<td><strong>MUFA</strong></td>
<td>26.35</td>
<td>12.34</td>
<td>8.18</td>
<td>10.64</td>
</tr>
<tr>
<td><strong>PUFA</strong></td>
<td>57.49</td>
<td>49.58</td>
<td>68.60</td>
<td>61.85</td>
</tr>
</tbody>
</table>

Abbreviations: P1, phase 1; P2, phase 2; P3, phase 3; DM, dry matter; c, cis; LA, linoleic acid; ALA, \(\alpha\)-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.
Table 2 Relationship between the proportion of hay and fresh grass in the diet and milk yield, milk main constituents and somatic cell count

<table>
<thead>
<tr>
<th>Diets†</th>
<th>G30 – P1</th>
<th>G20 – P2</th>
<th>G10 – P3</th>
<th>Significance‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 18</td>
<td>n = 18</td>
<td>n = 18</td>
<td></td>
</tr>
<tr>
<td>Milk yield (kg head⁻¹ day⁻¹)</td>
<td>3.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Fat (g 100g⁻¹)</td>
<td>3.12</td>
<td>2.81</td>
<td>2.87</td>
<td>ns</td>
</tr>
<tr>
<td>Protein (g 100g⁻¹)</td>
<td>3.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Lactose (g 100g⁻¹)</td>
<td>4.24</td>
<td>4.02</td>
<td>4.10</td>
<td>ns</td>
</tr>
<tr>
<td>SCC§ (n*10³ mL⁻¹)</td>
<td>446.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>552.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>690.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>*</td>
</tr>
</tbody>
</table>

Abbreviations: P1, phase 1; P2, phase 2; P3, phase 3; SCC, somatic cell count.

† 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. 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.

‡ Probability: * P≤0.05; ** P≤0.01; *** P≤0.001; ns, not significant (P>0.05). Different letters within rows indicate statistically significant differences between diets.
Table 3 Relationship between the proportion of hay and fresh grass in the diet and fatty acid profile (g 100g\(^{-1}\) methyl esters) of goat milk fat

<table>
<thead>
<tr>
<th>Diets(\dag)</th>
<th>G30 – P1</th>
<th>G20 – P2</th>
<th>G10 – P3</th>
<th>Significance(\ddag)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 18</td>
<td>n = 18</td>
<td>n = 18</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>1.15</td>
<td>1.43</td>
<td>1.13</td>
<td>ns</td>
</tr>
<tr>
<td>C8</td>
<td>1.95</td>
<td>2.01</td>
<td>1.94</td>
<td>ns</td>
</tr>
<tr>
<td>C10</td>
<td>7.85(^c)</td>
<td>9.42(^a)</td>
<td>8.62(^b)</td>
<td>***</td>
</tr>
<tr>
<td>C10:1 c9</td>
<td>0.13(^b)</td>
<td>0.19(^a)</td>
<td>0.17(^ab)</td>
<td>**</td>
</tr>
<tr>
<td>C12</td>
<td>3.87(^c)</td>
<td>5.27(^b)</td>
<td>6.73(^a)</td>
<td>***</td>
</tr>
<tr>
<td>C12:1 c9</td>
<td>0.18</td>
<td>0.22</td>
<td>0.17</td>
<td>ns</td>
</tr>
<tr>
<td>C14</td>
<td>10.03(^c)</td>
<td>11.04(^b)</td>
<td>13.11(^a)</td>
<td>***</td>
</tr>
<tr>
<td>C14:1 c9</td>
<td>0.30(^a)</td>
<td>0.32(^a)</td>
<td>0.25(^b)</td>
<td>**</td>
</tr>
<tr>
<td>C15</td>
<td>0.47(^a)</td>
<td>0.49(^a)</td>
<td>0.32(^b)</td>
<td>***</td>
</tr>
<tr>
<td>C15:1</td>
<td>1.03(^b)</td>
<td>1.19(^a)</td>
<td>0.86(^c)</td>
<td>***</td>
</tr>
<tr>
<td>C16</td>
<td>26.34(^b)</td>
<td>31.28(^a)</td>
<td>30.95(^a)</td>
<td>***</td>
</tr>
<tr>
<td>C16:1 c9</td>
<td>0.44(^a)</td>
<td>0.40(^a)</td>
<td>0.33(^b)</td>
<td>**</td>
</tr>
<tr>
<td>C17</td>
<td>0.85(^b)</td>
<td>1.07(^a)</td>
<td>1.08(^a)</td>
<td>***</td>
</tr>
<tr>
<td>C17:1 c9</td>
<td>0.77(^a)</td>
<td>0.77(^a)</td>
<td>0.45(^b)</td>
<td>***</td>
</tr>
<tr>
<td>C18</td>
<td>14.77(^a)</td>
<td>9.31(^b)</td>
<td>8.12(^a)</td>
<td>***</td>
</tr>
<tr>
<td>C18:1 c9</td>
<td>21.19(^a)</td>
<td>18.83(^b)</td>
<td>19.86(^ab)</td>
<td>*</td>
</tr>
<tr>
<td>C18:1 t6-11</td>
<td>3.62(^a)</td>
<td>1.91(^b)</td>
<td>1.85(^b)</td>
<td>***</td>
</tr>
<tr>
<td>C18:2 c9c12 (LA)</td>
<td>2.63(^b)</td>
<td>2.93(^a)</td>
<td>2.23(^c)</td>
<td>***</td>
</tr>
<tr>
<td>C20</td>
<td>0.30(^a)</td>
<td>0.29(^a)</td>
<td>0.17(^b)</td>
<td>***</td>
</tr>
<tr>
<td>CLA c9t11 (CLA)</td>
<td>0.95(^a)</td>
<td>0.72(^b)</td>
<td>0.67(^b)</td>
<td>***</td>
</tr>
<tr>
<td>C18:3 c9c12c15 (ALA)</td>
<td>1.16(^a)</td>
<td>0.91(^b)</td>
<td>0.89(^b)</td>
<td>**</td>
</tr>
<tr>
<td>SFA</td>
<td>67.60(^b)</td>
<td>71.61(^a)</td>
<td>72.26(^a)</td>
<td>***</td>
</tr>
<tr>
<td>MUFA</td>
<td>27.63(^a)</td>
<td>23.83(^b)</td>
<td>23.93(^b)</td>
<td>***</td>
</tr>
<tr>
<td>PUFA</td>
<td>4.73(^a)</td>
<td>4.56(^a)</td>
<td>3.78(^b)</td>
<td>***</td>
</tr>
<tr>
<td>SFA / UFA</td>
<td>2.11(^b)</td>
<td>2.60(^a)</td>
<td>2.72(^a)</td>
<td>***</td>
</tr>
<tr>
<td>AI(^§)</td>
<td>2.28(^b)</td>
<td>3.01(^a)</td>
<td>3.52(^a)</td>
<td>***</td>
</tr>
<tr>
<td>TI(^§)</td>
<td>2.72(^b)</td>
<td>3.22(^a)</td>
<td>3.38(^a)</td>
<td>**</td>
</tr>
<tr>
<td>DI(_{14})</td>
<td>0.03(^a)</td>
<td>0.03(^a)</td>
<td>0.02(^b)</td>
<td>***</td>
</tr>
<tr>
<td>HSFA(^\dagger)</td>
<td>40.27(^b)</td>
<td>47.59(^a)</td>
<td>50.90(^a)</td>
<td>***</td>
</tr>
</tbody>
</table>

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

\(\dag\) 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. 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.

‡ Probability: * P≤0.05; ** P≤0.01; *** P≤0.001; ns, not significant (P>0.05). Different letters within rows indicate statistically significant differences between diets.

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

# Calculated as C14:1 C9/C14:0.

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