TAX EXPENDITURES:
DEFINIZIONE E APPLICAZIONE

Changes in goat milk fatty acids during abrupt transition from indoor to pasture diet

Manuela Renna, Carola Lussiana, Paolo Cornale, Riccardo Fortina, Antonio Mimosi

Dipartimento di Scienze Zootecniche, via L. da Vinci 44, 10095 Grugliasco (TO), Italy

Corresponding author: Manuela Renna, Dipartimento di Scienze Zootecniche, via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Telephone: +39 011 6708576. Fax: +39 011 6708563. manuela.renna@unito.it

Email addresses: manuela.renna@unito.it, carola.lussiana@unito.it, paolo.cornale@unito.it, riccardo.fortina@unito.it, antonio.mimosi@unito.it

ABSTRACT

Goal of this study was to evaluate the kinetics of goat milk fatty acids during abrupt transition from indoor to pasture-based diets. Twelve Valdostana goats in mid-lactation reared indoors and fed hay and concentrates for 40 days were abruptly brought outdoors on natural pasture and fed fresh grass *ad libitum*. Feed samples and individual milk samples were collected for fatty acids analysis on the last day of indoor feeding (day 0) and after 1, 2, 3, 4, 6, 9, 13, 18 and 23 days of fresh grass feeding. Milk fatty acid composition was significantly affected by sampling day. Significant changes already took place few days after transition. The most marked and consistent variations occurred at the expense of some unsaturated fatty acids. Total *trans*-octadecenoic and *trans*-octadecadienoic acids, conjugated linoleic acids (CLA) and omega-3 fatty acids constantly increased, reaching concentrations 4.0, 3.0, 3.9, and 2.2 times higher at the end of the trial than at its beginning, respectively. On the last sampling day the omega-6/omega-3 fatty

acids ratio was two times lower than its initial value. Considering individual fatty acids, the most consistent and remarkable increasing trends throughout the trial were observed for C18:1 *t*6-11, C18:1 *t*12-14+*c*6-8, C18:1 *c*14+*t*16, C18:2 *t*11*c*15, C18:2 *c*9*t*13+*t*8*c*12, CLA isomers *c*9*t*11+*t*7*c*9+*t*8*c*10 and *t*11*c*13+*c*9*c*11. Alpha-linolenic and eicosapentaenoic acids also increased significantly, but to a lesser extent. In view of the many beneficial biological effects that have been attributed to vaccenic acid (C18:1 *t*11), rumenic acid (C18:2 *c*9*t*11), and omega-3 fatty acids, results showed that, from a human health perspective, goat milk fatty acid composition consistently improved after transition from indoor to pasture feeding. Such improvements, mainly due to the high content of α-linolenic acid in pasture plants, were already significant after two or three days of fresh grass feeding. Further increases of beneficial fatty acids in milk fat were observed till about thirteen (vaccenic acid and CLA) or twenty-three (omega-3 fatty acids) days after transition. These results show that pasture can be considered a natural feeding strategy to quickly enhance the healthfulness of goat milk fat.

Keywords: goat milk, fatty acids, transition, grazing

INTRODUCTION

The positive effects of fresh grass-based diets on the fatty acid (FA) profile and the nutritional quality of dairy fat have been recognized broadly (Morand-Fehr et al., 2007). Such improvement is related to both increased milk concentrations of polyunsaturated FA (e.g., omega-3 FA and conjugated linoleic acid) which are known to exert many putative beneficial effects for human health (Barcelo-Coblijn and Murphy, 2009; Benjamin and Spener, 2009) and decreased concentrations of saturated FA (particularly lauric, myristic, and palmitic acids) able to raise risk factors for cardiovascular diseases (Ohlsson, 2010). Modifications and persistency of ruminant milk FA concentrations determined by the

53 transition from indoor to fresh grass feeding have been investigated in a limited number of
54 studies in cows and sheep only. Kuzdzal-Savoie and Kuzdzal (1961) first reported a
55 notable and fast increase in the amount of conjugated dienes and α-linolenic acid (C18:3
56 *c*9*c*12*c*15, ALA) in butter from dairy cows as an effect of either sudden or gradual change
57 from a winter ration to a pasture-based diet. Similarly, Decaen and Ghadaki (1970)
58 observed that a gradual change from a winter diet consisting of hay, silage, and
59 concentrate to a zero-grazing system induced fast modifications in the FA composition of
60 cow milk fat. Particularly, they observed a decrease in the proportion of short- (SCFA)
61 and medium-chain (MCFA) fatty acids and contemporarily an increase in long-chain fatty
62 acids (LCFA). More recently, Khanal et al. (2008) reported stearic acid (C18:0), oleic acid
63 (C18:1 *c*9), vaccenic acid (C18:1 *t*11, VA), conjugated linoleic acid (CLA) and ALA to
64 increase, and most of the SCFA and MCFA to decrease, during abrupt transition of dairy
65 cows from a total mixed ration to a full-grazing diet. In their study, the majority of FA
66 changed daily before stabilizing around 22-23 days after transition. Coppa et al. (2011)
67 also investigated the kinetics of milk FA in dairy cows during rapid or progressive
68 transition from hay- to alpine pasture-based diets. These authors showed that many FA
69 (almost all saturated ones, linoleic acid – C18:2 *c*9*c*12, LA - and ALA) became stable
70 after five days in both transitions, while both VA and the most abundant among CLA
71 isomers (C18:2 *c*9*t*11, rumenic acid, RA) reached maximum and stable concentrations
72 about two weeks after the maximum fresh herbage intake.
73 In sheep, Biondi et al. (2008) reported that the major changes in milk FA occurred during
74 the first three days following an abrupt transition from indoor to pasture diet, being
75 predominantly attributable to unsaturated fatty acids (UFA). Particularly, they observed
76 significant increases of VA, RA, and ALA, as well as a notable decrease in the
77 concentration of LA and in the omega-6/omega-3 fatty acids ratio.
78 To the best of our knowledge, no information is currently available on the rate of change

and persistency in goat milk FA during transition from indoor to fresh grass feeding. The objective of this work was, therefore, to examine the kinetics of responses of goat milk FA during abrupt change from a winter diet based on hay and concentrates to a full-grazing diet on natural pasture.

MATERIALS AND METHODS

Animals feeding and management

The experiment was carried out in a farm located in North-Western Italy (latitude: 45°02'51'' N; longitude: 07°19'10'' E; altitude: 643 m a.s.l.) and breeding a flock of 40 Valdostana goats. The experimental period covered a total of 24 days, from March 29 to April 21, 2011.

Twelve goats were used in the experiment (days in milk at the beginning of the trial: 126±6; number of lactation: 2.2±0.4), with an average body condition score of 3.0±0.5 (Hervieu and Morand-Fehr, 1999). The goats were maintained indoor for 40 days (February 18 to March 29, pre-experimental period and first day of experimental period) when they were fed exclusively with 1.5 kg head^{-1} day^{-1} of hay (first cut) and 0.4 kg head^{-1} day^{-1} of commercial concentrate containing maize and wheat bran. Means and standard deviations of milk yield and milk fat, protein and lactose percentages at the beginning of the pre-experimental period were equal to 1.17±0.306 kg head^{-1} day^{-1}, 42.2±0.52 g kg^{-1}, 31.3±0.22 g kg^{-1} and 43.4±0.26 g kg^{-1}, respectively. From midday of March 30 (day 1) the goats were abruptly brought outdoor and exclusively fed with fresh grass from natural pasture (main species: *Lolium perenne* L., *Trifolium pratense* L., and *Poa* spp.) *ad libitum*. The pasture area was flat and adjacent to the farm. The goats were manually milked indoors twice a day (at 6.00 h and 18.00 h). They were allowed to graze during the milking interval while they were maintained indoors during the night. The goats had free access to water and mineralized salt blocks during both pasture and indoor housing.

105 **Sampling procedure and laboratory analyses of milk**

106 Individual milk yield was recorded on a daily basis; individual milk samples were
107 collected at the afternoon milkings ten times during the trial: on the last day of indoor
108 feeding (day 0) and on days 1, 2, 3, 4, 6, 9, 13, 18 and 23 of pasture feeding. Two aliquots
109 of each sample were taken for laboratory analysis. One aliquot (50 mL) was immediately
110 stored at 4°C with a preservative and subsequently transported to the laboratory for the
111 analysis of fat, protein, and lactose (MilkoScan FT 6000, Foss Electric, Hillerød,
112 Denmark). The second aliquot (150 mL) was frozen at –20°C and successively analyzed
113 for FA composition as previously reported by Renna et al. (2012). Peaks were identified
114 by injecting pure FAME standards (Sigma-Aldrich, Milano, Italy; Matreya Inc., Pleasant
115 Gap, PA, USA and Restek Corporation, Bellefonte, PA, USA) and by comparison with the
116 chromatogram published by Collomb and Bühler (2000). Quantification was assessed by
117 using nonanoic acid as internal standard. The results are expressed as absolute values as g
118 kg^{-1} fat.

119 **Sampling procedure and laboratory analyses of feed**

120 The hay and concentrate fed to the goats during the indoor period were collected on day 0.
121 For herbage sampling, the pasture area was subdivided in plots of 200 m^2 each. A sub-area
122 of 200 cm^2 was considered for sampling at every intersection among plots. Pasture
123 samples were collected following the same time schedule (days 1, 2, 3, 4, 6, 9, 13, 18, and
124 23) as for the collection of milk samples. The grazing behavior of the goats was observed
125 at each sampling date. After closely observation, hand-plucked forage samples, simulating
126 plants parts consumed by the goats, were collected from pasture. Two aliquots of each
127 herbage sample were transported to the laboratory in a portable refrigerator at 4°C and
128 then frozen at -80°C. Before the chemical analysis, the first aliquot of each herbage
129 sample was dried at 40°C for 24 h; hay, concentrate and dried herbage samples were then
130 ground with a cutting mill to pass a 1-mm screen sieve (Pulverisette 15 - Fritsch GmbH,

Idar-Oberstein, Germany). Samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF) according to AOAC procedures (2000). The second aliquot of herbage samples was freeze-dried (Edwards MF 1000, Milano, Italy) and ground. This aliquot as well as both grounded hay and concentrate were used for the assessment of the FA composition as reported by Alves et al. (2008). FAME were separated and quantified by using the same analytical instruments and temperature program described for the analysis of milk samples. The injection volume was 0.5 µL. Peaks were identified by injecting pure FAME standards (Restek Corporation, Bellefonte, PA, USA) and by comparison with the chromatogram published by Alves et al. (2008). Quantification was assessed by using heptadecanoic acid as internal standard. The results are expressed as absolute values as mg $100g^{-1}$ DM.

Statistical analysis

The goat was considered as the experimental unit. Changes in milk yield, main constituents and FA were analyzed using the PROC MIXED procedure of SAS (2006) according to the following model:

$$Y_{ijk} = \mu + D_i + G_j + \varepsilon_{ijk},$$

where Y_{ijk} = mean of response variable, μ = population mean, D_i = effect of day, G_j = random effect of goat, and ε_{ijk} = experimental error. Pairwise multiple comparisons were assessed by using the PDIFF option in SAS. Significance was declared at $P \leq 0.05$.

RESULTS

Characteristics of the feedstuffs

The chemical and FA compositions of hay, commercial concentrate, and fresh grass are presented in Table 1.

In fresh grass samples, DM and fiber contents increased while CP decreased from day 1 to day 23, following the advance of plants' age.

Hay, concentrate and fresh grass strongly differed in their FA amounts and compositions. The concentrate showed 1.8-fold and 4.0-fold higher total FA amounts when compared to fresh grass and hay, respectively. On average, the fresh grass had a total FA concentration approximately doubled than that detected in hay. Alpha-linolenic acid was the most abundant FA in fresh grass, accounting on average for the 62% of total FA. Palmitic and linoleic acids showed similar concentrations, accounting for the 16 and 14% of total FA, respectively. Alpha-linolenic, palmitic and linoleic acids were also the most representative FA of hay, together accounting for about the 83% of total FA. However, the relative contribution of ALA was lower (33% of total FA) in hay if compared to fresh grass. Linoleic acid was, instead, the prevailing FA in the concentrate, accounting for about the 57% of total FA. It was followed by oleic and palmitic acids, both representing about the 18-19% of total detected FA. ALA represented only about the 2% of total FA in the concentrate. The total FA content and both concentration and proportion of ALA decreased from the beginning till the end of the trial (data not shown).

Milk yield and gross composition

Milk yield and gross composition were significantly affected by sampling day ($P \leq 0.001$; Table 2). Milk yield maintained similar values as that observed the last day of stall feeding till day 9 (showing the absolute minimum levels on days 2 and 3 after transition), and then slightly increased reaching the absolute highest value on day 23. Fat production started to increase significantly the second day after transition and continued to increase till the end of the experiment. The fat percentage of milk significantly increased the second day after transition as well, but no significant variations were observed for this parameter in the following sampling days. Protein percentage, protein production and lactose production tended to increase as well, while no clear increasing or decreasing trend was observed for

182 the percentage of lactose in milk.

183 **Milk fatty acid composition**

184 Results of groups of FA and individual FA are reported in Tables 3 and 4, respectively.
185 The FA profile of milk fat was affected by days on pasture to a great extent. Only two FA
186 (C18:1 *t*5 and C18:2 *t*10*c*12) were not significantly influenced by sampling day.
187 Total SCFA and MCFA started to decrease significantly the second day after transition
188 (day 2) and they continued to decrease till day 4 (1.2 and 1.5 times lower values on day 4
189 relative to values on day 0, respectively). Subsequently SCFA rose again, reaching at the
190 end of the trial concentrations that did not significantly differ from the concentration
191 observed the last day of stall feeding. A similar trend also occurred for MCFA, but their
192 concentration was still significantly lower at the end of the trial than at its beginning.
193 Conversely, LCFA rapidly and markedly increased the second day after diet change and
194 then significantly declined from day 6 till the end of the trial. Starting from day 9 milk
195 LCFA concentrations were not statistically different from the value recorded on day 0.
196 Total saturated fatty acids (SFA) underwent a conspicuous drop from day 1 to day 4
197 subsequent to transition. From day 6 SFA increased again, but their levels remained
198 generally lower if compared to day 0. Total branched-chain fatty acids (BCFA) declined
199 as well from day 1 to day 4. From day 4 to day 9 they remained quite constant and finally
200 slightly increased, so that in the period from day 13 to day 23 their levels did not
201 statistically differ from the value observed on day 0.
202 Temporal changes in the concentration of total monounsaturated fatty acids (MUFA)
203 showed a sharp increase from day 1 to days 2-3. MUFA levels then significantly declined
204 till the end of the trial when they reached their absolute minimum levels. The trend
205 observed for total polyunsaturated fatty acids (PUFA) was much clearer, as this group of
206 FA continuously rose until the sixth day after transition, thereafter remaining constant.
207 PUFA were 1.7 times higher on day 23 than on day 0.

208 On day 3 total *trans*-octadecenoic (Σ C18:1 *trans*) and *trans*-octadecadienoic (Σ C18:2
209 *trans*) acids showed values already 1.8 and 1.6 times higher than those observed on day 0,
210 respectively. These groups of FA continued to increase markedly till the end of the
211 experiment. The highest values (about four and three times higher than the values
212 recorded the last day of stall feeding, respectively) were observed the last two (Σ C18:1
213 *trans*) or three (Σ C18:2 *trans*) sampling days.
214 Under the chromatographic conditions applied in this trial, the most abundant among
215 *trans*-octadecenoic acids in milk fat (vaccenic acid - C18:1 *t*11) coeluted with other C18:1
216 *trans*-isomers (C18:1 *t*6-10). This sum (C18:1 *t*6-11) as well as the values recorded for
217 other detected *trans*-octadecenoic isomers, particularly C18:1 *t*12-14 (which coeluted with
218 C18:1 *c*6-8 isomers) and C18:1 *t*16 (which coeluted with C18:1 *c*14), started to increase
219 significantly three days after turning out to pasture. C18:1 *t*6-11 isomers continued to
220 increase till day 13 and then maintained constant values (approximately four times higher
221 than the value observed on day 0). C18:1 *t*12-14+*c*6-8 and C18:1 *c*14+*t*16 isomers reached
222 their absolute highest concentrations on days 18 and 23, with values 2.8 and 2.1 times
223 higher than those recorded the last day of indoor feeding, respectively.
224 Considering individual CLA isomers, in the applied chromatographic conditions C18:2
225 *c*9*t*11, C18:2 *t*7*c*9 and C18:2 *t*8*c*10 coeluted in a single peak in the chromatogram. Their
226 sum represented on average the 97% of total CLA. They started to increase the third day
227 after transition and continued to increase significantly until day 13, thereafter maintaining
228 constant concentrations. The raise was conspicuous: values observed the last three
229 sampling days were up to 3.6 times higher than the value observed on day 0. The sum of
230 these three CLA isomers showed high individual variability among the goats involved in
231 the trial. In fact, it varied between 3.33 and 7.77 g kg^{-1} fat at the beginning of the trial (day
232 0) and between 11.79 and 22.96 g kg^{-1} fat the last sampling day. About two-fold variation
233 was constantly maintained among individual goats all along the experiment. However, the

ranking of individual goats for CLA content was not stable throughout the trial. The sums CLA *c*9*t*11+*t*7*c*9+*t*8*c*10 and C18:1 *t*6-11 were strongly correlated each other (r=0.97; P≤0.001).

CLA isomers *t*11*c*13 and *c*9*c*11 coeluted in the chromatogram. The third day after transition from the winter diet to full grazing their sum was already about nine times higher than the initial value. Their sum continued to increase until day 13 when it reached the highest absolute concentration. The contribution of these isomers to the total CLA content of milk varied from 0.42% on day 0 to 2.7% on day 23. Sampling day significantly affected the concentration of CLA isomer *t*9*t*11 as well. Its lowest value was observed the last day of stall feeding. From day 1 to day 23 its concentration remained quite constant, with the exception of days 13 and 18 when the highest values were reached (about 3 times higher than the value observed on day 0).

Linoleic acid significantly increased until the third day after transition to pasture feeding. Then it decreased, reaching concentrations up to 1.5 fold lower than those observed on day 0.

Total omega-3 FA as well as ALA (which is the most abundant detected FA in this group) significantly and constantly increased from the last day of stall feeding to the last sampling day. At the end of the trial total omega-3 FA and ALA concentrations were about two-fold higher than values recorded on day 0. Considering other omega-3 FA, a clear increasing trend was also observed for C18:2 *t*11*c*15. A first significant raise (approximately doubled values with respect to concentrations detected the last day of stall feeding) was observed the third day after variation of the diet. Then it continued to rise constantly till the last sampling day, doubling again its concentrations so that on day 23 it was about four times higher than on day 0. A significant and positive correlation was found between this octadecadienoic acid and the sum of CLA isomers *t*11*c*13 and *c*9*c*11 (r=0.75; P≤0.001). Among long-chain omega-3 FA, eicosapentaenoic acid (C20:5

260 *c*5*c*8*c*11*c*14*c*17, EPA) followed a similar trend as that observed for ALA and C18:2
261 *t*11*c*15. However, a significant increase was detected only after thirteen days after
262 transition. Its highest concentrations were observed the last two sampling days (with
263 values that almost doubled with respect to those observed at the beginning of the trial).
264 Conversely, no clear positive trend was observed for docosapentaenoic acid (C22:5
265 *c*7*c*10*c*13*c*16*c*19, DPA).
266 The omega-6/omega-3 FA ratio started to decline the third day after switching from stall
267 to pasture feeding. This ratio continued to decrease significantly throughout the trial up to
268 day 13. The lowest absolute values were observed the last sampling day, when it was two
269 times lower than its initial value.
270 Concerning Δ9-desaturase activity (estimated as the ratios of C16:1 *c*9 to C16:0 – DI_{16} –
271 and C18:1 *c*9 to C18:0 – DI_{18}), a significant increase was observed until day 4 after
272 transition ($P \leq 0.001$). However, from day 6 these ratios significantly decreased again
273 reaching the absolute lowest values at the end of the trial.
274
275 **DISCUSSION**
276 **Milk yield and gross composition**
277 The observed slight and progressive raise in milk yield and protein after transition from
278 indoor to pasture feeding could be related to increased ingested energy as reported to
279 occur in case of turning out, particularly when fresh grass is at an early growth stage.
280 Similarly, an early phenological phase of pasture plants resulted in high milk fat content
281 due to the increased intake of highly digestible fiber (Morand-Fehr et al., 2007). Besides
282 the vegetative stage of pasture plants, the diet fed during the indoor period can
283 significantly affect milk main constituents after turning out to pasture. If compared to
284 maize silage, both hay or grass silage used as winter rations were found to determine an
285 increase in milk fat content (Hoden et al., 1985). Such findings seem to be confirmed in

286 the current trial as milk fat significantly increased after diet change from a hay-concentrate
287 based diet to a pasture based diet with fresh grass at early vegetative stage.

288 **Milk fatty acid composition**

289 The main changes in goat milk FA during lactation have been shown to occur in early
290 lactation and have been mainly attributed to lipid mobilization as the consequence of a
291 negative energy balance phase for the animals. A relatively stable FA pattern is instead
292 generally observed in mid and late lactation (Ataşoğlu et al., 2009; Chilliard et al., 2003).
293 For this reason, a confounding effect due to lactation stage on milk FA can be reasonably
294 excluded in the current study and the observed variations are likely to be attributable to
295 feeding aspects only.

296 Switching from a winter indoor diet (on average 70% hay and 30% concentrate) to a full
297 grazing diet resulted in a higher availability of FA (particularly polyunsaturated) to be
298 used for the synthesis of milk fat. It is known that the haymaking process notably reduces
299 the total FA and the ALA concentrations in forages (Kalač and Samková, 2010).
300 Consequently the availability of FA, and above all of ALA, increased switching the goats
301 from a prevalent hay-based diet to a fresh grass-based diet. An increased supply of LCFA
302 (especially with a high level of unsaturation) and some of their biohydrogenation
303 intermediate products (e.g., C18:1 *trans* and C18:2 *trans* isomers) have been shown to
304 inhibit the *de novo* synthesis of C8:0 to C16:0 FA within the bovine mammary gland, by
305 exerting direct and/or indirect effects on the lipogenic enzymes acetyl-CoA carboxilase
306 (ACC) and fatty acid synthase (FAS) and by reducing acetate and 3-hydroxibutyrate
307 bioavailability for mammary lipogenesis (Chilliard and Ferlay, 2004). Nonetheless, milk
308 fat synthesis and FA responses to dietary PUFA supplies are known to vary considerably
309 among ruminant species (Chilliard et al., 2007). Mammary ACC and FAS mRNA
310 abundance and/or activity have been reported to be much less affected by dietary PUFA in
311 dairy goats than cows (Bernard et al., 2009). In the current study, the results we obtained

312 concerning SCFA and MCFA suggest a valuable inhibition of *de novo* synthesis in the
313 goat mammary gland by switching from an indoor to a grazing diet. Such inhibitory effect
314 was evident just the day after the diet change and was quite remarkable as these FA
315 decreased respectively by about 20 and 30% from day 0 to day 4. Since caproic and
316 caprylic acids are partly synthesized by metabolic pathways that are independent of ACC
317 (Chilliard and Ferlay, 2004), our results seem to confirm the existence of factors other
318 than altered mammary gene expression or lipogenic enzymes (particularly ACC) activities
319 able to exert an inhibitory effect of *de novo* synthesis of SCFA and MCFA in dairy goats.
320 The concentrations of the above-mentioned FA (particularly caproic, caprylic, and capric
321 acids) did not stabilize but significantly increased again beginning from day 6. Such
322 results suggest a possible temporal adaptation in mammary metabolism, which has already
323 been observed to occur in dairy cows even if at slower rate (Ferlay et al., 2006). In
324 addition, the observed subsequent increase in *de novo* synthesized FA could be also
325 related to changes in the physical characteristics of the sward grazed by the goats due to
326 maturation of plants with consequent notable changes in their FA concentrations and
327 proportions (particularly reductions in the total FA concentrations and in the proportion
328 and concentration of ALA) (Boufaïed et al., 2003; Cabiddu et al., 2009). Similar
329 proportions in the reduction of total FA and ALA in different grassland species with the
330 advance of the phenological phase (in a comparable period of time) have been also
331 reported by Wyss and Collomb (2010). Further investigations will be needed to better
332 elucidate both the mechanisms involved in the inhibition of *de novo* synthesis of SCFA
333 and MCFA and the persistency of such an effect in lactating goats.
334 Concerning LCFA, the trend observed for stearic acid could be ascribed to a decreasing
335 UFA content in pasture plants during the grazing period. The decrease in oleic acid seems
336 to be at least partly related to the lower availability of stearic acid. In fact, it is known that
337 more than the 50% of oleic acid is formed within the mammary gland by the activity of

338 Δ9-destaurase on stearic acid. Moreover, the decreased Δ9-desaturase activity (estimated
339 by DI_{18} and probably due to a higher availability of *trans* FA (Chilliard et al., 2007)) also
340 contributed to the decrease in the concentration of oleic acid in milk.
341 As previously reported for dairy ewes (Biondi et al., 2008), the most remarkable and
342 consistent variations in goat milk FA concentrations after transition from a winter indoor
343 to a pasture diet occurred at the expense of some long chain unsaturated FA and have to be
344 mainly related to a different FA supply from the ingested feeds. The observed increasing
345 concentrations of all *trans*-octadecenoic isomers (with the exception of C18:1 *t*5), of many
346 non-conjugated *trans*-octadecadienoic isomers (particularly C18:2 *t*11*c*15, C18:2
347 *c*9*t*13+*t*8*c*12, C18:2 *c*9*t*12, and C18:2 *tt*NMID+*t*9*t*12) and CLA isomers
348 *c*9*t*11+*t*7*c*9+*t*8*c*10, *t*11*c*13+*c*9*c*11, and *t*9*t*11 after the diet change are the consequence of
349 the high content of ALA in pasture plants. In fact, ALA is well known to undergo within
350 the rumen an intense and complex biohydrogenation process carried out by the anaerobic
351 microbial flora. Many octadecatrienoic isomers (C18:3 *c*9*t*11*c*15 and C18:3 *c*9*t*13*c*15
352 among others) have been shown to be formed during the initial step of ruminal
353 biohydrogenation of ALA. These trienes are subsequently hydrogenated to a multitude of
354 non conjugated and conjugated dienes, which are in turn mainly hydrogenated to
355 monoenoic FA. C18:2 *t*11*c*15 is thought to be the major non conjugated octadecadienoic
356 isomer deriving from the biohydrogenation of ALA (hydrogenation of C18:3 *c*9*t*11*c*15).
357 Other non conjugated C18:2 *trans*-isomers are expected to be formed similarly. For
358 example, the C18:2 *c*9*t*13 isomer probably derives from reduction of the *c*15 double bond
359 in C18:3 *c*9*t*13*c*15 (Lee and Jenkins, 2011). The Δ9,12 C18:2 isomers are thought to be
360 mainly formed during the biohydrogenation of LA, even if a putative metabolic pathway
361 for their formation from ALA has been also suggested (Chilliard et al., 2007). In the
362 current trial, the observed increased concentrations of C18:2 *tt*NMID+*t*9*t*12 and C18:2
363 *c*9*t*12 after diet change suggest their partly formation from dietary ALA. On the contrary,

since C18:2 *t*9*c*12 tended to decrease after turning out to pasture, we could hypothesize that ALA biohydrogenation would not be an important metabolic way for the ruminal formation of this diene.

Concerning conjugated linoleic isomers, the increase in the sum of C18:2 *c*9*t*11+*t*7*c*9+*t*8*c*10 after turning out to pasture is presumably attributable to rumenic acid, the main CLA isomer in ruminant milk fat. In fact, the other two isomers (*t*7*c*9 and *t*8*c*10) were previously reported to correlate positively and significantly with dietary oleic and linoleic acids, respectively, while no significant correlation was found with dietary ALA (Collomb et al., 2004). The increase in rumenic acid is due to similar increased concentrations of C18:1 *t*6-11, among which the precursor (C18:1 *t*11) for CLA *c*9*t*11 *de novo* synthesis within the mammary gland belongs. C18:1 *t*11 is an intermediate of the biohydrogenation processes and is formed from both dietary LA and ALA. The higher supply of ALA from pasture if compared to the indoor diet suggests a higher formation of vaccenic acid in the rumen as well as a higher absorption into the bloodstream and consequently a higher availability for desaturation mediated by the Δ9-desaturase enzyme within the mammary gland.

Referring to minor CLA isomers, as only very low amounts of CLA *c*9*c*11 are usually present in dairy fat (Ferlay et al., 2008), the sum CLA *t*11*c*13+*c*9*c*11 can be almost completely attributed to the *t*11*c*13 isomer. Kraft et al. (2003) first hypothesized that CLA *t*11*c*13 could be formed within the rumen at the third step of the biohydrogenation of dietary ALA, by means of an isomerization at the expense of C18:2 *t*11*c*15. Our results corroborate the hypothesis by Kraft et al. (2003) since both C18:2 *t*11*c*15 and CLA *t*11*c*13 significantly and rapidly increased after transition and they were significantly and positively correlated each other. However, the rapid increase we observed in the sum of CLA isomers *t*11*c*13 and *c*9*c*11 could be attributed to the latter isomer as well. In fact, more than 50% of CLA *c*9*c*11 has also been recently reported to derive directly from the

biohydrogenation of dietary ALA (Lee and Jenkins, 2011). The same authors also reported that CLA *t*9*t*11 partly derives from ALA, which could explain the observed increasing trend of this CLA isomer in goat milk fat after transition from indoor to pasture diet.

As usually occurs with high-forage diets, CLA *t*10*c*12 was detected only in traces (concentrations ≤0.01 g kg^{-1} fat) and was not significantly affected by the feeding change.

Besides vaccenic acid, other *trans*-octadecenoic fatty acids are known to be formed by means of various isomerizations occurring during different steps of the biohydrogenation of dietary UFA. In particular, the observed increase in the concentration of C18:1 *t*12-14+*c*6-8 could be attributed to C18:1 *t*13 and *t*14 isomers, which were found to be formed during the biohydrogenation of both C18:2 *c*9*t*13 and CLA Δ11,13 (Chilliard et al., 2007).

The increased concentrations of ALA and EPA (the latter formed by means of ALA desaturation and elongation processes (Barcelo-Coblijn and Murphy, 2009)) in milk fat have to be related as well to the higher ALA supply from fresh grass. Their concentrations in milk remained however quite low since the extent of the raise was less pronounced if compared to those observed for C18:2 *t*11*c*15, C18:2 *t*11*c*13, C18:2 *tt*NMID+*t*9*t*12, C18:2 *c*9*t*13+*t*8*c*12, C18:1 *t*6-11, and C18:2 *c*9*t*11+*t*7*c*9+*t*8*c*10, probably because the disappearance of ALA in the rumen is very high (usually >90% in case of high-forage diets) while only a little part of ALA is absorbed intact in the gut and secreted into milk (Chilliard and Ferlay, 2004). The observed consistent increasing concentrations of ALA despite the lower ALA supply due to the advance of the phenological phase of the grazed pasture plants suggests that the rate of disappearance of ALA probably decreased during the trial.

Overall, the observed kinetics of responses of goat milk fatty acids to transition from indoor to pasture feeding were more similar to changes already observed in dairy ewes (Biondi et al., 2008) rather than dairy cows (Coppa et al., 2011; Khanal et al., 2008) under comparable feeding conditions.

CONCLUSION

A sudden transition of dairy goats from winter indoor to fresh grass feeding significantly affected the concentrations of FA in milk already two or three days after the diet change. Short- and medium-chain FA rapidly decreased by about 17% and 33% until day 4 after transition, suggesting that fresh grass feeding inhibited their *de novo* synthesis within the mammary gland. An adaptation to the new dietary conditions is hypothesized since from day 6 these FA significantly increased again. The higher availability of α-linolenic acid from pasture plants determined a notable increase in milk concentrations of its ruminal biohydrogenation intermediates, particularly conjugated (*t*11*c*13+*c*9*c*11, *t*9*t*11) and non-conjugated (*t*11*c*15, *c*9*t*13+*t*8*c*12, *c*9*t*12, *tt*NMID+*t*9*t*12) *trans*-octadecadienoic acids, and *trans*-octadecenoic acids (*t*6-11, *t*12-14+*c*6-8, *c*14+*t*16). The sum of CLA isomers *c*9*t*11+*t*7*c*9+*t*8*c*10 also markedly increased (up to 261% at day 13), due to the higher absorption and availability of C18:1 *trans* isomers in the mammary gland as substrates for Δ9-desaturase activity. Omega-3 FA (particularly α-linolenic and eicosapentaenoic acids) increased to a lesser extent (up to 93% and 85% at days 23 and 18, respectively), probably because of the high rate of disappearance of dietary ALA in the rumen. The increase in milk concentration of FA considered beneficial for human health went on till about thirteen (vaccenic and rumenic acids) or twenty-three (omega-3 fatty acids) days after transition.

ACKNOWLEDGMENTS

This research was supported by MIUR grants (ex 60%). The authors gratefully acknowledge their colleague Vanda Malfatto, Dr. Alessandro Moschietto and the farmers for their careful technical assistance.

REFERENCES

Alves, S.P., Cabrita, A.R.J., Fonseca, A.J.M., Bessa, R.J.B., 2008. Improved method for fatty acid analysis in herbage based on direct transesterification followed by solid-phase extraction. J. Chromatogr. A 1209, 212–219.

Association of Official Analytical Chemists (AOAC), 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Ataşoğlu, C., Uysal-Pala, Ç., Karagül-Yüceer, Y., 2009. Changes in milk fatty acid composition of goats during lactation in a semi-intensive production system. Arch. Anim. Breed. 52(6), 627–636.

Barcelo-Coblijn, G., Murphy, E.J., 2009. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. Prog. Lipid Res. 48, 355–374.

Benjamin, S., Spener, F., 2009. Conjugated linoleic acids as functional food: an insight into their health benefits. Nutr. Metab. 6, 36 - doi:10.1186/1743-7075-6-36.

Bernard, L., Leroux, C., Faulconnier, Y., Durand, D., Shingfield, K.J., Chilliard, Y., 2009. Effect of sunflower-seed oil or linseed oil on milk fatty acid secretion and lipogenic gene expression in goats fed hay-based diets. J. Dairy Res. 76, 241–248.

Biondi, L., Valvo, M.A., Di Gloria, M., Scinadro Tenghi, E., Galofaro, V., Priolo, A., 2008. Changes in ewe milk fatty acids following turning out to pasture. Small Ruminant Res. 75, 17–23.

Boufaïed, H., Chouinard, P.Y., Tremblay, G.F., Petit, H.V., Michaud, R., Bélanger, G., 2003. Fatty acids in forages. I. Factors affecting concentrations. Can. J. Anim. Sci. 83, 501–511.

Cabiddu, A., Decandia, M., Salis, L., Scanu, G., Fiori, M., Addis, M., Sitzia, M., Molle, G., 2009. Effect of species, cultivar and phenological stage of different forage legumes on herbage fatty acid composition. Ital. J. Anim. Sci. 8(Suppl. 2), 277–279.

468 Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., Doreau, M., 2007. Diet, rumen
469 biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci.
470 Tech. 109, 828–855.
471 Chilliard, Y., Ferlay, A., Rouel, J., Lamberet, G., 2003. A review of nutritional and
472 physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86,
473 1751–1770.
474 Chilliard, Y., Ferlay, A., 2004. Dietary lipids and forages interactions on cow and goat
475 milk fatty acid composition and sensory properties. Reprod. Nutr. Dev. 44, 467–492.
476 Collomb, M., Bühler, T., 2000. Analyse de la composition en acides gras de la graisse de
477 lait. Optimisation et validation d'une méthode générale à haute résolution. Travaux de
478 chimie alimentaire et d'hygiène 91, 306–332.
479 Collomb, M., Sieber, R., Bütikofer, U., 2004. CLA isomers in milk fat from cows fed diets
480 with high levels of unsaturated fatty acids. Lipids 39, 355–364.
481 Coppa, M., Gorlier, A., Lonati, M., Lombardi, G., 2011. Changes in cow milk fatty acids
482 during transition from hay- to pasture-based diets. In: Proceedings of the 10th
483 International Meeting on Mountain Cheese, Dronero (CN), Italy, September 14-15, pp.
484 57-58.
485 Decaen, C., Ghadaki, M.B., 1970. Variation de la sécrétion des acides gras des matières
486 grasses du lait de vache à la mise à l'herbe et au cours des six premières semaines
487 d'exploitation du fourrage vert. Ann. Zootech. 19, 399–411.
488 Ferlay, A., Agabriel, C., Sibra, C., Journal, C., Martin, B., Chilliard, Y., 2008. Tanker
489 milk variability in fatty acids according to farm feeding and husbandry practices in a
490 French semi-mountain area. Dairy Sci. Technol. 88, 193–215.
491 Ferlay, A., Martin, B., Pradel, Ph., Coulon, J.B., Chilliard, Y., 2006. Influence of grass-
492 based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and
493 Montbéliarde cow breeds. J. Dairy Sci. 89, 4026–4041.

494 Hervieu, J., Morand-Fehr, P., 1999. Comment noter l'état corporel des chèvres. Réussir La
495 Chèvre 231, 26–32 (in French).
496 Hoden, A., Coulon, J.B., Dulphy, J.P., 1985. Influence de l'alimentation sur la
497 composition du lait. 3. Effet des régimes alimentaires sur les taux butyreux et protéique
498 (Influence of feeding on milk composition. 3. Effect of diets on milk fat and protein
499 contents). Bull. Techn. CRZV. Theix INRA, No. 62, pp. 69–79.
500 Kalač, P., Samková, E., 2010. The effects of feeding various forages on fatty acid
501 composition of bovine milk fat: A review. Czech J. Anim. Sci. 55, 521–537.
502 Khanal, R.C., Dhiman, T.R., Boman, R.L., 2008. Changes in fatty acid composition of
503 milk from lactating dairy cows during transition to and from pasture. Livest. Sci. 114,
504 164–175.
505 Kraft, J., Collomb, M., Möckel, P., Sieber, R., Jahreis, G., 2003. Differences in CLA
506 isomer distribution of cow's milk lipids. Lipids 38, 657–664.
507 Kuzdzal-Savoie, S., Kuzdzal, W., 1961. Influence de la mise à l'herbe des vaches laitières
508 sur les indices de la matière grasse du beurre et sur les teneurs en différents acides gras
509 poly-insaturés. Lait 41, 369–398.
510 Lee, Y.-J., Jenkins, T.C., 2011. Biohydrogenation of linolenic acid to stearic acid by the
511 rumen microbial population yields multiple intermediate conjugated diene isomers. J.
512 Nutr. 141, 1445–1450.
513 Morand-Fehr, P., Fedele, V., Decandia, M., Le Frileux, Y., 2007. Influence of farming and
514 feeding systems on composition and quality of goat and sheep milk. Small Ruminant
515 Res. 68, 20–34.
516 Ohlsson, L., 2010. Dairy products and plasma cholesterol levels. Food & Nutrition
517 Research 54, 5124 - doi: 10.3402/fnr.v54i0.5124.
518 Renna, M., Cornale, P., Lussiana, C., Malfatto, V., Fortina, R., Mimosi, A., Battaglini
519 L.M., 2012. Use of *Pisum sativum* (L.) as alternative protein resource in diets for dairy

sheep: effects on milk yield, gross composition and fatty acid profile. Small Ruminant Res 102, 142–150.

SAS, 2006. SAS User's Guide: Statistics, Version 9.1.3. SAS Institute Inc., Cary, NC, USA.

Wyss, U., Collomb, M., 2010. Fatty acid composition of different grassland species. Grassland Science in Europe 15, 631–633.

Table 1

Table 1. Chemical composition (g kg^{-1} DM, unless otherwise stated) and fatty acid profile (mg $100g^{-1}$ DM) of the feed consumed by the goats.[a]

	Indoor feeding		Pasture[c]
	Hay	Concentrate[b]	
Main nutrients			
DM (g kg^{-1})	891	886	198 ± 11.2
Ash	78	31	90 ± 9.5
CP	103	136	152 ± 25.6
EE	35	39	26 ± 2.8
NDF	568	202	458 ± 48.8
ADF	322	68	229 ± 16.9
ADL	56	17	41 ± 0.5
NE_L (MJ kg DM^{-1})	4.8	7.7	5.8
Fatty acids			
C12	7.9	n.d.	1.2 ± 0.48
C14	22.6	6.4	7.7 ± 1.18
C15	n.d.	n.d.	2.6 ± 0.26
C16	364.5	809.2	387.2 ± 29.03
C16:1 *t*3	33.1	4.9	57.4 ± 12.64
C16:1 *c*9	3.3	8.9	2.5 ± 1.18
C18	49.6	56.3	32.9 ± 2.86
C18:1 *c*9	70.5	823.3	46.4 ± 8.81
C18:1 *c*11	2.9	41.2	6.2 ± 1.37
C19	n.d.	n.d.	1.2 ± 0.31
C18:2 *c*9*c*12 (LA)	188.4	2489.1	344.0 ± 65.49
C20	2.4	10.1	12.6 ± 1.59
C18:3 *c*6*c*9*c*12	n.d.	n.d.	6.1 ± 1.00
C18:3 *c*9*c*12*c*15 (ALA)	354.6	130.5	1507.3 ± 395.34
C22	n.d.	n.d.	0.4 ± 0.15
ΣSFA	446.8	881.9	445.7 ± 32.72
ΣMUFA	109.8	878.2	112.5 ± 8.56
ΣPUFA	543.1	2619.6	1857.4 ± 459.35
TFA	1099.7	4379.7	2415.6 ± 492.04

[a] Abbreviations: DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; NE_L, net energy for lactation; LA, linoleic acid; ALA, α-linolenic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; n.d., not detected.

[b] Based on maize and wheat bran.

[c] Means and standard deviations of nine collected samples.

Table 2

Table 2. Goat milk yield and gross composition during abrupt transition from indoor (hay and concentrate; day 0) to pasture (days 1 to 23) feeding.[a]

	Days on pasture										Significance
	0	1	2	3	4	6	9	13	18	23	
Yield (kg head^{-1} day^{-1})	1.02^{DE}	1.05^{DE}	0.99^{E}	0.99^{E}	1.03^{DE}	1.09^{D}	1.17^{CD}	1.18^{BC}	1.25^{AB}	1.27^{A}	***
Fat (g kg^{-1})	41.8^{B}	40.2^{B}	47.3^{A}	45.4^{A}	46.6^{A}	45.8^{A}	45.2^{A}	46.4^{A}	45.3^{A}	48.4^{A}	***
Fat (g head^{-1} day^{-1})	40.7^{F}	41.7^{F}	46.8^{DE}	44.7^{EF}	46.8^{DE}	50.9^{CD}	52.9^{BC}	54.2^{BC}	57.3^{AB}	60.7^{A}	***
Protein (g kg^{-1})	30.5^{D}	31.3^{CD}	31.8^{BC}	31.7^{BC}	32.2^{BC}	33.6^{A}	32.4^{B}	34.1^{A}	32.5^{B}	34.2^{A}	***
Protein (g head^{-1} day^{-1})	31.1^{E}	32.5^{E}	31.3^{E}	31.1^{E}	32.4^{E}	36.9^{D}	37.7^{CD}	40.2^{BC}	40.7^{B}	43.2^{A}	***
Lactose (g kg^{-1})	40.4^{F}	40.8^{DEF}	40.3^{F}	40.1^{F}	41.4^{DE}	43.7^{A}	41.8^{CD}	42.6^{BC}	40.6^{EF}	43.2^{AB}	***
Lactose (g head^{-1} day^{-1})	41.0^{DE}	42.8^{D}	39.7^{DE}	39.3^{E}	41.7^{DE}	47.8^{C}	48.8^{BC}	50.1^{BC}	51.2^{B}	54.6^{A}	***

[a] Total number of samples analyzed equal to 120 (12 goats × 10 sampling days).

$^{A-F}$ Means within a row with different superscripts differ significantly. Probability: *** P≤0.001.

Table 3

Table 3. Groups of fatty acids (g kg^{-1} fat) in goat milk during abrupt transition from indoor (hay and concentrate; day 0) to pasture (days 1 to 23) feeding.[a,b]

	Days on pasture										Significance
	0	1	2	3	4	6	9	13	18	23	
Σ short chain[1]	125.2^{B}	123.8^{B}	108.3^{C}	97.7^{D}	103.4^{CD}	128.4^{AB}	129.3^{AB}	122.7^{B}	134.2^{A}	130.6^{AB}	***
Σ medium chain[2]	367.9^{A}	376.1^{A}	307.2^{BC}	266.1^{D}	246.6^{D}	295.6^{C}	299.9^{BC}	319.2^{BC}	318.9^{BC}	320.7^{B}	***
Σ long chain[3]	416.8^{D}	428.5^{CD}	513.3^{A}	526.6^{A}	486.8^{AB}	465.8^{BC}	422.8^{CD}	435.6^{CD}	405.6^{D}	415.5^{D}	***
Σ saturated[4]	590.2^{AB}	598.2^{A}	534.2^{CDE}	480.9^{F}	450.9^{G}	523.4^{DE}	518.6^{E}	534.0^{CDE}	551.3^{CD}	554.9^{BC}	***
Σ branched chain[5]	26.8^{ABC}	28.5^{A}	27.4^{AB}	24.7^{CDE}	22.3^{F}	22.9^{EF}	23.2^{DEF}	25.5^{BCD}	25.6^{BC}	25.8^{BC}	***
Σ monounsaturated[6]	285.6^{CD}	294.4^{C}	353.1^{A}	362.3^{A}	337.1^{AB}	312.3^{BC}	282.7^{CDE}	287.3^{C}	254.3^{E}	255.3^{DE}	***
Σ C18:1[7]	273.3^{CDE}	281.5^{CD}	340.6^{A}	349.7^{A}	324.8^{AB}	299.4^{BC}	269.4^{DEF}	273.1^{CDE}	242.0^{F}	243.4^{EF}	***
Σ C18:1 *trans*[8]	12.0^{G}	11.8^{G}	15.9^{G}	21.6^{F}	26.0^{E}	37.6^{D}	38.7^{CD}	42.7^{BC}	47.8^{A}	46.1^{AB}	***
Σ polyunsaturated[9]	34.6^{E}	37.0^{E}	42.5^{D}	47.9^{C}	49.4^{C}	54.7^{AB}	51.4^{BC}	57.2^{A}	54.0^{AB}	57.7^{A}	***
Σ C18:2[10]	26.6^{E}	28.5^{E}	32.7^{D}	37.2^{C}	38.6^{C}	42.6^{AB}	40.8^{BC}	45.5^{A}	42.7^{AB}	43.7^{AB}	***
Σ C18:2 *trans*[11]	10.9^{D}	11.1^{D}	13.9^{D}	17.8^{C}	20.2^{C}	26.0^{B}	27.4^{B}	32.3^{A}	31.9^{A}	31.4^{A}	***
Σ *trans* without CLA[12]	33.1^{F}	33.5^{F}	43.8^{E}	57.5^{D}	66.1^{D}	91.4^{C}	94.1^{C}	104.9^{B}	116.5^{A}	113.1^{AB}	***
Σ n3 FA[13]	7.5^{F}	8.1^{EF}	9.4^{E}	11.1^{D}	11.2^{D}	12.8^{BC}	11.9^{CD}	13.6^{B}	13.4^{BC}	16.3^{A}	***
Σ n6 FA[14]	23.1^{E}	25.1^{BCDE}	27.7^{AB}	28.9^{A}	27.6^{AB}	26.7^{ABC}	23.6^{DE}	24.3^{CDE}	25.2^{BCDE}	26.1^{BCD}	***
n6/n3	3.28^{A}	3.26^{A}	3.04^{A}	2.65^{B}	2.51^{B}	2.11^{C}	2.02^{CD}	1.79^{DE}	1.88^{CDE}	1.61^{E}	***
Σ CLA[15]	5.5^{F}	5.5^{F}	7.1^{F}	9.6^{E}	12.1^{D}	17.0^{C}	18.2^{BC}	21.4^{A}	19.6^{AB}	19.4^{AB}	***
Σ unsaturated[16]	320.3^{DE}	331.4^{DE}	395.6^{AB}	410.2^{A}	386.5^{AB}	367.0^{BC}	334.1^{CDE}	344.5^{CD}	308.3^{E}	313.0^{DE}	***
HSFA[17]	568.2^{A}	582.8^{A}	445.8^{B}	369.2^{C}	345.8^{C}	459.5^{B}	449.1^{B}	477.4^{B}	478.7^{B}	481.8^{B}	***

[a] Total number of samples analyzed equal to 120 (12 goats × 10 sampling days).

[b] Abbreviations: CLA, conjugated linoleic acid; FA, fatty acids; HSFA, hypercholesterolemic saturated fatty acids.

[A-G] Means within a row with different superscripts differ significantly. Probability: *** P≤0.001.

[1] C4, C5, C6, C7, C8, C10, C10:1.
[2] C12, C13 *iso*, C13 *aiso*, C12:1 *c*, C13, C14 *iso*, C14, C15 *iso*, C14:1 *t*, C15 *aiso*, C14:1 *c*+C15, C16 *iso*, C16, C17 *iso*, C16:1 *t*, C17 *aiso*, C16:1 *c*.
[3] C17, C17:1 *t*, C18 *aiso*, C18, Σ C18:1, Σ C18:2, C20, C20:1 *t*, C18:3 *c*6*c*9*c*12, C20:1 *c*9, C20:1 *c*11, C18:3 *c*9*c*12*c*15, C18:2 *c*9*t*11+*t*7*c*9+*t*8*c*10, C18:2 *t*10*c*12, C18:2 *t*11*c*13+*c*9*c*11, C18:2 *t*9*t*11, C20:2 *c*,*c* n6, C22, C20:3 n6, C20:3 n3, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA).
[4] C4, C5, C6, C7, C8, C10, C12, Σ branched chain, C13, C14, C14:1 *c*+C15, C16, C17, C18, C20, C22.
[5] C13 *iso*, C13 *aiso*, C14 *iso*, C15 *iso*, C15 *aiso*, C16 *iso*, C17 *iso*, C17 *aiso*, C18 *aiso*.
[6] C10:1, C12:1 *c*, C14:1 *t*, C16:1 *t*, C16:1 *c*, C17:1 *t*, Σ C18:1, C20:1 *t*, C20:1 *c*9, C20:1 *c*11.
[7] C18:1 *t*5, *t*6-11, *t*12-14+*c*6-8, *c*9, *c*11, *c*12, *c*14+*t*16.
[8] C18:1 *t*5, *t*6-11, *t*12-14+*c*6-8.
[9] Σ C18:2, C18:3 *c*6*c*9*c*12, C18:3 *c*9*c*12*c*15, C20:2 *c*,*c* n6, C20:3 n6, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA).
[10] C18:2 *t*,*t*-NMID+*t*9*t*12, *c*9*t*13+*t*8*c*12, *c*9*t*12, *c*,*c*-MID+*t*8*c*13, *t*11*c*15, *t*9c12, *c*9*c*12, *c*9*c*15, *c*9*t*11+*t*7*c*9+*t*8*c*10, *t*10*c*12, *t*11*c*13+*c*9*c*11, *t*9*t*11.

[11] C18:2 *t*,*t*-NMID+*t*9*t*12, *c*9*t*13+*t*8*c*12, *c*9*t*12, *c*,*c*-MID+*t*8*c*13, *t*11*c*15, *t*9*c*12, C18:2 *c*9*t*11+*t*7*c*9+*t*8*c*10, C18:2 *t*10*c*12, C18:2 *t*11*c*13+*c*9*c*11, C18:2 *t*9*t*11.

[12] C14:1 *t*, C16:1 *t*, C17:1 *t*, Σ C18:1 *t*, Σ C18:2 *t* (without CLA *trans*), C20:1 *t*.

[13] C18:2 *t*11*c*15, C18:2 *c*9*c*15, C18:3 *c*9*c*12*c*15, C20:5 n3 (EPA), C22:5 n3 (DPA).

[14] C18:1 *t*12-14+*c*6-8, C18:1 *c*12, C18:2 *t*,*t*-NMID+*t*9*t*12, C18:2 *c*9*t*12, C18:2 *t*9*c*12, C18:2 *c*9*c*12, C18:3 *c*6*c*9*c*12, C20:2 *c*,*c* n6, C20:3 n6, C20:4 n6 (AA).

[15] C18:2 *c*9*t*11+*t*7*c*9+*t*8*c*10, *t*10*c*12, *t*11*c*13+*c*9*c*11, *t*9*t*11.

[16] C10:1, C12:1 *c*, C14:1 *t*, C16:1 *t*, C16:1 *c*, C17:1 *t*, Σ C18:1, Σ C18:2, C20:1 *t*, C18:3 *c*6*c*9*c*12, C20:1 *c*9, C20:1 *c*11, C18:3 *c*9*c*12*c*15, C18:2 *c*9*t*11+*t*7*c*9+*t*8*c*10, C18:2 *t*10*c*12, C18:2 *t*11*c*13+*c*9*c*11, C18:2 *t*9*t*11, C20:2 *c*,*c* n6, C20:3 n6, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA).

[17] Calculated as C12+4*C14+C16.

Table 4

Table 4.
(a) Individual short- and medium-chain fatty acids (g kg^{-1} fat) in goat milk during abrupt transition from indoor (hay and concentrate; day 0) to pasture (days 1 to 23) feeding.[a,b]

	Days on pasture										Significance
	0	1	2	3	4	6	9	13	18	23	
C4	20.52^{CD}	19.09^{E}	20.26^{CDE}	21.13^{BCD}	22.96^{A}	22.42^{AB}	22.36^{AB}	19.91^{DE}	20.71^{CD}	21.36^{BC}	***
C5	0.07^{CDE}	0.11^{ABC}	0.12^{AB}	0.08^{BCDE}	0.07^{DE}	0.06^{E}	0.10^{BCD}	0.11^{ABC}	0.14^{A}	0.11^{ABC}	**
C6	18.71^{BC}	17.91^{CDE}	17.56^{DE}	16.96^{E}	18.31^{CD}	20.50^{A}	20.32^{A}	18.54^{CD}	19.72^{AB}	19.82^{A}	***
C7	0.17^{BCD}	0.20^{AB}	0.16^{CD}	0.14^{DE}	0.11^{E}	0.17^{BCD}	0.19^{BC}	0.18^{BCD}	0.24^{A}	0.21^{AB}	***
C8	20.57^{CD}	20.16^{DE}	18.93^{EF}	17.42^{G}	18.51^{FG}	22.61^{A}	22.12^{AB}	20.83^{BCD}	22.70^{A}	22.00^{ABC}	***
C10	62.36^{B}	63.41^{AB}	49.21^{C}	40.38^{D}	41.79^{D}	60.36^{B}	61.78^{B}	60.84^{B}	68.38^{A}	65.03^{AB}	***
C10:1	2.83^{A}	2.93^{A}	2.05^{D}	1.60^{E}	1.66^{E}	2.27^{BCD}	2.40^{B}	2.33^{BC}	2.28^{BCD}	2.08^{CD}	***
C12	28.83^{A}	29.99^{A}	22.47^{D}	17.36^{E}	16.68^{E}	23.69^{CD}	25.46^{BC}	25.22^{CD}	30.68^{A}	28.19^{AB}	***
C13 *iso*	0.24^{BC}	0.27^{AB}	0.17^{DE}	0.12^{E}	0.12^{E}	0.17^{DE}	0.21^{CD}	0.24^{BC}	0.25^{BC}	0.30^{A}	***
C13 *aiso*	0.41^{B}	0.51^{A}	0.29^{CD}	0.19^{E}	0.18^{E}	0.25^{DE}	0.30^{CD}	0.32^{CD}	0.33^{C}	0.32^{CD}	***
C12:1 *c*	0.69^{AB}	0.76^{A}	0.47^{EF}	0.38^{FG}	0.32^{G}	0.41^{EF}	0.45^{EF}	0.51^{DE}	0.63^{BC}	0.58^{CD}	***
C13	0.74^{AB}	0.80^{A}	0.51^{D}	0.31^{E}	0.31^{E}	0.56^{CD}	0.67^{ABC}	0.63^{BCD}	0.75^{AB}	0.69^{ABC}	***
C14 *iso*	1.23^{A}	1.31^{A}	1.02^{BC}	0.69^{D}	0.64^{D}	0.71^{D}	0.92^{C}	0.97^{C}	1.19^{A}	1.17^{AB}	***
C14	80.90^{A}	83.79^{A}	59.26^{C}	45.83^{D}	43.44^{D}	66.00^{BC}	62.31^{BC}	66.68^{BC}	67.41^{B}	67.91^{B}	***
C15 *iso*	2.09^{B}	2.32^{AB}	1.80^{C}	1.45^{D}	1.23^{D}	1.47^{D}	1.75^{C}	2.16^{AB}	2.29^{AB}	2.40^{A}	***
C14:1 *t*	0.05^{E}	0.08^{DE}	0.11^{CD}	0.17^{AB}	0.17^{AB}	0.14^{BC}	0.15^{ABC}	0.19^{A}	0.18^{AB}	0.15^{ABC}	***
C15 *aiso*	3.96^{BC}	4.29^{B}	3.26^{D}	2.41^{EF}	2.06^{F}	2.74^{E}	3.71^{CD}	4.16^{BC}	4.94^{A}	4.87^{A}	***
C14:1 *c*+C15	11.01^{A}	11.27^{A}	7.99^{D}	6.01^{EF}	5.13^{F}	6.15^{E}	7.81^{D}	9.01^{C}	9.92^{B}	10.41^{AB}	***
C16 *iso*	2.90^{B}	3.37^{A}	2.82^{B}	2.31^{DE}	1.93^{F}	2.07^{EF}	2.08^{EF}	2.46^{CD}	2.61^{BCD}	2.73^{BC}	***
C16	215.83^{A}	217.60^{A}	186.30^{B}	168.54^{CD}	155.33^{D}	171.84^{BC}	174.45^{BC}	185.44^{B}	178.37^{BC}	181.95^{BC}	***
C17 *iso*	3.86^{BCDE}	4.20^{ABC}	4.42^{A}	4.26^{AB}	3.86^{BCDE}	3.98^{BCDE}	3.83^{CDE}	4.09^{ABCD}	3.73^{DE}	3.63^{E}	**
C16:1 *t*	0.89^{F}	0.86^{F}	1.04^{F}	1.46^{E}	1.92^{D}	3.05^{C}	3.58^{B}	4.26^{A}	3.45^{BC}	3.48^{B}	***
C17 *aiso*	8.11^{AB}	8.28^{AB}	8.59^{A}	7.85^{ABC}	7.14^{CD}	6.99^{D}	7.06^{CD}	7.58^{BCD}	7.66^{BCD}	7.69^{BCD}	***
C16:1 *c*	6.24^{A}	6.36^{A}	6.66^{A}	6.73^{A}	6.14^{A}	5.36^{B}	5.13^{B}	5.34^{B}	4.47^{C}	4.22^{C}	***

Table 4.
(b) Individual long-chain fatty acids (g kg^{-1} fat) in goat milk and desaturase indexes during abrupt transition from indoor (hay and concentrate; day 0) to pasture (days 1 to 23) feeding.[a,b]

	Days on pasture										Significance
	0	1	2	3	4	6	9	13	18	23	
C17	6.97^{DE}	6.85^{DE}	8.59^{AB}	8.71^{A}	7.83^{BC}	7.05^{CD}	6.19^{E}	6.44^{DE}	6.33^{DE}	6.57^{DE}	***
C17:1 *t*	0.53^{DEF}	0.74^{BC}	0.99^{A}	1.14^{A}	1.05^{A}	0.80^{B}	0.67^{BCD}	0.59^{CDE}	0.44^{F}	0.50^{EF}	***
C18 *aiso*	4.06^{CD}	3.92^{CDE}	5.02^{AB}	5.46^{A}	5.16^{A}	4.49^{BC}	3.37^{E}	3.49^{DE}	2.60^{F}	2.69^{F}	***
C18	93.94^{B}	94.99^{B}	112.71^{A}	111.27^{A}	96.47^{B}	97.28^{B}	89.68^{B}	92.35^{B}	97.49^{B}	101.72^{AB}	**
C18:1 *t*5	0.06	0.08	0.10	0.11	0.07	0.08	0.02	0.06	0.04	0.10	ns
C18:1 *t*6-11	9.57^{E}	9.41^{E}	12.98^{E}	18.37^{D}	22.73^{C}	33.66^{B}	34.34^{B}	38.30^{A}	40.87^{A}	39.54^{A}	***
C18:1 *t*12-14+*c*6-8	2.33^{E}	2.29^{E}	2.86^{DE}	3.16^{CD}	3.16^{CD}	3.83^{BC}	4.30^{B}	4.38^{B}	6.87^{A}	6.51^{A}	***
C18:1 *c*9	253.68^{D}	261.88^{CD}	316.09^{AB}	318.77^{A}	290.12^{BC}	253.22^{D}	222.81^{E}	222.06^{E}	185.03^{F}	188.00^{F}	***
C18:1 *c*11	4.59^{BCD}	4.58^{BCD}	5.13^{AB}	5.49^{A}	5.30^{A}	4.89^{ABC}	4.29^{DE}	4.41^{CDE}	3.93^{E}	3.87^{E}	***
C18:1 *c*12	0.69^{ABC}	0.84^{A}	0.81^{AB}	0.85^{A}	0.75^{ABC}	0.67^{BC}	0.59^{C}	0.70^{ABC}	0.81^{AB}	0.81^{AB}	*
C18:1 *c*14+*t*16	2.21^{D}	2.38^{D}	2.65^{CD}	2.96^{BC}	2.64^{CD}	3.03^{BC}	3.02^{BC}	3.23^{B}	4.41^{A}	4.59^{A}	***
C18:2 *t*,*t*-NMID+*t*9*t*12	0.39^{F}	0.53^{F}	0.69^{E}	0.94^{D}	0.86^{DE}	1.02^{CD}	0.97^{D}	1.25^{BC}	1.51^{A}	1.42^{AB}	***
C18:2 *c*9*t*13+*t*8*c*12	0.15^{G}	0.19^{FG}	0.22^{EFG}	0.30^{EF}	0.34^{E}	0.50^{D}	0.53^{CD}	0.64^{C}	0.91^{A}	0.78^{B}	***
C18:2 *c*9*t*12	1.45^{G}	1.49^{FG}	1.81^{EF}	2.03^{DE}	1.95^{DE}	2.06^{DE}	2.24^{CD}	2.44^{BC}	2.89^{A}	2.75^{AB}	***
C18:2 *c*,*c*-MID+*t*8*c*13	1.33^{D}	1.37^{D}	1.76^{C}	2.06^{BC}	2.03^{BC}	2.07^{BC}	2.13^{B}	2.36^{AB}	2.51^{A}	2.51^{A}	***
C18:2 *t*11*c*15	0.97^{E}	1.09^{E}	1.31^{DE}	1.91^{CD}	1.99^{C}	2.56^{BC}	2.84^{B}	3.57^{A}	3.76^{A}	3.85^{A}	***
C18:2 *t*9*c*12	1.04^{AB}	0.98^{AB}	1.08^{A}	0.97^{AB}	0.90^{BC}	0.76^{CD}	0.46^{E}	0.69^{D}	0.68^{D}	0.66^{D}	***
C18:2 *c*9*c*12 (LA)	15.69^{D}	17.08^{BCD}	18.58^{AB}	19.19^{A}	18.18^{ABC}	16.50^{CD}	13.28^{E}	12.96^{E}	10.70^{F}	12.17^{EF}	***
C18:2 *c*9*c*15	0.04^{D}	0.27^{A}	0.15^{BCD}	0.19^{ABC}	0.19^{ABC}	0.10^{BCD}	0.10^{BCD}	0.20^{AB}	0.08^{CD}	0.19^{ABC}	***
C20	2.27^{AB}	2.33^{A}	1.73^{D}	1.31^{EF}	1.06^{G}	1.23^{FG}	1.19^{FG}	1.48^{E}	1.84^{CD}	2.04^{BC}	***
C20:1 *t*	0.21^{CD}	0.19^{CD}	0.23^{BC}	0.28^{AB}	0.31^{A}	0.29^{AB}	0.22^{C}	0.16^{D}	0.17^{CD}	0.17^{CD}	***
C18:3 *c*6*c*9*c*12	0.14^{CD}	0.10^{DE}	0.15^{BCD}	0.10^{CDE}	0.11^{CDE}	0.08^{E}	0.20^{AB}	0.15^{BC}	0.22^{A}	0.21^{A}	***
C20:1 *c*9	0.23^{CDE}	0.26^{CD}	0.32^{BC}	0.16^{E}	0.19^{DE}	0.17^{DE}	0.39^{AB}	0.32^{BC}	0.41^{A}	0.29^{C}	***
C20:1 *c*11	0.70^{A}	0.69^{A}	0.62^{AB}	0.66^{A}	0.55^{BC}	0.48^{CD}	0.32^{E}	0.43^{D}	0.34^{E}	0.39^{DE}	***
C18:3 *c*9*c*12*c*15 (ALA)	5.16^{CD}	4.84^{D}	6.23^{C}	7.49^{B}	7.56^{B}	8.39^{B}	7.47^{B}	8.02^{B}	7.59^{B}	9.94^{A}	***
CLA *c*9*t*11+*t*7*c*9+*t*8*c*10	5.48^{F}	5.36^{F}	6.89^{F}	9.29^{E}	11.78^{D}	16.46^{C}	17.56^{BC}	19.76^{A}	18.95^{AB}	18.76^{AB}	***
CLA *t*10*c*12	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	0.01	ns
CLA *t*11*c*13+*c*9*c*11	0.02^{D}	0.04^{D}	0.08^{D}	0.19^{C}	0.26^{C}	0.45^{B}	0.52^{B}	0.64^{A}	0.53^{B}	0.51^{B}	***
CLA *t*9*t*11	0.05^{D}	0.10^{C}	0.10^{C}	0.11^{BC}	0.10^{C}	0.14^{ABC}	0.14^{ABC}	0.16^{AB}	0.16^{A}	0.13^{ABC}	***
C20:2 *c*,*c* n6	0.07^{F}	0.14^{EF}	0.20^{DE}	0.26^{CD}	0.30^{BC}	0.30^{BC}	0.31^{BC}	0.42^{A}	0.36^{AB}	0.39^{AB}	***
C22	0.97^{BC}	1.22^{A}	1.06^{ABC}	0.75^{DE}	0.64^{E}	0.64^{E}	0.73^{DE}	0.91^{CD}	0.98^{BC}	1.15^{AB}	***

C20:3 n6	0.13^{BC}	0.16^{AB}	0.19^{A}	0.17^{AB}	0.16^{AB}	0.13^{BC}	0.08^{D}	0.10^{CD}	0.07^{D}	0.08^{CD}	***
C20:4 n6	1.17^{CD}	1.45^{A}	1.36^{ABC}	1.19^{CD}	1.19^{CD}	1.38^{AB}	1.13^{D}	1.22^{BCD}	1.08^{D}	1.08^{D}	**
C20:5 n3 (EPA)	0.46^{DEF}	0.50^{DEF}	0.47^{DEF}	0.41^{F}	0.43^{EF}	0.60^{CD}	0.56^{CDE}	0.65^{BC}	0.85^{A}	0.75^{AB}	***
C22:5 n3 (DPA)	0.94^{D}	1.36^{AB}	1.19^{BC}	1.08^{CD}	1.06^{CD}	1.18^{BC}	0.92^{D}	1.17^{BC}	1.17^{BC}	1.53^{A}	***
DI_{16}[c]	0.29^{C}	0.29^{C}	0.36^{B}	0.40^{A}	0.40^{A}	0.31^{C}	0.29^{C}	0.29^{C}	0.25^{D}	0.23^{D}	***
DI_{18}[d]	27.4^{BC}	28.0^{BC}	28.4^{AB}	29.1^{AB}	30.4^{A}	26.0^{CD}	25.3^{D}	24.3^{D}	19.1^{E}	18.6^{E}	***

[a] Total number of samples analyzed equal to 120 (12 goats × 10 sampling days).

[b] Abbreviations: *c*, *cis*; *t*, *trans*; NMID, non methylene interrupted diene; MID, methylene interrupted diene; LA, linoleic acid; ALA, α-linolenic acid; CLA, conjugated linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DI, desaturase index.

[c] Calculated as: C16:1 *c*9/C16:0.

[d] Calculated as: C18:1 *c*9/C18:0.

[A-G] Means within a row with different superscripts differ significantly. Probability: * P≤0.05; ** P≤0.01; *** P≤0.001; ns, not significant (P≥0.10).