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

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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 t6-11, C18:1 t12-14+c6-8, C18:1 c14+t16, C18:2 t11c15, C18:2 c9t13+t8c12, CLA isomers c9t11+t7c9+t8c10 and t11c13+c9c11. 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 t11), rumenic acid (C18:2 c9t11), 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

transition from indoor to fresh grass feeding have been investigated in a limited number of studies in cows and sheep only. Kuzdzal-Savoie and Kuzdzal (1961) first reported a notable and fast increase in the amount of conjugated dienes and α -linolenic acid (C18:3 c9c12c15, ALA) in butter from dairy cows as an effect of either sudden or gradual change from a winter ration to a pasture-based diet. Similarly, Decaen and Ghadaki (1970) observed that a gradual change from a winter diet consisting of hay, silage, and concentrate to a zero-grazing system induced fast modifications in the FA composition of cow milk fat. Particularly, they observed a decrease in the proportion of short- (SCFA) and medium-chain (MCFA) fatty acids and contemporarily an increase in long-chain fatty acids (LCFA). More recently, Khanal et al. (2008) reported stearic acid (C18:0), oleic acid (C18:1 c9), vaccenic acid (C18:1 t11, VA), conjugated linoleic acid (CLA) and ALA to increase, and most of the SCFA and MCFA to decrease, during abrupt transition of dairy cows from a total mixed ration to a full-grazing diet. In their study, the majority of FA changed daily before stabilizing around 22-23 days after transition. Coppa et al. (2011) also investigated the kinetics of milk FA in dairy cows during rapid or progressive transition from hay- to alpine pasture-based diets. These authors showed that many FA (almost all saturated ones, linoleic acid – C18:2 c9c12, LA - and ALA) became stable after five days in both transitions, while both VA and the most abundant among CLA isomers (C18:2 c9t11, rumenic acid, RA) reached maximum and stable concentrations about two weeks after the maximum fresh herbage intake. In sheep, Biondi et al. (2008) reported that the major changes in milk FA occurred during the first three days following an abrupt transition from indoor to pasture diet, being predominantly attributable to unsaturated fatty acids (UFA). Particularly, they observed significant increases of VA, RA, and ALA, as well as a notable decrease in the concentration of LA and in the omega-6/omega-3 fatty acids ratio.

To the best of our knowledge, no information is currently available on the rate of change

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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.

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MATERIALS AND METHODS

Animals feeding and management

The experiment was carried out in a farm located in North-Western Italy (latitude: 86 45°02'51" N; longitude: 07°19'10" E; altitude: 643 m a.s.l.) and breeding a flock of 40 87 88 Valdostana goats. The experimental period covered a total of 24 days, from March 29 to 89 April 21, 2011. 90 Twelve goats were used in the experiment (days in milk at the beginning of the trial: 91 126±6; number of lactation: 2.2±0.4), with an average body condition score of 3.0±0.5 92 (Hervieu and Morand-Fehr, 1999). The goats were maintained indoor for 40 days 93 (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 94 day⁻¹ of commercial concentrate containing maize and wheat bran. Means and standard 95 96 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, 97 31.3±0.22 g kg⁻¹ and 43.4±0.26 g kg⁻¹, respectively. From midday of March 30 (day 1) the 98 99 goats were abruptly brought outdoor and exclusively fed with fresh grass from natural 100 pasture (main species: Lolium perenne L., Trifolium pratense L., and Poa spp.) ad libitum. 101 The pasture area was flat and adjacent to the farm. The goats were manually milked 102 indoors twice a day (at 6.00 h and 18.00 h). They were allowed to graze during the 103 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.

Sampling procedure and laboratory analyses of milk

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

Sampling procedure and laboratory analyses of feed

The hay and concentrate fed to the goats during the indoor period were collected on day 0. For herbage sampling, the pasture area was subdivided in plots of 200 m² each. A sub-area of 200 cm² was considered for sampling at every intersection among plots. Pasture samples were collected following the same time schedule (days 1, 2, 3, 4, 6, 9, 13, 18, and 23) as for the collection of milk samples. The grazing behavior of the goats was observed at each sampling date. After closely observation, hand-plucked forage samples, simulating plants parts consumed by the goats, were collected from pasture. Two aliquots of each herbage sample were transported to the laboratory in a portable refrigerator at 4°C and then frozen at -80°C. Before the chemical analysis, the first aliquot of each herbage sample was dried at 40°C for 24 h; hay, concentrate and dried herbage samples were then 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

- 144 The goat was considered as the experimental unit. Changes in milk yield, main
- 145 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_i =
- random effect of goat, and ε_{iik} = experimental error. Pairwise multiple comparisons were
- assessed by using the PDIFF option in SAS. Significance was declared at $P \le 0.05$.

RESULTS

Characteristics of the feedstuffs

- 154 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 hav, 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

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Milk yield and gross composition were significantly affected by sampling day (P≤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

the percentage of lactose in milk.

Milk fatty acid composition

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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 t5 and C18:2 t10c12) were not significantly influenced by sampling day. Total SCFA and MCFA started to decrease significantly the second day after transition 187 188 (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 guite 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 t11) coeluted with other C18:1 216 trans-isomers (C18:1 t6-10). This sum (C18:1 t6-11) as well as the values recorded for 217 other detected trans-octadecenoic isomers, particularly C18:1 t12-14 (which coeluted with 218 C18:1 c6-8 isomers) and C18:1 t16 (which coeluted with C18:1 c14), started to increase 219 significantly three days after turning out to pasture. C18:1 t6-11 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 t12-14+c6-8 and C18:1 c14+t16 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 c9t11, C18:2 t7c9 and C18:2 t8c10 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 the trial. In fact, it varied between 3.33 and 7.77 g kg⁻¹ fat at the beginning of the trial (day 231 0) and between 11.79 and 22.96 g kg⁻¹ fat the last sampling day. About two-fold variation 232 233 was constantly maintained among individual goats all along the experiment. However, the 234 ranking of individual goats for CLA content was not stable throughout the trial. The sums 235 CLA c9t11+t7c9+t8c10 and C18:1 t6-11 were strongly correlated each other (r=0.97; 236 P≤0.001). 237 CLA isomers t11c13 and c9c11 coeluted in the chromatogram. The third day after 238 transition from the winter diet to full grazing their sum was already about nine times 239 higher than the initial value. Their sum continued to increase until day 13 when it reached 240 the highest absolute concentration. The contribution of these isomers to the total CLA 241 content of milk varied from 0.42% on day 0 to 2.7% on day 23. Sampling day 242 significantly affected the concentration of CLA isomer t9t11 as well. Its lowest value was 243 observed the last day of stall feeding. From day 1 to day 23 its concentration remained 244 quite constant, with the exception of days 13 and 18 when the highest values were reached 245 (about 3 times higher than the value observed on day 0). 246 Linoleic acid significantly increased until the third day after transition to pasture feeding. 247 Then it decreased, reaching concentrations up to 1.5 fold lower than those observed on 248 day 0. 249 Total omega-3 FA as well as ALA (which is the most abundant detected FA in this group) 250 significantly and constantly increased from the last day of stall feeding to the last 251 sampling day. At the end of the trial total omega-3 FA and ALA concentrations were 252 about two-fold higher than values recorded on day 0. Considering other omega-3 FA, a 253 clear increasing trend was also observed for C18:2 t11c15. A first significant raise 254 (approximately doubled values with respect to concentrations detected the last day of stall 255 feeding) was observed the third day after variation of the diet. Then it continued to rise 256 constantly till the last sampling day, doubling again its concentrations so that on day 23 it 257 was about four times higher than on day 0. A significant and positive correlation was 258 found between this octadecadienoic acid and the sum of CLA isomers t11c13 and c9c11 259 (r=0.75; P≤0.001). Among long-chain omega-3 FA, eicosapentaenoic acid (C20:5

c5c8c11c14c17, EPA) followed a similar trend as that observed for ALA and C18:2 t11c15. However, a significant increase was detected only after thirteen days after transition. Its highest concentrations were observed the last two sampling days (with values that almost doubled with respect to those observed at the beginning of the trial). Conversely, no clear positive trend was observed for docosapentaenoic acid (C22:5 *c*7*c*10*c*13*c*16*c*19, DPA). The omega-6/omega-3 FA ratio started to decline the third day after switching from stall to pasture feeding. This ratio continued to decrease significantly throughout the trial up to day 13. The lowest absolute values were observed the last sampling day, when it was two times lower than its initial value. Concerning $\Delta 9$ -desaturase activity (estimated as the ratios of C16:1 c9 to C16:0 – DI_{16} – and C18:1 c9 to C18:0 - DI₁₈), a significant increase was observed until day 4 after transition (P≤0.001). However, from day 6 these ratios significantly decreased again

DISCUSSION

Milk yield and gross composition

reaching the absolute lowest values at the end of the trial.

The observed slight and progressive raise in milk yield and protein after transition from indoor to pasture feeding could be related to increased ingested energy as reported to occur in case of turning out, particularly when fresh grass is at an early growth stage. Similarly, an early phenological phase of pasture plants resulted in high milk fat content due to the increased intake of highly digestible fiber (Morand-Fehr et al., 2007). Besides the vegetative stage of pasture plants, the diet fed during the indoor period can significantly affect milk main constituents after turning out to pasture. If compared to maize silage, both hay or grass silage used as winter rations were found to determine an increase in milk fat content (Hoden et al., 1985). Such findings seem to be confirmed in

the current trial as milk fat significantly increased after diet change from a hay-concentrate

based diet to a pasture based diet with fresh grass at early vegetative stage.

Milk fatty acid composition

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The main changes in goat milk FA during lactation have been shown to occur in early lactation and have been mainly attributed to lipid mobilization as the consequence of a negative energy balance phase for the animals. A relatively stable FA pattern is instead generally observed in mid and late lactation (Ataşoğlu et al., 2009; Chilliard et al., 2003). For this reason, a confounding effect due to lactation stage on milk FA can be reasonably excluded in the current study and the observed variations are likely to be attributable to feeding aspects only. Switching from a winter indoor diet (on average 70% hay and 30% concentrate) to a full grazing diet resulted in a higher availability of FA (particularly polyunsaturated) to be used for the synthesis of milk fat. It is known that the haymaking process notably reduces the total FA and the ALA concentrations in forages (Kalač and Samková, 2010). Consequently the availability of FA, and above all of ALA, increased switching the goats from a prevalent hay-based diet to a fresh grass-based diet. An increased supply of LCFA (especially with a high level of unsaturation) and some of their biohydrogenation intermediate products (e.g., C18:1 trans and C18:2 trans isomers) have been shown to inhibit the *de novo* synthesis of C8:0 to C16:0 FA within the bovine mammary gland, by exerting direct and/or indirect effects on the lipogenic enzymes acetyl-CoA carboxilase (ACC) and fatty acid synthase (FAS) and by reducing acetate and 3-hydroxibutyrate bioavailability for mammary lipogenesis (Chilliard and Ferlay, 2004). Nonetheless, milk fat synthesis and FA responses to dietary PUFA supplies are known to vary considerably among ruminant species (Chilliard et al., 2007). Mammary ACC and FAS mRNA abundance and/or activity have been reported to be much less affected by dietary PUFA in dairy goats than cows (Bernard et al., 2009). In the current study, the results we obtained

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

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339 by DI₁₈ 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 t5), of many 346 non-conjugated trans-octadecadienoic isomers (particularly C18:2 t11c15, C18:2 347 c9t13+t8c12. C18:2 c9t12. and C18:2 *tt*NMID+*t*9*t*12) and CLA isomers 348 c9t11+t7c9+t8c10, t11c13+c9c11, and t9t11 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 c9t11c15 and C18:3 c9t13c15 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 t11c15 is thought to be the major non conjugated octadecadienoic 356 isomer deriving from the biohydrogenation of ALA (hydrogenation of C18:3 c9t11c15). 357 Other non conjugated C18:2 trans-isomers are expected to be formed similarly. For 358 example, the C18:2 c9t13 isomer probably derives from reduction of the c15 double bond 359 in C18:3 c9t13c15 (Lee and Jenkins, 2011). The $\Delta 9,12$ C18:2 isomers are thought to be mainly formed during the biohydrogenation of LA, even if a putative metabolic pathway 360 361 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 ttNMID+t9t12 and C18:2 363 c9t12 after diet change suggest their partly formation from dietary ALA. On the contrary,

 $\Delta 9$ -destaurase on stearic acid. Moreover, the decreased $\Delta 9$ -desaturase activity (estimated

364 since C18:2 t9c12 tended to decrease after turning out to pasture, we could hypothesize 365 that ALA biohydrogenation would not be an important metabolic way for the ruminal 366 formation of this diene. 367 Concerning conjugated linoleic isomers, the increase in the sum of C18:2 368 c9t11+t7c9+t8c10 after turning out to pasture is presumably attributable to rumenic acid, 369 the main CLA isomer in ruminant milk fat. In fact, the other two isomers (t7c9 and t8c10) 370 were previously reported to correlate positively and significantly with dietary oleic and 371 linoleic acids, respectively, while no significant correlation was found with dietary ALA 372 (Collomb et al., 2004). The increase in rumenic acid is due to similar increased 373 concentrations of C18:1 t6-11, among which the precursor (C18:1 t11) for CLA c9t11 de 374 novo synthesis within the mammary gland belongs. C18:1 t11 is an intermediate of the 375 biohydrogenation processes and is formed from both dietary LA and ALA. The higher 376 supply of ALA from pasture if compared to the indoor diet suggests a higher formation of 377 vaccenic acid in the rumen as well as a higher absorption into the bloodstream and 378 consequently a higher availability for desaturation mediated by the $\Delta 9$ -desaturase enzyme 379 within the mammary gland. 380 Referring to minor CLA isomers, as only very low amounts of CLA c9c11 are usually 381 present in dairy fat (Ferlay et al., 2008), the sum CLA t11c13+c9c11 can be almost 382 completely attributed to the t11c13 isomer. Kraft et al. (2003) first hypothesized that CLA 383 t11c13 could be formed within the rumen at the third step of the biohydrogenation of 384 dietary ALA, by means of an isomerization at the expense of C18:2 t11c15. Our results 385 corroborate the hypothesis by Kraft et al. (2003) since both C18:2 t11c15 and CLA t11c13 386 significantly and rapidly increased after transition and they were significantly and 387 positively correlated each other. However, the rapid increase we observed in the sum of 388 CLA isomers t11c13 and c9c11 could be attributed to the latter isomer as well. In fact, 389 more than 50% of CLA c9c11 has also been recently reported to derive directly from the

390 biohydrogenation of dietary ALA (Lee and Jenkins, 2011). The same authors also reported 391 that CLA t9t11 partly derives from ALA, which could explain the observed increasing 392 trend of this CLA isomer in goat milk fat after transition from indoor to pasture diet. 393 As usually occurs with high-forage diets, CLA t10c12 was detected only in traces (concentrations ≤ 0.01 g kg⁻¹ fat) and was not significantly affected by the feeding change. 394 Besides vaccenic acid, other trans-octadecenoic fatty acids are known to be formed by 395 396 means of various isomerizations occurring during different steps of the biohydrogenation 397 of dietary UFA. In particular, the observed increase in the concentration of C18:1 t12-398 14+c6-8 could be attributed to C18:1 t13 and t14 isomers, which were found to be formed during the biohydrogenation of both C18:2 c9t13 and CLA $\Delta11,13$ (Chilliard et al., 2007). 399 400 The increased concentrations of ALA and EPA (the latter formed by means of ALA 401 desaturation and elongation processes (Barcelo-Coblin and Murphy, 2009)) in milk fat 402 have to be related as well to the higher ALA supply from fresh grass. Their concentrations 403 in milk remained however quite low since the extent of the raise was less pronounced if 404 compared to those observed for C18:2 t11c15, C18:2 t11c13, C18:2 ttNMID+t9t12, C18:2 405 c9t13+t8c12, C18:1 t6-11, and C18:2 c9t11+t7c9+t8c10, probably because the 406 disappearance of ALA in the rumen is very high (usually >90% in case of high-forage 407 diets) while only a little part of ALA is absorbed intact in the gut and secreted into milk 408 (Chilliard and Ferlay, 2004). The observed consistent increasing concentrations of ALA 409 despite the lower ALA supply due to the advance of the phenological phase of the grazed 410 pasture plants suggests that the rate of disappearance of ALA probably decreased during 411 the trial. 412 Overall, the observed kinetics of responses of goat milk fatty acids to transition from 413 indoor to pasture feeding were more similar to changes already observed in dairy ewes 414 (Biondi et al., 2008) rather than dairy cows (Coppa et al., 2011; Khanal et al., 2008) under 415 comparable feeding conditions.

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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 (t11c13+c9c11, t9t11) and nonconjugated (t11c15, c9t13+t8c12, c9t12, ttNMID+t9t12) trans-octadecadienoic acids, and trans-octadecenoic acids (t6-11, t12-14+c6-8, c14+t16). The sum of CLA isomers c9t11+t7c9+t8c10 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 Δ 9-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.

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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

	Indoo	r feeding	D4C
	Hay	Concentrate ^b	– Pasture ^c
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 <i>t</i> 3	33.1	4.9	57.4 ± 12.64
C16:1 <i>c</i> 9	3.3	8.9	2.5 ± 1.18
C18	49.6	56.3	32.9 ± 2.86
C18:1 <i>c</i> 9	70.5	823.3	46.4 ± 8.81
C18:1 <i>c</i> 11	2.9	41.2	6.2 ± 1.37
C19	n.d.	n.d.	1.2 ± 0.31
C18:2 <i>c</i> 9 <i>c</i> 12 (LA)	188.4	2489.1	344.0 ± 65.49
C20	2.4	10.1	12.6 ± 1.59
C18:3 c6c9c12	n.d.	n.d.	6.1 ± 1.00
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15 (ALA)	354.6	130.5	1507.3 ± 395.34
C22	n.d.	n.d.	0.4 ± 0.15
Σ SFA	446.8	881.9	445.7 ± 32.72
Σ MUFA	109.8	878.2	112.5 ± 8.56
ΣΡυγΑ	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.

^{8 &}lt;sup>b</sup> Based on maize and wheat bran.

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 p	Days on pasture										
	0	1	2	3	4	6	9	13	18	23	Significance	
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)	$40.7^{\rm F}$	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	***	

 $^{^{\}rm a}$ Total number of samples analyzed equal to 120 (12 goats \times 10 sampling days).

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

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										
	0	1	2	3	4	6	9	13	18	23	Significance
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 [°]	49.4 ^C	54.7^{AB}	51.4 ^{BC}	57.2 ^A	54.0^{AB}	57.7 ^A	***
$\Sigma C18:2^{10}$	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	$7.1^{\rm F}$	9.6^{E}	12.1^{D}	17.0°	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: *** $P \le 0.001$.

¹C4, C5, C6, C7, C8, C10, C10:1.

²C12, 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.

 $^{^{3}\}text{ C17, C17:1 } t, \text{ C18 } \textit{aiso}, \text{ C18; } \Sigma \text{ C18:1, } \Sigma \text{ C18:2, C20, C20:1 } t, \text{ C18:3 } \textit{c6c9c12, C20:1 } \textit{c9, C20:1 } \textit{c11, C18:3 } \textit{c9c12c15, C18:2 } \textit{c9t11+t7c9+t8c10, C18:2 } t11\textit{c13+c9c11, C18:2 } \textit{t9t11, C20:2 } \textit{c,c} \text{ n6, C22, C20:3 } \textit{n6, C20:3 } \textit{n3, C20:4 } \textit{n6 (AA), C20:5 } \textit{n3 (EPA), C22:5 } \textit{n3 (DPA).}$

⁴C4, C5, C6, C7, C8, C10, C12, Σ branched chain, C13, C14, C14:1 c+C15, C16, C17, C18, C20, C22.

⁵C13 iso, C13 aiso, C14 iso, C15 iso, C15 aiso, C16 iso, C17 iso, C17 aiso, C18 aiso.

⁶C10:1, C12:1 c, C14:1 t, C16:1 t, C16:1 c, C17:1 t, Σ C18:1, C20:1 t, C20:1 c9, C20:1 c11.

⁷C18:1 t5, t6-11, t12-14+c6-8, c9, c11, c12, c14+t16.

⁸C18:1 t5, t6-11, t12-14+c6-8.

⁹Σ C18:2, C18:3 c6c9c12, C18:3 c9c12c15, C20:2 c,c n6, C20:3 n6, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA).

¹⁰C18:2 t,t-NMID+t9t12, c9t13+t8c12, c9t12, c,c-MID+t8c13, t11c15, t9c12, c9c12, c9c15, c9t11+t7c9+t8c10, t10c12, t11c13+c9c11, t9t11.

¹¹C18:2 t,t-NMID+t9t12, c9t13+t8c12, c9t12, c,c-MID+t8c13, t11c15, t9c12, C18:2 c9t11+t7c9+t8c10, C18:2 t10c12, C18:2 t11c13+c9c11, C18:2 t9t11.

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 t11c15, C18:2 c9c15, C18:3 c9c12c15, C20:5 n3 (EPA), C22:5 n3 (DPA).

¹⁴C18:1 *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).

¹⁵C18:2 *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 t, C16:1 t, C18:1, Σ C18:1, Σ C18:2, C20:1 t, C18:3 c6c9c12, C20:1 c9, C20:1 c11, C18:3 c9c12c15, C18:2 c9t11+t7c9+t8c10, C18:2 t10c12, C18:2 t11c13+c9c11, C18:2 t9t11, 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.

(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 p	Days on pasture										
	0	1	2	3	4	6	9	13	18	23	Significance	
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}	$0.07^{ m DE}$	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 <i>c</i>	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}	$0.97^{\rm C}$	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}	$1.80^{\rm C}$	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}	$0.08^{ m DE}$	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 <i>c</i> +C15	11.01 ^A	11.27 ^A	7.99^{D}	6.01^{EF}	$5.13^{\rm F}$	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}	$1.04^{\rm F}$	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 <i>c</i>	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 p	pasture									Cionificanos
	0	1	2	3	4	6	9	13	18	23	Significance
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}	$2.60^{\rm F}$	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 <i>t</i> 5	0.06	0.08	0.10	0.11	0.07	0.08	0.02	0.06	0.04	0.10	ns
C18:1 <i>t</i> 6-11	9.57^{E}	9.41^{E}	12.98^{E}	18.37^{D}	$22.73^{\rm C}$	33.66^{B}	34.34^{B}	38.30^{A}	40.87^{A}	39.54 ^A	***
C18:1 <i>t</i> 12-14+ <i>c</i> 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 <i>c</i> 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}	$185.03^{\rm F}$	$188.00^{\rm F}$	***
C18:1 <i>c</i> 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 <i>c</i> 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 <i>c</i> 14+ <i>t</i> 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 <i>t,t</i> -NMID+ <i>t</i> 9 <i>t</i> 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 <i>c</i> 9 <i>t</i> 13+ <i>t</i> 8 <i>c</i> 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 <i>c</i> 9 <i>t</i> 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 <i>c,c</i> -MID+ <i>t</i> 8 <i>c</i> 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 <i>t</i> 11 <i>c</i> 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 <i>t</i> 9 <i>c</i> 12	1.04^{AB}	0.98^{AB}	1.08^{A}	0.97^{AB}	0.90^{BC}	$0.76^{\rm CD}$	0.46^{E}	0.69^{D}	0.68^{D}	0.66^{D}	***
C18:2 <i>c</i> 9 <i>c</i> 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}	$10.70^{\rm F}$	12.17^{EF}	***
C18:2 <i>c</i> 9 <i>c</i> 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}	$1.73^{\rm D}$	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}	$0.17^{\rm CD}$	0.17^{CD}	***
C18:3 <i>c6c</i> 9 <i>c</i> 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 <i>c</i> 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 <i>c</i> 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 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15 (ALA)	5.16 ^{CD}	4.84^{D}	6.23 ^C	7.49^{B}	$7.56^{\rm B}$	8.39^{B}	7.47^{B}	8.02^{B}	7.59^{B}	9.94 ^A	***
CLA <i>c</i> 9 <i>t</i> 11+ <i>t</i> 7 <i>c</i> 9+ <i>t</i> 8 <i>c</i> 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 t10c12	< 0.01	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01	0.01	0.01	ns
CLA t11c13+c9c11	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 <i>t</i> 9 <i>t</i> 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 <i>c</i> , <i>c</i> n6	0.07^{F}	0.14^{EF}	$0.20^{ m DE}$	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}	$1.08^{\rm D}$	1.08^{D}	**
C20:5 n3 (EPA)	$0.46^{ m DEF}$	$0.50^{ m DEF}$	0.47^{DEF}	$0.41^{\rm F}$	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}	$1.17^{\rm BC}$	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}	***
$\mathrm{DI}_{18}^{\mathrm{d}}$	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: * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$; ns, not significant ($P \ge 0.10$).