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Application of a linear regression model to study the origin of C17 branched-chain fatty acids in caprine milk fat

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Research Article

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Abstract

This research communications addresses the hypothesis that a part of *iso* 17:0 and *anteiso* 17:0 in milk fat could come from endogenous extraruminal tissue synthesis. In order to confirm this a linear regression model was applied to calculate the proportions of *iso* 17:0 and *anteiso* 17:0 in milk fat that could come from elongation of their putative precursors *iso* 15:0 and *anteiso* 15:0, respectively. Sixteen dairy goats were allocated to two simultaneous experiments, in a crossover design with four animals per treatment and two experimental periods of 25 d. In both experiments, alfalfa hay was the sole forage and the forage to concentrate ratio (33 : 67) remained constant. Experimental diets differed on the concentrate composition, either rich in starch or neutral detergent fibre, and they were administered alone or in combination with 30 g/d of linseed oil. *Iso* 15:0, *anteiso* 15:0, *iso* 17:0 and *anteiso* 17:0, the most abundant branched-chain fatty acids in milk fat, were determined by gas chromatography using two different capillary columns. The regression model resolved that 49% of *iso* 17:0 and 60% of *anteiso* 17:0 in milk fat was formed extraruminally from *iso* 15:0 and *anteiso* 15:0 elongation.

Branched-chain fatty acids (**BCFA**) constitute only some 2% of total fatty acids (**FA**) in milk fat, but they are major lipids of rumen bacterial membranes, where they are incorporated after *de novo* synthesis from different available substrates. There is a growing interest in BCFA, as their contents in milk fat could be used as biomarkers of ruminal function and their variations could reflect changes of rumen bacteria populations induced by diet composition (Vlaeminck *et al.*, 2006; Fievez *et al.*, 2012; Cívico *et al.*, 2017).

Although the presence of BCFA in milk fat has largely been ascribed to ruminal microorganisms, a recent study has shown that endogenous synthesis of certain BCFA should not be dismissed (Vlaeminck *et al.*, 2015). These authors suggested that a postruminal 2-carbon elongation may occur on BCFA converting *iso* 15:0 and *anteiso* 15:0 into *iso* 17:0 and *anteiso* 17:0, respectively, which are the four most abundant BCFA in milk fat. Thus, the BCFA profile in milk fat would probably reflect the BCFA absorption in the small intestine combined with certain metabolic reactions that would occur prior to their incorporation into milk fat.

However, the contribution of the endogenous elongation to the milk BCFA C17 content is still poorly understood. The aim of this work was to apply a linear regression model to quantify the postruminal contribution of *iso* and *anteiso* 17:0 to goat milk fat. In order to achieve this objective, we examined by chemometrics the BCFA profile of milk fat samples from dairy goats fed with different basal diets.

Materials and methods

The current investigation was carried out at the University of Córdoba facilities in accordance with the EU Directive 2010/63/EU for animal studies (European Commission, 2010). Sixteen Malagueña goats were allocated to two simultaneous experiments in a crossover design with two treatments, four animals per treatment and two experimental periods of 25 d. Goats were blocked by BW and milk production (4 animals per block). Within each block, animals were randomly assigned to dietary treatments and all four diets were represented in all blocks. During the whole study, goats were placed in individual cages and machine milked (DeLaval, Madrid, Spain) before morning feeding. In both experiments, the basal diet consisted of alfalfa hay and concentrate in a 33 : 67 ratio. In experiment 1, the concentrate consisted of (g/kg, as fed): maize, 356.0; barley, 356.0; and soybean meal, 250.0. The concentrate of experiment 2 included (g/kg, as fed): soybean hulls, 356.0; maize, 178.0; barley, 178.0; and soybean meal, 250.0. As a result, the starch-to-nonforage neutral detergent fibre (NDF) ratio of the diet was 3.1 and 0.8 in the experiments 1 and 2, respectively. In both experiments, basal diets were offered either alone or in

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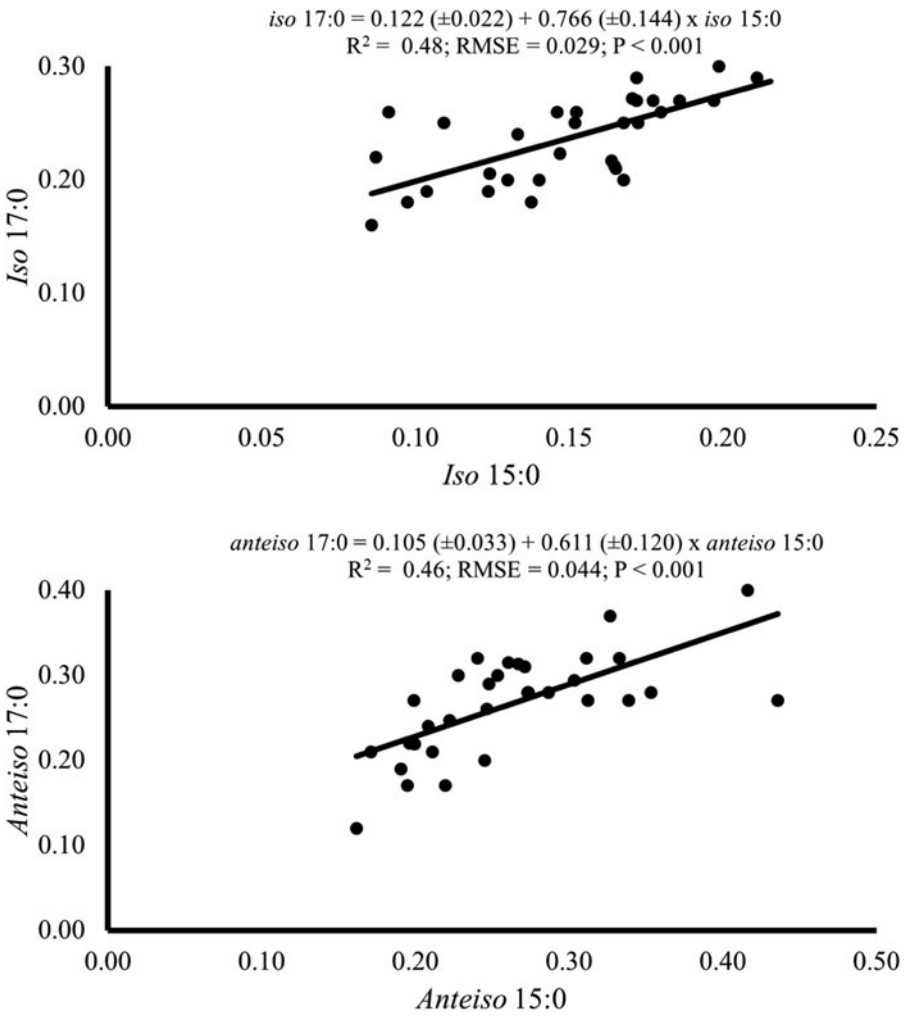

Fig. 1. Relationship between *iso* 15:0 and *iso* 17:0 as well as *anteiso* 15:0 and *anteiso* 17:0 contents (g/100 g total fatty acid methyl esters) in milk fat from goats fed with different concentrates and supplemented or not with linseed oil. RMSE, root mean square error.

combination with 30 g/d of linseed oil. Body weight of the animals was recorded to the nearest 0.1 kg at the beginning and at the end of each experimental period. Milk samples were collected the last day of each experimental period and stored at −20 °C until analysis.

A total of 32 milk fat samples were obtained and analysed according to Bichi *et al.* (2012). Milk fat extraction was carried out by two consecutive centrifugations and fatty acid methyl esters were prepared by base-catalysed methanolysis of glycerides with KOH in methanol. Two capillary columns (CP-Sil88 100m and SLB-IL111 100m) were used in the chromatographic analysis, in order to prevent possible coelutions and to carry out a proper BCFA quantification.

The regression model proposed by Palmquist *et al.* (2004) was used to calculate the proportions of *iso* 17:0 and *anteiso* 17:0 that were postruminally synthesized from their putative precursors *iso* 15:0 and *anteiso* 15:0. Data were analysed with the REG procedure of SAS 3.5 University Edition (SAS Institute Inc., Cary, NC, USA). The dependent variables were *iso* 17:0 and *anteiso* 17:0 and the independent variables were *iso* 15:0 and *anteiso* 15:0, respectively. According to Palmquist *et al.* (2004), the intercept of the regression equation would correspond to the rumen origin fraction of the dependent variable (C17 BCFA). For our purposes, the regression coefficient allows calculation of the fraction of the independent variable (C15 BCFA) that is converted to the dependent variable (C17 BCFA) by applying the relationship $\beta1/(1+\beta1)$, where β1 is the regression coefficient.

Results and discussion

The contents of *iso* 15:0, *anteiso* 15:0, *iso* 17:0 and *anteiso* 17:0 in milk fat were 0.15 ± 0.036, 0.26 ± 0.066, 0.24 ± 0.039 and 0.27 ± 0.060 g/100 g total fatty acid methyl esters. The pairs *iso* 15:0/*iso* 17:0 and *anteiso* 15:0/*anteiso* 17:0 showed a strictly linear relationship (Fig. 1). The regression coefficients allowed us to calculate that 43% of *iso* 15:0 and 38% of *anteiso* 15:0 would have been converted to *iso* 17:0 and *anteiso* 17:0, respectively. Correspondingly 49% of total *iso* 17:0 and 60% of total *anteiso* 17:0 in milk fat derived from *iso* 15:0 and *anteiso* 15:0. These results would be in line with those derived from in vivo data obtained in dairy cows by Vlaeminck *et al.* (2015). These authors found that the proportions *iso* 15:0/*iso* 17:0 and *anteiso* 15:0/*anteiso* 17:0 in milk fat were lower than those observed in duodenal digesta, which suggest that chain elongation processes on BCFA would occur in extraruminal tissues. Furthermore, the lower ratio *iso* 15:0 to *iso* 17:0 and *anteiso* 15:0 to *anteiso* 17:0 that those authors observed in plasma triacylglycerols and non-esterified fatty acids as compared with duodenal samples might indicate that elongation would take place before uptake by the mammary gland, as discussed by Vlaeminck *et al.* (2015). The extramammary elongation of *iso* 15:0 and *anteiso* 15:0 with 2 carbon atoms would be also supported by the fact that ruminal infusions of isovalerate and 2-methylbutyrate, the primers for the *de novo* synthesis in the

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mammary gland, did not result in increased secretions of *iso* or *anteiso* 17:0 in milk (French *et al*., 2012).

Enrichment of plasma non-esterified fatty acids with C17 BCFA would also be possible during mobilization of adipose tissue in negative energy balance periods, thus providing more *iso* 17:0 and *anteiso* 17:0 to the mammary gland (Craninx *et al*., 2008). Nevertheless, that confounding factor was not present in the present work because no weight changes were observed in the animals and they were well after the peak of lactation (118 ± 16 d in milk).

Although fatty acid elongase 6 (ELOVL6) activity has been well recognized in humans (Wang *et al*., 2017), its role and importance in ruminant tissues remain less clear. Moreover, from the current research there is not enough evidence to make a definitive conclusion about the origin of the extraruminal C17 BCFA in milk. In this regard, Castro-Carrera *et al*. (2015) observed in dairy ewes that the mRNA abundance of ELOVL6 genes was higher in subcutaneous and perirenal adipose tissues than in the mammary tissue. However, the possibility that endogenous BCFA elongation could also take place, at least partially, in mammary tissue should not be ruled out. The genes coding ELOVL6 have been detected in mammary epithelial cells of goats (Xu *et al*., 2016) and, an in vitro study has directly assessed the role of ELOVL6 in the elongation of fatty acids in caprine mammary cells (Shi *et al*., 2017).

The elongation indexes of *iso* 17:0 and *anteiso* 17:0, calculated as [product/(substrate + product)], that were obtained in the present research did not differ ($P > 0.05$) between diets supplemented and unsupplemented with linseed oil (0.60 *vs*. 0.62 for *iso* 17:0 and 0.49 *vs*. 0.51 for *anteiso* 17:0). These results agree with the absence of effects on the expression of genes coding ELOVL6 in adipose and mammary tissues of dairy ewes due to sunflower oil supplementation that was found by Castro-Carrera *et al*. (2015).

In conclusion, the linear regression model applied in the current study supports the idea that a relevant fraction of 17C BCFA in milk fat would be synthesized in endogenous tissues *via* the elongation of 15C BCFA.

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References

Bichi E, Toral PG, Hervás G, Frutos P, Gómez-Cortés P, Juárez M and De la Fuente MA (2012) Inhibition of Δ9-desaturase activity with sterculic acid: effect on the endogenous synthesis of cis-9 18:1 and cis-9 trans-11 18:2 in dairy sheep. *Journal of Dairy Science* **95**, 5242–5252.

Castro-Carrera T, Frutos P, Leroux C, Chilliard Y, Hervás G, Belenguer A, Bernard L and Toral PG (2015) Dietary sunflower oil modulates milk fatty acid composition without major changes in adipose and mammary tissue fatty acid profile or related gene mRNA abundance in sheep. *Animal: An International Journal of Animal Bioscience* **9**, 582–591.

Cívico A, Núñez Sánchez N, Gómez-Cortés P, De la Fuente MA, Peña Blanco F, Juárez M, Schiavone A and Martínez Marín AL (2017) Odd- and branched-chain fatty acids in goat milk as indicators of the diet composition. *Italian Journal of Animal Science* **16**, 68–74.

Craninx M, Steen A, Van Laar H, Van Nespen T, Martin-Tereso J, De Baets B and Fievez V (2008) Effect of lactation stage on the odd-and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. *Journal of Dairy Science* **91**, 2662–2677.

European Commission (2010) Directive 2010/63/EU of the European Parliament and of 58 the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of European Union* **276**, 33–79.

Fievez V, Colman E, Castro-Montoya JM, Stefanov I and Vlaeminck B (2012) Milk odd-and branched-chain fatty acids as biomarkers of rumen function—an update. *Animal Feed Science and Technology* **172**, 51–65.

French EA, Bertics SJ and Armentano LE (2012) Rumen and milk odd-and branched-chain fatty acid proportions are minimally influenced by ruminal volatile fatty acid infusions. *Journal of Dairy Science* **95**, 2015–2026.

Palmquist DL, St. Pierre N and McClure KE (2004) Tissue fatty acid profiles can be used to quantify endogenous rumenic acid synthesis in lambs. *Journal of Nutrition* **134**, 2407–2414.

Shi HB, Wu M, Zhu JJ, Zhang CH, Yao DW, Luo J and Loor JJ (2017) Fatty acid elongase 6 plays a role in the synthesis of long-chain fatty acids in goat mammary epithelial cells. *Journal of Dairy Science* **100**, 4987–4995.

Vlaeminck B, Fievez V, van Laar H, Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM and Dewhurst RJ (2006) Factors affecting odd- and branched-chain fatty acids in milk: A review. *Animal Feed Science and Technology* **131**, 389–417.

Vlaeminck B, Gervais R, Rahman MM, Gadeyne F, Gorniak M, Doreau M and Fievez V (2015) Postruminal synthesis modifies the odd- and branched-chain fatty acid profile from the duodenum to milk. *Journal of Dairy Science* **98**, 4829–4840.

Xu HF, Luo J, Zhao WS, Yang YC, Tian HB, Shi HB and Bionaz M (2016) Overexpression of SREBP1 (sterol regulatory element binding protein 1) promotes de novo fatty acid synthesis and triacylglycerol accumulation in goat mammary epithelial cells. *Journal of Dairy Science* **99**, 783–795.

Wang Z, Park HG, Wang DH, Goykhman Y, Lawrence P, Kothapalli KSD and Brenna JT (2017) ELOVL3, 6 and 7 specifically elongate saturated branched chain fatty acids and compete with normal fatty acids. *FASEB Journal* **31**(suppl. 1), Abs. 137.8.

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