Journal of Dairy Research (2017) **84** 264–271. doi:10.1017/S0022029917000371

Mammary gland and milk fatty acid composition of two dairy goat breeds under feed-restriction

Mariana Palma[1]†, Susana P. Alves[2], Lorenzo E. Hernández-Castellano[3,4], Juan Capote[5], Noemí Castro[4], Anastasio Argüello[4], Manolis Matzapetakis[1], Rui J. B. Bessa[2] and André M. de Almeida[6]*‡

[1] ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
[2] CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária da Universidade de Lisboa, Lisboa, Portugal
[3] Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland
[4] Animal Production and Biotechnology Group, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, Arucas, Gran Canaria, Spain
[5] ICIA – Instituto Canario de Investigaciones Agrarias, Valle Guerra, Tenerife, Spain
[6] Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

Received 7 December 2016; accepted for publication 4 July 2017

Goat dairy products are an important source of animal protein in the tropics. During the dry season, pasture scarcity leads animals to lose up to 40% of their body weight, a condition known as Seasonal Weight Loss (SWL) that is one of the major constraints in ruminant production. Breeds with high tolerance to SWL are relevant to understand the physiological responses to pasture scarcity so they could be used in programs for animal breeding. In the Canary Islands there are two dairy goat breeds with different levels of tolerance to SWL: the *Palmera*, susceptible to SWL; and the *Majorera*, tolerant to SWL. Fat is one of the milk components most affected by environmental and physiological conditions. This study hypothesises that feed-restriction affects *Majorera* and *Palmera* breeds differently, leading to different fatty acid profiles in the mammary gland and milk. An interaction between breed and feed-restriction was observed in the mammary gland. Feed-restriction was associated with an increase in oleic acid and a decrease in palmitic acid percentage in the *Palmera* breed whereas no differences were observed in the *Majorera* breed. Palmitic and oleic acids together constituted around 60% of the total fatty acids identified, which suggests that *Palmera* breed is more susceptible to SWL. In milk, feed-restriction affected both breeds similarly. Regarding the interaction of the breed with the treatment, we also observed similar responses in both breeds, but this influence affects only around 2% of the total fatty acids. In general, *Majorera* breed is more tolerant to feed-restriction.

Keywords: Goat, milk, mammary gland, fatty acids, feed-restriction.

Goats (*Capra hircus*) have particular importance in tropical and sub-tropical environments, especially for milk and dairy products production. Goat dairy products are an important source of animal protein and a significant income in developing countries (Haenlein, 2001; Boyazoglu et al. 2005). These products have also an increased demand as substitute of cow milk and dairy products (Sánchez-Macías et al. 2012, 2013), particularly as a healthier gourmet product.

In the tropics and the Mediterranean, milk yields are affected by pasture availability and nutritional quality. During the dry season, pasture scarcity leads animals to lose up to 40% of their body weight, a condition known as seasonal weight loss – SWL; (Cardoso & Almeida, 2013). Seasonal feed scarcity is a major limitation in extensive ruminant production in various regions around the World (Almeida et al. 2013; Lérias et al. 2013). However, some breeds from drought-prone regions have evolved high tolerance to SWL. Those breeds have special relevance in breeding programs with special interest in food supply and economics (Cardoso & Almeida, 2013).

†Present address: Department of Life Sciences, CFE – Center for Functional Ecology, University of Coimbra, Coimbra, Portugal.
*For correspondence; e-mail: aalmeida@fmv.utl.pt
‡Present address: Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal.

The Canary Islands are comprised of a subtropical archipelago with different rain patterns among the islands. Generally speaking, the eastern islands are drier than the western islands, affecting agriculture and animal production. In the Canary Islands, two major goat breeds evolved from a common ancestry of African and Iberian origin with different levels of tolerance to SWL (Fresno et al. 1994; Amills et al. 2004). The *Palmera* breed (from the Westernmost Palma Island) is adapted to the rainy climate and is considered to be susceptible to SWL (Escuder et al. 2006), while the *Majorera* breed (from the Easternmost Island of Fuerteventura) is very well adapted to arid environments and is thus expected to be tolerant to SWL (Fresno et al. 1994; Fernández et al. 2011). We conducted an experiment to study the effects of feed-restriction on fatty acid composition of the mammary gland and milk of the aforementioned goat breeds. Previous studies from our group have reported the effects of feed-restriction in milk yields and body live weight (Lérias et al. 2013), in mammary gland proteomics (Cugno et al. 2016; Hernández-Castellano et al. 2016a) and in metabolome profiles (Lérias et al. 2015; Palma et al. 2016a). In this work we study how *Palmera* and *Majorera* breeds react to feed-restriction, by profiling fatty acid in the mammary gland and in the milk.

These results will contribute to a more detailed systematic interpretation of the responses associated with feed-restriction and SWL, adding valuable information about its fatty acid composition and nutritional characteristics. Fat is one of the milk components that highly influence the organoleptic properties of milk and dairy products (Bauman & Griinari, 2003). Additionally, fat is also one of the components most affected by environmental and physiological conditions (Bauman et al. 2006).

Climate changes are unbalancing the global climate equilibrium and drylands could be pushed to a drier condition. Moreover, some current non-dryland regions could also evolve for a drought-prone situation increasing the global arid area. Breed selection of SWL tolerant animals could also be an interesting approach to consider for land and water use management. In this context, the effects of SWL in ruminants could affect regions that are not currently affected by this issue, including developed countries (Huang et al. 2016).

Therefore, outcomes from the present study will also help to define strategies in dairy product optimisation and selection with application to other ruminant species in drought-prone regions. Breeds with higher tolerance to SWL effects, like Majorera breed, could have a special role to cope with futures issues related to water scarcity and pasture availability.

Material and methods

Animal experiment

The study was conducted at the experimental farm of the Faculty of Veterinary Medicine of the Universidad de Las Palmas de Gran Canaria (Arucas, Gran Canaria, Spain). The study period consisted of 23 d during May and June of 2012 and included nine *Majorera* and ten *Palmera* adult dairy goats. During this time of the year temperatures ranged 23–29 °C and relative humidy was about 75–80%. Animals were housed in a park with dirt floor. Half the area was covered with a roof to shade the animals from the sun. Detailed information about animals, diet, experimental design and management were described before (Lérias et al. 2013). Briefly, adult goats (three lactations with kidding in late February) were divided in two groups per breed: a control group (*Majorera* $n = 4$ and *Palmera* $n = 6$) and a restricted-fed group (*Majorera* $n = 5$, *Palmera* $n = 4$).

Control groups were fed following the guidelines by the *Institut National de la Recherche Agronomique*, with maize, soy 44 (crude protein 44%), dehydrated lucerne, dehydrated beetroot, lucerne hay, and a vitamin-mineral supplement. The composition of the ration based on dry matter was 6·2% ash, 10·6% crude protein, 10·2% crude fibre and 2% ether extract. The control diet provided 1·81 kg of dry matter, 1·46 UFL, 133 g of metabolisable protein, 12 g of calcium and 6 g of phosphorus. Restricted-fed groups were fed ad-libitum with standard wheat straw, corresponding to a low-level of crude protein (approximately 30 g/kg dry matter), high amounts of fibre (420 g/kg dry matter), and a low energy contents (5·5 MJ/kg dry matter); and vitamin-mineral supplement. Restricted-fed animals were fed to achieve 15–20% reduction of their initial live weight in the end of the trial period. For more details about diet and nutritional information, kindly refer to Lérias et al. (2013, 2015).

The means of the absolute live weight and the means of the daily milk yield, of Majorera and Palmera control and restricted-fed groups were described at Lérias et al. (2013). Mean of the absolute live weight of each group at day zero were as follows: Majorera control: 45·5 ± 7·74 kg; Palmera control: 32·8 ± 4·91 kg; Majorera restricted-fed: 50·6 ± 3·64 kg ; and Palmera restricted-fed: 40·6 ± 2·05 kg. Mean of the absolute live weight of each group at day 23 were as follows: Majorera control: 48·2 ± 7·51 kg; Palmera control: 33·9 ± 5·00 kg; Majorera restricted-fed: 44·1 ± 3·42 kg; and Palmera restricted-fed: 35·4 ± 1·45 kg.

Mammary gland biopsies were collected, after milking, from the left half udder, in the last day of the trial, as previously described (Palma et al. 2016a). Goats were milked once daily at a vacuum pressure of 42 kPa, a pulsation rate of 90 pulses/min, and a pulsation ratio of 60/40 (Hernández-Castellano et al. 2011; Torres et al. 2013), and a 2 ml sample were collected from the whole available milk of each animal of the last day of the experiment. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until processing.

Spanish and European Union guidelines and legislation on care, use and handling of experimental farm animals were followed. Author AM Almeida holds a FELASA grade C certificate enabling the design and conduction of animal experimentation in the European Union.

https://doi.org/10.1017/S0022029917000371 Published online by Cambridge University Press

Fatty acids analysis

Lipids from mammary gland were extracted as described by Folch et al. (1957) and slightly modified by using dichloromethane and methanol (2 : 1, vol/vol). Total lipids were measured gravimetrically, weighing the residue after evaporation of solvents at 37 °C. Extracted lipids were converted to fatty acid methyl esters (FAME) using sodium methoxide in anhydrous methanol (0·5 N) followed by hydrochloric acid in methanol (1 : 1, vol/vol) and 1 mg of nonadecanoic acid was used as internal standard. The FAME from lyophilised milk fat samples were prepared by direct transesterification using KOH in methanol (2 N) and extracted with hexane (Molkentin & Precht, 2000).

FAME from mammary gland and milk samples were then analysed by gas chromatography using a Shimadzu 2010Plus (Shimadzu, Kyoto, Japan), equipped with a flame-ionisation detector and a fused silica capillary column (SP-2560, 100 m, 0·2 mm internal diameter, and 0·20 μM film thickness; Supelco Inc., Bellefonte, PA, USA). Initial oven temperature of 50 °C was held for 1 min, increased at 50 °C/min to 150 °C and held for 20 min, increased at 1 °C/min to 190 °C and then increased at 2 °C/min to 220 °C and held for 18 min. The injector and detector temperatures were maintained at 250 °C. Helium was used as carrier gas at a flow rate of 1 ml/min and 1 μl of sample was injected. Identification of FAME was achieved by comparison of the FAME retention times with those of commercial standard mixtures (FAME mix 37 components from Supelco Inc., Bellefonte, PA, USA) and by electron impact mass spectrometry using a Shimadzu GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan).

Statistical analysis

For univariate analysis, data from both samples were evaluated using a Proc MIXED in SAS (SAS Inst., Cary, NC, USA) with a model that included the treatment (control vs. restricted) and the breed and their interaction as fixed effects. Values are presented as percentage of the total fatty acids identified, with the standard error of the mean, and significance was considered for $P < 0{\cdot}05$.

Multivariate analysis for Principal Component Analysis (PCA) was performed to mammary gland and milk samples, using SIMCA 13.0.3.0 software (Umetrics AB, Umeå, Sweden). For multivariate analysis were considered the percentage of the total fatty acids identified for each fatty acid, in each animal.

Results

The lipid and fatty acid content (mg/g of dry tissue) and fatty acid composition of mammary gland tissue, expressed in percentage of the total fatty acids, is presented in Table 1.

The most representative fatty acids in the mammary gland tissue from all experimental groups were oleic acid (18 : 1*cis*-9), palmitic acid (16 : 0) and stearic acid (18 : 0). These three fatty acids represented 69% of the total fatty acids in the *Palmera* control group and 77% in the *Palmera* restricted-fed group. In the *Majorera* breed, these three fatty acids together (18 : 1*cis*-9 + 16 : 0 + 18 : 0) represent the 74 and 76% of the total fatty acids identified in control and restricted-fed groups, respectively. It is noteworthy that 16 : 0 and 18 : 1*cis*-9 were the only two fatty acids that presented significant interactions between breed and feed-restriction ($P < 0{\cdot}05$). Although the proportions of 16 : 0 and 18 : 1*cis*-9 numerically decreased and increased respectively with feed-restriction in both breeds, those changes were only significant in the *Palmera* breed. Concerning the effect of feed-restriction only, the proportions of 12 : 0, 14 : 0, 14 : 1*cis*-9, 15 : 0, iso-16 : 0, 16 : 0, 18 : 3*n*-3, 18 : 2 *cis*-9, *trans*-11 and a few 18 : 1-*trans* were lower, and only the 17 : 1*cis*-9, 18 : 1*cis*-9 and 20 : 4*n*-6 were higher in the restricted-fed groups compared to the control ones. Thus, the effect of feed-restriction was also observed in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) sums, with the *cis*-MUFA presenting greater proportions in the restricted-fed groups relative to the control groups. However, looking to the indexes used to estimate the activity of stearoyl-CoA desaturase (SCD), only SCD-17 (17 : 1*cis*-9/(17 : 1*cis*-9 + 17 : 0) tended to be higher for the restricted-fed groups compared to the control ones ($P = 0{\cdot}078$).

A PCA of the four experimental groups did not reveal any clear separation (Fig. 1). A slight tendency of sample clustering by breed was observed on the first Principal Component (PC1), however, the results were not robust enough to discuss this separation. Loadings list of this model is presented in online Supplemental Table S1.

The fatty acid composition in milk, expressed as percentage of the total fatty acids, is presented in Table 2. From the ten more abundant fatty acids, eight are SFA representing around 75% of the total fatty acids. This set is completed by linoleic acid (18 : 2*n*-6) and oleic acid (18 : 1*cis*-9), which is the second more abundant component in milk. Margaric acid (17 : 0) and heptadecenoic acid (17 : 1*cis*-9) in milk showed differences due to the interaction between breed and feed-restriction ($P < 0{\cdot}05$). In both breeds the proportions of 17 : 0 and 17 : 1*cis*-9 were higher in restricted-fed groups compared to control groups, however *Majorera* showed the greatest increase in both fatty acids. In this study, the majority of the 46 identified fatty acids were affected by feed-restriction ($P < 0{\cdot}05$), while 10 fatty acids were not affected ($P > 0{\cdot}05$). Eleven fatty acids also showed significant differences between breeds ($P < 0{\cdot}05$), with the short chain-fatty acids, iso-14 : 0, 18 : 0, 20 : 0 and 22 : 0 presenting the highest percentages in the *Palmera* breed and the 14 : 0, 14 : 1*cis*-9, 16 : 1*cis*-9 and 18 : 2 isomers the highest proportions in the *Majorera* breed. Total SFA decreased between control and restricted-fed groups in *Majorera* breed, however no difference was observed in *Palmera* breed. Concerning the content of *cis*-MUFA, restricted-fed groups had higher percentages than control groups. The opposite response was observed to the total *trans*-MUFA,

https://doi.org/10.1017/S0022029917000371 Published online by Cambridge University Press

Table 1. Mean ± SEM of lipids and fatty acid (FA) content (mg/g of tissue DM) and fatty acid composition (% of total FA) in the mammary gland from Majorera and Palmera goat breeds, in control and restricted-fed groups

	Majorera		Palmera		*P*		
	Control	Restricted	Control	Restricted	*B*	*T*	*B* × *T*
Lipids (mg/g MS)	280 ± 37·3	208 ± 33·4	256 ± 30·5	218 ± 37·3	0·854	0·132	0·645
FA (mg/g MS)	153 ± 20·3	85 ± 18·2	122 ± 16·6	80 ± 20·3	0·362	0·011	0·500
8 : 0	0·26 ± 0·091	0·14 ± 0·048	0·42 ± 0·209	0·28 ± 0·088	0·272	0·342	0·937
10 : 0	2·2 ± 0·87	1·1 ± 0·16	3·1 ± 0·96	1·6 ± 0·34	0·291	0·086	0·753
12 : 0	1·43 ± 0·564	0·71 ± 0·128	2·09 ± 0·460	0·76 ± 0·143	0·370	0·024	0·443
i-14 : 0	0·04 ± 0·013	0·04 ± 0·012	0·07 ± 0·011	0·04 ± 0·013	0·220	0·464	0·196
14 : 0	4·0 ± 1·06	3·1 ± 0·26	6·0 ± 0·86	2·6 ± 0·29	0·320	0·014	0·116
i-15 : 0	0·10 ± 0·018	0·10 ± 0·016	0·12 ± 0·015	0·11 ± 0·018	0·297	0·677	0·967
a-15 : 0	0·16 ± 0·034	0·13 ± 0·031	0·22 ± 0·028	0·12 ± 0·034	0·501	0·062	0·304
14 : 1 *cis*-9	0·12 ± 0·019	0·10 ± 0·017	0·13 ± 0·015	0·06 ± 0·019	0·309	0·014	0·184
15 : 0	$0·53^{ab}$ ± 0·064	$0·40^{b}$ ± 0·057	$0·68^{a}$ ± 0·052	$0·33^{b}$ ± 0·064	0·534	0·001	0·078
i-16 : 0	0·21 ± 0·031	0·17 ± 0·028	0·23 ± 0·025	0·13 ± 0·031	0·780	0·034	0·295
16 : 0	$24·2^{ab}$ ± 1·09	$21·4^{b}$ ± 0·98	$27·8^{a}$ ± 0·89	$20·6^{b}$ ± 1·09	0·213	<0·001	0·050
i-17 : 0	$0·26^{a}$ ± 0·028	$0·26^{a}$ ± 0·025	$0·32^{a}$ ± 0·022	$0·22^{a}$ ± 0·028	0·706	0·098	0·076
16 : 1 *cis*-7	0·27 ± 0·038	0·29 ± 0·034	0·28 ± 0·031	0·34 ± 0·038	0·457	0·221	0·611
16 : 1 *cis*-9	1·25 ± 0·314	1·18 ± 0·281	1·29 ± 0·256	0·90 ± 0·314	0·677	0·447	0·590
a-17 : 0	0·47 ± 0·050	0·49 ± 0·044	0·43 ± 0·041	0·36 ± 0·050	0·084	0·570	0·370
17 : 0	0·87 ± 0·133	1·02 ± 0·12	0·72 ± 0·109	0·80 ± 0·133	0·168	0·364	0·785
17 : 1 *cis*-9	0·42 ± 0·104	0·88 ± 0·093	0·45 ± 0·085	0·65 ± 0·104	0·308	0·004	0·204
18 : 0	13·8 ± 2·75	13·7 ± 2·46	11·8 ± 1·28	13·5 ± 1·57	0·614	0·668	0·699
18 : 1 *trans*-6/7/8	0·11 ± 0·017	0·07 ± 0·015	0·13 ± 0·014	0·08 ± 0·017	0·362	0·013	0·776
18 : 1 *trans*-9	0·19 ± 0·024	0·16 ± 0·021	0·19 ± 0·019	0·11 ± 0·024	0·409	0·023	0·256
18 : 1 *trans*-10	0·20 ± 0·029	0·16 ± 0·019	0·29 ± 0·109	0·09 ± 0·025	0·859	0·078	0·255
18 : 1 *trans*-11	$0·49^{a}$ ± 0·067	$0·35^{a}$ ± 0·041	$0·89^{a}$ ± 0·174	$0·32^{a}$ ± 0·074	0·107	0·008	0·066
18 : 1 *cis*-9	$36·0^{ab}$ ± 2·2	$41·8^{a}$ ± 1·9	$30·9^{b}$ ± 1·8	$44·0^{a}$ ± 2·2	0·312	0·001	0·022
18 : 1 *cis*-11	1·04 ± 0·074	1·04 ± 0·067	1·00 ± 0·061	0·93 ± 0·074	0·297	0·622	0·601
18 : 1 *cis*-12	0·33 ± 0·572	0·29 ± 0·051	0·33 ± 0·047	0·20 ± 0·057	0·427	0·124	0·463
18 : 2 isomers	0·33 ± 0·046	0·28 ± 0·042	0·27 ± 0·038	0·17 ± 0·046	0·064	0·098	0·577
18 : 2 *n*-6	4·1 ± 0·55	4·7 ± 0·49	4·3 ± 0·45	4·06 ± 0·55	0·724	0·732	0·441
20 : 0	0·13 ± 0·023	0·17 ± 0·061	0·23 ± 0·019	0·24 ± 0·069	0·120	0·548	0·780
18 : 3 *n*-3	0·39 ± 0·045	0·36 ± 0·040	0·46 ± 0·036	0·28 ± 0·045	0·855	0·024	0·121
18 : 2 *cis*-9,*trans*-11	0·52 ± 0·104	0·28 ± 0·093	0·72 ± 0·085	0·32 ± 0·10	0·237	0·005	0·436
20 : 4 *n*-6	1·2 ± 0·37	3·0 ± 0·33	1·5 ± 0·30	2·36 ± 0·37	0·658	0·001	0·178
22 : 4 *n*-6	$0·24^{a}$ ± 0·060	$0·42^{a}$ ± 0·054	$0·32^{a}$ ± 0·049	$0·25^{a}$ ± 0·373	0·500	0·427	0·098
22 : 5 *n*-3	0·76 ± 0·201	1·20 ± 0·180	0·93 ± 0·164	1·18 ± 0·201	0·706	0·085	0·633
Other	2·38 ± 0·389	1·43 ± 0·348	2·52 ± 0·318	1·89 ± 0·389	0·427	0·046	0·674
SFA	48 ± 2·5	43 ± 2·3	53 ± 2·1	41 ± 2·5	0·382	0·002	0·169
cis-MUFA	41 ± 2·5	45 ± 2·2	34 ± 2·1	48 ± 2·5	0·309	0·002	0·058
trans-MUFA	0·99 ± 0·225	0·74 ± 0·201	1·50 ± 0·184	0·61 ± 0·225	0·375	0·015	0·149
PUFA	6·9 ± 1·13	9·5 ± 1·01	7·8 ± 0·92	8·2 ± 1·13	0·859	0·191	0·311
SCD_i-14	3·1 ± 0·71	3·2 ± 0·638	2·6 ± 0·58	2·2 ± 0·71	0·272	0·846	0·697
SCD_i-16	4·9 ± 1·09	5·3 ± 0·97	4·5 ± 0·89	4·2 ± 1·09	0·476	0·999	0·755
SCD_i-17	34 ± 5·5	47 ± 5·0	37 ± 4·5	44 ± 5·5	0·994	0·078	0·588
SCD_i-18	74 ± 3·1	75 ± 2·8	73 ± 2·5	77 ± 3·1	0·972	0·343	0·569
SCD_i-*t*11	51 ± 3·7	44 ± 3·3	46 ± 3·0	49 ± 3·7	0·938	0·581	0·149

Key: (SEM) standard error of mean; (B) breed; (T) treatment; (FA) fatty acids; (DM) dry matter; (other) sum of other fatty acids; (SFA) saturated fatty acids; (MUFA) monounsaturated fatty acids; (PUFA) polyunsaturated fatty acids; (SCD) estimated stearoyl-Coa desaturase activity indexes; SCD_i-14, (14 : 1/(14 : 1 + 14 : 0) *100); SCD_i-16, (16 : 1/(16 : 1 + 16 : 0)*100); SCD_i-17, (17 : 1/(17 : 1 + 17 : 0)*100); SCD_i-18, (18 : 1/(18 : 1 + 18 : 0)*100). (a, b) within a row, means without a common letter differ ($P < 0·05$).

for which restricted-fed groups showed lower percentage than the control groups. No significant differences were observed between control and restricted-fed groups in the total polyunsaturated fatty acids. Regarding the indexes to estimate SCD activity, with the exception of SCD_i-14, all indexes were higher ($P < 0·001$) in restricted-fed animals relative to the control ones. In addition, *Majorera* breed showed higher indexes compared to *Palmera* breed ($P < 0·05$). No interaction between breed and feed-restriction was detected in any of the estimated indexes ($P > 0·05$).

https://doi.org/10.1017/S0022029917000371 Published online by Cambridge University Press

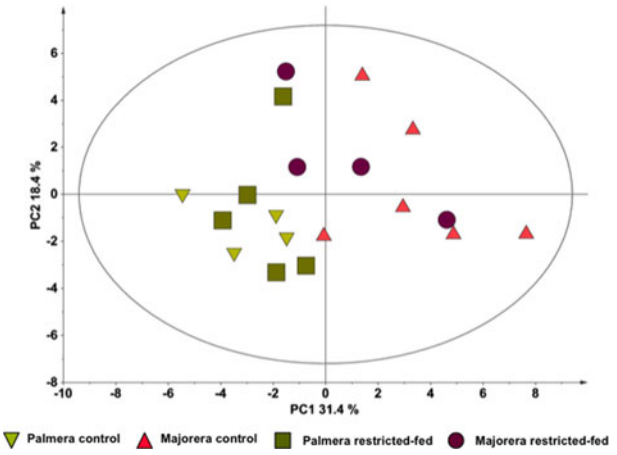

Fig. 1. Multivariate analysis of mammary gland fatty acids percentages: Principal Component Analysis scores of the four experimental groups (NC = 4; PC1 = 31·4%; PC2 = 18·4%; ellipse: Hostelling's T2 (95%)).

A PCA of the four experimental groups revealed separation due to feed-restriction, along to the first PC1 (Fig. 2). The loadings values for this model (online Supplemental Table S2), allowed us to identify the fatty acids responsible for the major separation between control and restricted groups. In this model, separation was due to differences in 18 : 1*cis*-9, 17 : 1cis-9, 15 : 0 and 10 : 0. It is noteworthy to mention that most relevant loadings were fatty acids with differences in univariate analysis, either due to feed-restriction (18 : 1*cis*-9 and 10 : 0) or the interaction between breed and feed-restriction (17 : 1*cis*-9 and 17 : 0).

Discussion

We have previously studied the effect of weight loss in these two breeds at the level of blood biochemistry parameters (Lérias et al. 2015), the mammary gland proteome (Cugno et al. 2016; Hernández-Castellano et al. 2016b) and the mammary gland metabolome (Palma et al. 2016a). These studies point to a differential response to weight loss, albeit relative live weight and milk yield changes seem to be minimal (Lérias et al. 2013). In the present study, we focus on the changes occurring in the mammary gland and milk, with a specific focus on fatty acid composition. The mammary gland presented significant interactions between breed and feed-restriction for 18 : 1*cis*-9 and 16 : 0, which also were the two major fatty acids identified in those samples. Apparently, *Majorera* goats were able to maintain the fatty acid composition of the mammary gland when they were under feed-restriction, whereas the *Palmera* breed goats clearly increased the concentration of 18 : 1*cis*-9 and decreased the concentration of 16 : 0 as a consequence of the feed-restriction. Due to the critical role of the mammary gland with respect to survival of the ruminant neonate (Hernández-Castellano et al. 2015, 2016b), it is likely that this tissue has developed significant tolerance to external influence, keeping its integrity in order to preserve functions. Thus, the superior ability of *Majorera* goats in maintaining the mammary gland fatty acid composition, compared to *Palmera* goats, may be attributed to its higher tolerance to SWL. On the contrary, as *Palmera* goats are more susceptive to SWL, the decrease in 16 : 0 and increase of 18 : 1*cis*-9 found in the fed-restricted *Palmera* group may be caused by an extensive fat mobilisation in order to compensate feed-restriction as a means to maintain milk production (Chilliard et al. 2000).

The fat mobilisation above described for the Palmera goats' mammary gland as well as the changes previously described for the same breed regarding the mammary proteome (Hernández-Castellano et al. 2016b), essentially related to an increase in apoptotic pathway in the Palmera breed and an expression increase in immune system related proteins in the Majorera breed, could be proposed to have effects in milk fatty acid composition. The milk fatty acid composition, however, seems to be largely affected by feed-restriction irrespective of the breed. In our experiment, interaction between breed and feed-restriction was only observed in two minor fatty acids (17 : 0 and 17 : 1 *cis*-9). These two fatty acids represent approximately 2% of the total milk fatty acids identified, indicating the small influence of breed in milk variations. In contrast, the more predominant fatty acids in milk such as 10 : 0, 12 : 0, 14 : 0, 16 : 0, 18 : 0 and 18 : 1 *cis*-9 were only affected by feed-restriction, suggesting the direct influence of the diet on milk composition. This is particularly important as the restricted diet is very poor from the quantitative and qualitative point of view. Milk fatty acid composition seems to be more affected by feed-restriction as observed in the multivariate analysis of milk fat percentages (Fig. 2). Milk fat can arise from two sources, mammary gland de novo lipogenesis (mainly the short and medium chain fatty acids, including about 50% of the 16 : 0) and uptake of fatty acids from circulation (mainly C16 : 0 and C18 : 0 fatty acids; Adewuyi et al. 2005). Uptake of fatty acids into the mammary gland mostly comprises the circulating lipoproteins, derived either from the digestive tract or from hepatic reassembly of non-esterified fatty acids (NEFA) mobilised from body fat reserves. Utilisation of fat depots are especially important in early lactation given that up to 40% of the milk fat is derived from mobilised fatty acids (Adewuyi et al. 2005). During early lactation and undernutrition periods, adipose tissues not only contributes to milk fat secretion, but also to supply energy to other tissues, sparing glucose and amino acids for the mammary gland (Chilliard et al. 2000). Milk fatty acid composition is quite susceptible to energy balance status (Bauman et al. 2006). In particular, when the energy balance is negative, animals mobilise stored lipids in the adipose tissue to be used for energy production, mainly the 16 : 0, 18 : 0 and 18 : 1*cis*-9 (Chilliard et al. 2003). In our study, the substantial reduction in the proportions of de novo synthetised fatty acids, including the 16 : 0, and the increased ratio of

https://doi.org/10.1017/S0022029917000371 Published online by Cambridge University Press

Table 2. Mean ± SEM of lipids and fatty acid (FA) content (mg/g of tissue DM) and fatty acid composition (% of total FA) in milk from Majorera and Palmera goat breeds, in control and restricted-fed groups

	Majorera		Palmera		*P*		
	Control	Restricted	Control	Restricted	*B*	*T*	*B* × *T*
4 : 0	1·80 ± 0·080	1·37 ± 0·071	1·89 ± 0·065	1·66 ± 0·080	0·023	<0·001	0·203
6 : 0	2·27 ± 0·094	1·54 ± 0·084	2·49 ± 0·077	1·79 ± 0·094	0·016	<0·001	0·845
8 : 0	2·80 ± 0·135	1·77 ± 0·121	3·18 ± 0·110	2·02 ± 0·135	0·025	<0·001	0·599
10 : 0	11·41 ± 0·402	5·44 ± 0·359	11·97 ± 0·328	5·74 ± 0·402	0·269	<0·001	0·734
12 : 0	5·67 ± 0·433	2·01 ± 0·387	5·38 ± 0·353	1·96 ± 0·433	0·678	<0·001	0·767
i-14 : 0	0·06 ± 0·080	0·07 ± 0·080	0·08 ± 0·080	0·08 ± 0·080	0·308	0·669	0·545
14 : 0	11·44 ± 0·405	6·12 ± 0·362	10·08 ± 0·330	5·76 ± 0·405	0·038	<0·001	0·209
i-15 : 0	0·13 ± 0·013	0·14 ± 0·012	0·16 ± 0·011	0·15 ± 0·013	0·149	0·789	0·262
a-15 : 0	0·31 ± 0·024	0·20 ± 0·021	0·33 ± 0·019	0·23 ± 0·024	0·347	<0·001	0·704
14 : 1 *cis*-9	0·24 ± 0·019	0·14 ± 0·017	0·14 ± 0·015	0·09 ± 0·019	<0·001	<0·001	0·123
15 : 0	1·10 ± 0·040	0·56 ± 0·036	1·04 ± 0·032	0·51 ± 0·040	0·148	<0·001	0·850
i-16 : 0	0·22 ± 0·020	0·19 ± 0·018	0·23 ± 0·017	0·18 ± 0·020	0·811	0·047	0·678
16 : 0	32·6 ± 0·99	21·5 ± 0·89	29·9 ± 0·81	21·9 ± 0·99	0·247	<0·001	0·106
i-17 : 0	0·24 ± 0·019	0·39 ± 0·017	0·27 ± 0·016	0·37 ± 0·019	0·737	<0·001	0·149
16 : 1 *cis*-7	0·24 ± 0·019	0·27 ± 0·017	0·27 ± 0·016	0·26 ± 0·019	0·631	0·578	0·216
16 : 1 *cis*-9	0·75 ± 0·051	0·99 ± 0·046	0·57 ± 0·042	0·88 ± 0·051	0·009	<0·001	0·494
a-17 : 0	0·35 ± 0·027	0·46 ± 0·024	0·37 ± 0·022	0·41 ± 0·027	0·509	0·010	0·208
17 : 0	$0·54^{c}$ ± 0·036	$1·03^{a}$ ± 0·032	$0·63^{c}$ ± 0·030	$0·87^{b}$ ± 0·036	0·324	<0·001	0·003
17 : 1 *cis*-9	$0·25^{c}$ ± 0·045	$0·80^{a}$ ± 0·040	$0·25^{c}$ ± 0·036	$0·57^{b}$ ± 0·045	0·012	<0·001	0·015
18 : 0	5·84 ± 0·534	7·88 ± 0·478	7·81 ± 0·436	9·50 ± 0·534	0·003	0·002	0·733
18 : 1 *trans*-6/7/8	0·15 ± 0·014	0·09 ± 0·012	0·17 ± 0·011	0·09 ± 0·014	0·706	<0·001	0·318
18 : 1 *trans*-9	0·16 ± 0·014	0·16 ± 0·012	0·17 ± 0·011	0·14 ± 0·014	0·826	0·245	0·281
18 : 1 *trans*-10	0·24 ± 0·030	0·16 ± 0·027	0·23 ± 0·025	0·13 ± 0·030	0·437	0·004	0·692
18 : 1 *trans*-11	0·53 ± 0·101	0·29 ± 0·090	0·85 ± 0·083	0·29 ± 0·101	0·115	<0·001	0·116
18 : 1 *trans*-12	0·33 ± 0·026	0·16 ± 0·023	0·32 ± 0·021	0·13 ± 0·026	0·464	<0·001	0·708
18 : 1 *cis*-9	15·0 ± 1·06	39·8 ± 0·95	15·7 ± 0·87	38·9 ± 1·06	0·902	<0·001	0·412
18 : 1 *trans*-15	0·15 ± 0·009	0·08 ± 0·008	0·14 ± 0·007	0·06 ± 0·009	0·159	<0·001	0·673
18 : 1 *cis*-11	0·42 ± 0·034	0·64 ± 0·031	0·42 ± 0·028	0·59 ± 0·034	0·451	<0·001	0·551
18 : 1 *cis*-12	0·27 ± 0·030	0·26 ± 0·027	0·24 ± 0·024	0·19 ± 0·030	0·106	0·319	0·510
18 : 1 *cis*-13	0·04 ± 0·003	0·05 ± 0·003	0·04 ± 0·003	0·05 ± 0·003	0·548	<0·001	0·699
18 : 1 *trans*-16	0·25 ± 0·015	0·14 ± 0·013	0·25 ± 0·012	0·12 ± 0·015	0·373	<0·001	0·442
18 : 1 *cis*-15	0·07 ± 0·048	0·05 ± 0·043	0·07 ± 0·039	0·04 ± 0·048	0·417	<0·001	0·167
18 : 2 isomers	0·43 ± 0·038	0·42 ± 0·034	0·35 ± 0·031	0·29 ± 0·038	0·006	0·287	0·547
18 : 2 *n*-6	2·10 ± 0·182	3·18 ± 0·163	2·13 ± 0·149	2·57 ± 0·182	0·111	<0·001	0·079
20 : 0	0·13 ± 0·008	0·13 ± 0·007	0·17 ± 0·007	0·15 ± 0·008	0·001	0·107	0·189
18 : 3 *n*-6	0·03 ± 0·048	0·04 ± 0·048	0·03 ± 0·048	0·04 ± 0·048	0·711	0·003	0·606
18 : 3 *n*-3	0·45 ± 0·062	0·38 ± 0·055	0·54 ± 0·050	0·26 ± 0·062	0·062	0·004	0·209
20 : 1	0·04 ± 0·005	0·07 ± 0·004	0·04 ± 0·004	0·07 ± 0·005	0·717	<0·001	0·977
18 : 2 *cis*-9,*trans*-11	0·42 ± 0·063	0·35 ± 0·056	0·51 ± 0·051	0·27 ± 0·063	0·910	0·019	0·177
21 : 0	0·05 ± 0·006	0·04 ± 0·005	0·06 ± 0·005	0·04 ± 0·006	0·159	0·004	0·377
22 : 0	0·05 ± 0·006	0·05 ± 0·006	0·07 ± 0·005	0·06 ± 0·006	0·034	0·416	0·363
20 : 4 *n*-6	0·21 ± 0·022	0·38 ± 0·020	0·22 ± 0·018	0·33 ± 0·022	0·409	<0·001	0·141
20 : 5 *n*-3	0·05 ± 0·004	0·05 ± 0·004	0·05 ± 0·003	0·05 ± 0·004	0·233	0·914	0·855
22 : 4 *n*-6	0·02 ± 0·004	0·03 ± 0·004	0·04 ± 0·003	0·03 ± 0·004	0·283	0·453	0·114
22 : 5 *n*-3	0·09 ± 0·013	0·14 ± 0·011	0·11 ± 0·010	0·11 ± 0·013	0·869	0·037	0·071
22 : 6 *n*-3	0·01 ± 0·004	0·03 ± 0·004	0·02 ± 0·003	0·02 ± 0·004	0·541	0·025	0·187
SFA	77·0 ± 1·26	50·9 ± 1·13	76·1 ± 1·03	77·0 ± 1·26	0·489	<0·001	0·157
cis-MUFA	17·3 ± 1·14	43·1 ± 1·02	17·8 ± 0·93	41·6 ± 1·14	0·630	<0·001	0·383
trans-MUFA	1·80 ± 0·178	1·07 ± 0·159	2·12 ± 0·145	0·95 ± 0·178	0·577	<0·001	0·204
PUFA	3·84 ± 0·306	4·97 ± 0·273	4·01 ± 0·249	4·00 ± 0·306	0·179	0·067	0·064
SCD_i-14	2·03 ± 0·178	2·20 ± 0·159	1·34 ± 0·145	1·65 ± 0·178	0·002	0·172	0·675
SCD_i-16	2·26 ± 0·178	4·39 ± 0·159	1·90 ± 0·145	3·86 ± 0·178	0·054	<0·001	0·694
SCD_i-17	31·9 ± 1·52	43·6 ± 1·37	28·3 ± 1·25	39·3 ± 1·52	0·015	<0·001	0·799
SCD_i-18	72·0 ± 1·38	83·5 ± 1·23	66·9 ± 1·25	39·3 ± 1·13	0·006	<0·001	0·482
SCD_i-*t*11	43·5 ± 1·82	54·2 ± 1·63	37·8 ± 1·49	48·9 ± 1·82	0·005	<0·001	0·909

Key: (SEM) standard error of mean; (B) breed; (T) treatment; (FA) fatty acids; (DM) dry matter; (other) sum of other fatty acids; (SFA) saturated fatty acids; (MUFA) monounsaturated fatty acids; (PUFA) polyunsaturated fatty acids; (SCD) estimated stearoyl-Coa desaturase activity indexes; SCD_i-14, (14 : 1/(14 : 1 + 14 : 0) *100); SCD_i-16, (16 : 1/(16 : 1 + 16 : 0)*100); SCD_i-17, (17 : 1/(17 : 1 + 17 : 0)*100); SCD_i-18, (18 : 1/(18 : 1 + 18 : 0)*100). (a, b) within a row, means without a common letter differ ($P < 0·05$).

https://doi.org/10.1017/S0022029917000371 Published online by Cambridge University Press

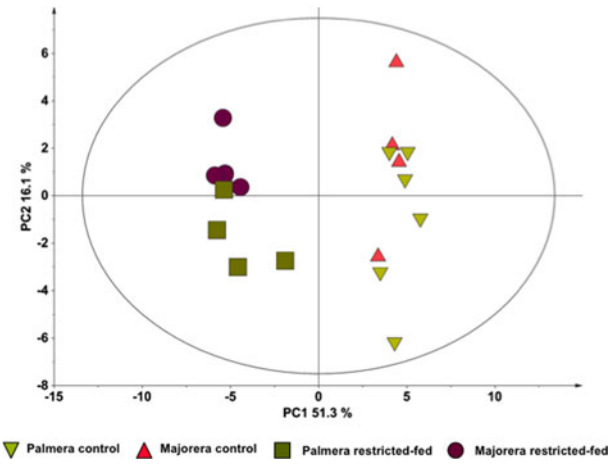

Fig. 2. Multivariate analysis of milk fatty acids percentages: Principal Component Analysis scores of the four experimental groups (NC = 3; PC1 = 51·3%; PC2 = 16·1%; ellipse: Hostelling's T2 (95%)).

18 : 1*cis*-9 and 18 : 0 in milk from the restricted-fed groups compared to the control ones are consistent with an extensive fat tissue mobilisation and lack of dietary derived precursors for the de novo fatty acid synthesis. Previous studies on the same animals, analysed the influence of feed-restriction in blood metabolites and protein expression in the mammary gland secretory tissue (Lérias et al. 2015; Hernández-Castellano et al. 2016a), supporting these conclusions. Namely, NEFA increased in blood from control to restricted-fed groups due to higher metabolisation of fatty acids depots (Lérias et al. 2015). Concerning protein expression, proteins related to fat biosynthesis decreased as a consequence of the feed-restriction in the *Majorera* and *Palmera* breeds (Hernández-Castellano et al. 2016a).

Cis-MUFA percentage in milk increased due to feed-restriction, in particular in the 16 : 1*cis*-9, 17 : 1*cis*-9 and 18 : 1*cis*-9. This suggests a high SCD activity, which is responsible for synthesising mostly the *cis*-9 MUFA from their respective saturated fatty acid. In fact, the SCD product/substrate ratio, computed with the fatty acid present in milk, used to estimate the overall SCD activity supports these findings. Moreover, the SCD seems to be more active in *Majorera* than in the *Palmera* breed.

Previous studies on the same animals reported no differences between breeds in body live weight and milk production yields (Lérias et al. 2013). However, we observed variations in mammary gland due to the interaction between breed and feed-treatment, and variations in milk due both to breed and the interaction between breed and the feed-restriction. Moreover, no differences between breeds were observed in the metabolic profiles of mammary gland and milk, of the same animals (Palma et al. 2016a). These results could mean that besides no differences between breeds in body weight, milk yields and metabolite profiles, the nutritional restriction lead to a differential response in mammary gland and milk fatty acid composition. Considering that animals were similar in the beginning of the trial, fatty acids pathways could then be one of the first affected by the feed-restriction.

The results presented here could also be interpreted in relation to the nutritional quality of the ration presented to the animals. Indeed, the restricted fed goats had access only to poor quality wheat straw, clearly below their nutritional needs from both the qualitative and the quantitative perspectives. Such nutritional quality changes will likely affect the milk output and the fatty acids metabolisms involved in milk secretion and at the level of lipid reserves in the whole body and the mammary gland. Results from proteomics studies conducted with these animals seem, however, to demonstrate that Palmera and Majorera goats react differently to weight loss by activating different metabolic pathways (see Hernández-Castellano et al. 2016a for more details). As such it looks particularly pertinent to extend the fatty acid composition analysis to other organs in order to relate fatty acid metabolism with the status of the lipid body reserves of these animals. Organs of interest would putatively include the skeletal muscle, the liver (Palma et al. 2016b) and adipose tissues (Alves et al. 2015). Such an approach would also be interesting to link the effect of feed restriction (quantitative and qualitative) on fatty acid profiles of the mammary gland and the milk.

In summary, the differential response patterns observed in this study between mammary gland tissue and milk could be attributed to the high level of organisation of the mammary epithelium. This tissue is specialised in converting circulating nutrients in milk components, and integrates several secretory and regulatory pathways of the mammary gland, which increases the adaptability of the tissue (McManaman & Neville, 2003; Bauman et al. 2006). In milk, the interaction of breed and restricted-fed was not significant, affecting less than 2% of the total fatty acids. In both breeds, mammary gland fatty acid composition responded differently according to feed restriction, which may indicate the higher tolerance of *Majorera* breed to SWL.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029917000371.

Authors acknowledge the collaboration of A. Morales-deLaNuez, A. Suárez-Trujillo, P. Delgado-delOlmo, D. Martell-Jaizme and G. Cugno for their collaboration in sample collection. Authors are members of COST action FA1308 – Dairy Care to whom networking support is acknowledged. This work was funded by research project PTDC/CVT/116499/2010 from *Fundação para a Ciência e Tecnologia* (FCT, Lisbon, Portugal). Authors M. Palma and S.P. Alves are funded by FCT grants SFRH/BD/85391/2012 and SFRH/BPD/76836/2011, respectively.

References

Adewuyi AA, Gruys E & van Eerdenburg FJ 2005 Non esterified fatty acids (NEFA) in dairy cattle. A review. *Veterinary Quarterly* **27** 117–126

Almeida AM, Kilminster T, Scanlon T, Araújo SS, Milton J, Oldham C & Greeff JC 2013 Assessing carcass and meat characteristics of Damara, Dorper and Australian Merino lambs under restricted feeding. *Tropical Animal Health and Production* **45** 1305–1311

Alves SP, Raundrup K, Cabo Â, Bessa RJ & Almeida AM 2015 Fatty acid composition of muscle, adipose tissue and liver from muskoxen (ovibos moschatus) living in West Greenland. *PLoS ONE* **10** e0145241

Amills M, Capote J, Tomàs A, Kelly L, Obexer-Ruff G, Angiolillo A & Sanchez A 2004 Strong phylogeographic relationships among three goat breeds from the Canary Islands. *Journal of Dairy Research* **71** 257–262

Bauman DE & Griinari JM 2003 Nutritional regulation of milk fat synthesis. *Annual Review of Nutrition* **23** 203–227

Bauman DE, Mather IH, Wall RJ & Lock AL 2006 Major advances associated with the biosynthesis of milk. *Journal of Dairy Science* **89** 1235–1243

Boyazoglu J, Hatziminaoglou I & Morand-Fehr P 2005 The role of the goat in society: past, present and perspectives for the future. *Small Ruminant Research* **60** 13–23

Cardoso LA & Almeida AM 2013 Seasonal weight loss – an assessment of losses and implications on animal welfare and production in the tropics: Southern Africa and Western Australia as case studies. Enhancing animal welfare and farmer income through strategic animal feeding. In *FAO Animal Production and Health Paper No. 175*, pp. 37–45 (Ed. HPS Makkar). Rome: Food and Agricultural Organization of the United Nations (FAO)

Chilliard Y, Ferlay A, Faulconnier Y, Bonnet M, Rouel J & Bocquier F 2000 Adipose tissue metabolism and its role in adaptations to undernutrition in ruminants. *Proceedings of the Nutrition Society* **59** 127–134

Chilliard Y, Ferlay A, Rouel J & Lamberet G 2003 A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science* **86** 1751–1770

Cugno G, Parreira JR, Ferlizza E, Hernández-Castellano LE, Carneiro M, Renaut J, Castro N, Arguello A, Capote J, Campos AM & Almeida AM 2016 The goat (Capra hircus) mammary gland mitochondrial proteome: a study on the effect of weight loss using blue-native PAGE and two-dimensional gel electrophoresis. *PLoS ONE* **11** e0151599

Escuder A, Fernández G & Capote J 2006 Characterization of Palmera dairy goat production systems. *Options Méditerranéennes* **70** 95–100

Fernández G, Navarro-Rios MJ & Perezgrovas R 2011 Characterization of Majorera goat production systems in the Canary Islands. *Options Méditerranéennes* **100** 205–210

Fresno MR, Gómez J, Molina A, Darmanin N, Capote JF & Delgado JV 1994 Estudio preliminar del rendimiento productivo de la cabra Majorera. *Archivos de Zootecnia* **43** 181–186

Folch J, Lees M & Stanley GHS 1957 A simple method for the isolation and purification of total lipides from animal tissues. *Journal Biological Chemistry* **226** 497–509

Haenlein G 2001 Past, present, and future perspectives of small ruminant dairy research. *Journal of Dairy Science* **84** 2097–2115

Hernández-Castellano L, Ferreira A, Nanni P, Grossmann J, Argüello A, Capote J, Cai G, Lippolis J, Castro N & Almeida AM 2016a The goat (*Capra hircus*) mammary gland secretory tissue proteome as influenced by weight loss: a study using label free proteomics. *Journal of Proteomics* **145** 60–69

Hernández-Castellano LE, Torres A, Alavoine A, Ruiz-Díaz MD, Argüello A, Capote J & Castro N 2011 Effect of milking frequency on milk immunoglobulin concentration (IgG, IgM and IgA) and chitotriosidase activity in Majorera goats. *Small Ruminant Research* **98** 70–72

Hernández-Castellano LE, Argüello A, Almeida AM, Castro N & Bendixen E 2015 Colostrum protein uptake in neonatal lambs examined by descriptive and quantitative liquid chromatography-tandem mass spectrometry. *Journal of Dairy Science* **98** 135–147

Hernández-Castellano LE, Almeida AM, Renaut J, Argüello A & Castro N 2016b A proteomics study of colostrum and milk from the two major small ruminant dairy breeds from the Canary Islands: a bovine milk comparison perspective. *Journal of Dairy Research* **83** 366–374

Huang J, Yu H, Guan X, Wang G & Guo R 2016 Accelerated dryland expansion under climate change. *Nature Climate Change* **6** 166–171

Lérias JR, Hernández-Castellano LE, Morales-Delanuez A, Araújo SS, Castro N, Argüello A, Capote J & Almeida AM 2013 Body live weight and milk production parameters in the Majorera and Palmera goat breeds from the Canary Islands: influence of weight loss. *Tropical Animal Health and Production* **45** 1731–1736

Lérias JR, Peña R, Hernández-Castellano LE, Capote J, Castro N, Argüello A, Araújo SS, Saco Y, Bassols A & Almeida AM 2015 Establishment of the biochemical and endocrine blood profiles in the Majorera and Palmera dairy goat breeds: the effect of feed restriction. *Journal of Dairy Research* **82** 416–425

McManaman J & Neville M 2003 Mammary physiology and milk secretion. *Advanced Drug Delivery Reviews* **55** 629–641

Molkentin J & Precht D 2000 Validation of a gas-chromatographic method for the determination of milk fat contents in mixed fats by butyric acid analysis. *European Journal of Lipid Science and Technology* **102** 194–201

Palma M, Hernández-Castellano LE, Castro N, Arguëllo A, Capote J, Matzapetakis M & Almeida AM 2016a NMR-metabolomics profiling of mammary gland secretory tissue and milk serum in two goat breeds with different levels of tolerance to seasonal weight loss. *Molecular Biosystems* **12** 2094–2107

Palma M, Scanlon T, Kilminster T, Milton J, Oldham C, Greeff J, Matzapetakis M & Almeida AM 2016b The hepatic and skeletal muscle ovine metabolomes as affected by weight loss: a study in three sheep breeds using NMR-metabolomics. *Scientific Reports* **6** 39120

Sánchez-Macías D, Morales-Delanuez A, Moreno-Indias I, Hernández-Castellano LE, Mendoza-Grimón V, Castro N & Argüello A 2012 Lipolysis and proteolysis profiles of fresh artisanal goat cheese made with raw milk with 3 different fat contents. *Journal of Dairy Science* **94** 5786–5793

Sánchez-Macías D, Morales-delaNuez A, Torres A, Hernández-Castellano LE, Jimenez-Flores R, Castro N & Arguello A 2013 Effects of addition of somatic cells to caprine milk on cheese quality. *International Dairy Journal* **29** 61–67

Torres A, Castro N, Hernández-Castellano LE, Argüello A & Capote J 2013 Short communication: effects of milking frequency on udder morphology, milk partitioning, and milk quality in 3 dairy goat breeds. *Journal of Dairy Science* **96** 1071–1074