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Fatty acids composition, lipids health indices and enzyme activities of *longissimus thoracis* muscle of six breeds of sheep produced on pasture in Northern region of Uruguay

Ácidos grasos, índices lipídicos de salud y actividades enzimáticas en el musculo *longissimus thoracis*, de seis razas de corderos producidos sobre pasturas en el norte de Uruguay

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ABSTRACT

The determination of fatty acids composition of glycerolipids and glycerophospholipids of meat from *longissimus thoracis* of six breeds of lamb produced on pasture in Uruguay was undertaken by gas chromatography. Also some lipids health indices and lipids metabolism enzymes were determined. The studied lambs were males aged of 11–12 months of breeds and biotypes Highlander® (H), Merino Dohne (MD), Corriedale (C), Corriedale Pro® (CPRO), a crossing between Corriedale × Australian Merino (C×AM) and Romney Marsh (RM). The animals were reared on pasture in identical conditions without supplementation. The grazing was rotational based on a winter annual crops oats (*Avena sativa* spp.), cocksfoot, (*Dactylis glomerata* spp.) and white clover (*Trifolium repens* spp.). The results of the study did not show substantial differences between breeds regarding the fatty acids composition of meat, except for few relevant fatty acids such as C16:0 (MD>C), C18:3n3 (H<C) and CLA (H<CPRO, CxAM) for glycerolipids. Also C18:1(H>CPRO, CxAM), C18:2n6 (H<CxAM) and C18:3n3 (H<C) for glycerophospholipids. Likewise, other differences were outlined such as the anteiso monomethyl fatty acid content (MD<RM), the hypocholesterolemic/hypercholesterolemic ratio (MD<C). For lipids metabolism enzymes indices, MD showed a lower Δ–9 desaturase enzyme for C16:0 than C, CPRO and CxAM. Also, H showed a lower Δ–6 desaturase enzyme activity than C, and both MD and CxAM showed a lower elongase enzyme activity than C. The results of the present investigation showed that the meat of the lamb of the different breeds overall present good lipids nutritional indicators, in comparison with the results of other research in lambs. That information could help lamb producers in Uruguay to promote their products based on scientific data.

Key words: Lamb meat; fatty acids; extensive system; pasture

RESUMEN

Se determinó la composición en ácidos grasos de los glicerolípidos y glicerofosfolípidos del músculo *longissimus thoracis* de seis razas de corderos producidos con pasturas en Uruguay, mediante el uso de cromatografía de gases. También se cuantificaron los ácidos grasos de cadena ramificada monometiles iso y anteiso, y el contenido de ácidos grasos impares de la carne. Se determinaron índices lipídicos de salud y actividades de las enzimas del metabolismo de los ácidos grasos. Los corderos estudiados fueron machos de 11–12 meses de razas y biotipos Highlander® (H), Merino Dohne (MD), Corriedale (C), Corriedale Pro® (CPRO), un cruce entre Corriedale × Australian Merino (C×AM) y Romney Marsh (RM). Los animales fueron criados sobre pasturas en condiciones idénticas sin suplementos. El pastoreo fue rotativo basado en una avena de cultivos anuales de invierno (*Avena sativa* spp.), cocksfoot (*Dactylis glomerata* spp.) y trébol blanco (*Trifolium repens* spp.). Los resultados no mostraron diferencias sustanciales entre razas en la composición en ácidos grasos de la carne, excepto por ácidos grasos relevantes como C16:0 (MD>C), C18:3n3 (H<C) y CLA (H<CPRO, C×AM) para glicerolípidos. También C18:1 (H>CPRO y C×AM), C18:2n6 (H<C×AM) y C18:3n3 (H< C) para glicerofosfolípidos. Asimismo, hay otras diferencias como el contenido de ácidos grasos anteiso (RM>MD) y la relación del índice hipocolesterolémico/hipercolesterolémico (MD<C). Para las actividades enzimáticas del metabolismo de los ácidos grasos, el MD mostró una menor enzima desaturasa Δ–9 para C16:0 que C, CPRO y CxAM. Además, H mostró una menor actividad de la enzima Δ–6 desaturasa que C, y tanto MD como CxAM mostraron una menor actividad de la enzima elongasa que C. Los resultados mostraron que la carne de cordero de las diferentes razas presenta en general buenos indicadores nutricionales de lípidos, en comparación con los resultados de otras investigaciones en corderos. Esa información podría ayudar a los productores de corderos del Uruguay a promover sus productos sobre la base de datos científicos.

Palabras clave: Carne de cordero; ácidos grasos; sistema extensivo; pastura

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INTRODUCTION

Sheep (*Ovis aries*) meat is since thousands of years, a valuable food for human nutrition [1] Sheep meat is available in many Countries, often produced and consumed locally. Approximatively 82% of sheep breed in the World are local breed well adapted to their particular biotope, most of them fed local pasture [2]. However, there are also commercial breeds that are the basis of the international sheep meat trade, often associated to wool trade. Sheep meat provides to consumers protein, lipids, minerals (particularly iron and zinc), and vitamins, all of them necessary to an adequate growth and metabolism function at all ages. Lipids, through their fatty acids composition, are particularly relevant since they are associated with some chronic diseases. Indeed, ruminant meat from sheep or lamb contains glycerolipids and glycerophospholipids composed by saturated fatty acids (SAT), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Some of SAT are associated with the occurrence of cardiovascular pathologies and cancer in human, meanwhile MUFA seems to have beneficial effects on health [3]. For other part, PUFA such as linoleic acid and α–linolenic acid are essential for human nutrition and metabolism, which means that they have to be present in the diet. The latter is precursor for the biosynthesis of EPA (C20:5n3) and DHA (C22:6n3), two n–3 fatty acids involved in the protection against cardiovascular diseases and cancer in human.

In Uruguay, sheep production is based on pasture and constitutes a relevant part for the meat market and the economical scheme of the Country. Various sheep breeds and crossing are present and producers have been always interested to improve their knowledge about the breeds that they produce, mainly in genetically aspects linked to the wool quality. The main breed present in Uruguay is the Corriedale (42% of total sheep breeds), because of its dual purpose characteristics to produce wool and meat. However, in the last few years, the incomes of sheep producers in Uruguay become depending much more to the meat, for both domestic and international trade, than wool one. This is probably due to the relatively better stability of international sheep meat market, compared to the wool market and the positive future perspective of sheep meat trade in the region [4]. In consequence, producers become now interested to known the nutritional quality of the sheep meat that they produce, to help themselves promote their products, mainly in the international sheep meat market. Therefore, the present study has been undertaken to known the fatty acids composition of meat obtained from six breeds and crossing produced in Uruguay, including Corriedale, and fed exclusively with pasture. Some of those breeds have been recently introduced in the country and scarce or no information, in our knowledge; about the nutritional values of their meat could be sourced in the scientific literature. Furthermore, the study will generate information about some lipid health indices for consumers, and fatty acid metabolism indices related to the enzymes desaturases, elongases and thioesterases.

MATERIAL AND METHODS

Animals and feeding

The meat studied in the work come from males of six breed produced in Uruguay on extensive system condition based on pasture.

1. Highlander® (H, n=15; slaughtered at 54.38 ± 4.45 kg of body weight, 339 ± 6.7 days of age). H is a composite breed (½ Romney, ¼ Texeland ¼ Finnish Landrace) introduced in Uruguay on year 2005.
2. Merino Dohne (MD, n=11; slaughtered at 55.05 ± 3.72 kg of body weight, 341 ± 5.8 days of age), a dual purpose breed originated in South Africa and introduced in Uruguay from Australia on year 2002.
3. Corriedale (C, n=11; slaughtered at 50.3 ± 5.35 kg of body weight, 339 ± 4.83 days of age), a dual purpose breed obtained by crossing Merino and Lincoln breeds in Australia and New Zealand around the years 1874–1880. C was introduced in Uruguay on 1916.
4. Corriedale Pro® (CPRO, n= 15; slaughtered at 46.54 ± 5.53 kg of body weight, 341 ± 7.57 days of age), CPRO is a composite crossbreed developed in Uruguay, based on a crossing of Freisian Milchschaf (25%) with Finnish Landrace (25%) and C (50%). CPRO has been developed principally to improve the prolificacy without the loss of double purpose attribute of C.
5. A crossbreed between Corriedale and Australian Merino breed (C×AM, n=15; slaughtered at 48.18 ± 7.04 kg of body weight, 334 ± 10.1 days of age). C×AM has been developed in Uruguay to improve the resistance to the gastrointestinal parasitism.
6. The last breed used in the study was Romney Marsh (RM, n=4; slaughtered at 48.92 ± 6.82 kg of body weight, 335 ± 2.5 days of age). RM is a dual purpose breed, developed in England, and introduced in Uruguay on year 1896. Although only 4 animals RM were obtained from producers, the results of the experiment with those animals have been anyway included in the study, taking into account the long presence of that breed in the productive scheme of the country and the lack of nutritional information of RM meat produced in Uruguay.

Animals were maintained in the facilities of the Experimental Station of the Faculty of Agronomy (Udelar) in Paysandú – Uruguay, following the regulations of the University's ethics committee. The investigation has been approved by the Honorary Commission for Animal Experimentation (CHEA, Universidad de la República, Udelar, Uruguay), recorded as protocol number 1401. Furthermore, the investigation has been also approved by the Ethical Commission for the Use of Animals (CEUA, CENUR, Udelar).

Animals have grazed pasture, without any supplementation, with a maximum animal density of 6 sheeps by hectare, and rotated in paddocks of 15 hectares. The animals were reared on pasture, in groups separated by breed. Pasture (P1) consisted by a winter annual crops oats (*Avena sativa* spp.) with the availability of forage of 2,743 kg DM·ha^{-1}, that pasture has been used in a rotational grazing. Furthermore, the lamb grazed another pasture (P2) principally constituted by cocksfoot, (*Dactylis glomerata* spp.) and white clover (*Trifolium repens* spp.) with an availability of forage of 2,756 kg DM·ha^{-1}.

All groups have been concomitantly transferred between P1 and P2 and inversely, depending of the availability of forage. For the sampling and the estimation of available forage and botanical composition in the grazing area, the cutting method "Sample Sward–cutting techniques" and Botanal was used [5]. The lipids and fatty acid composition of pasture was presented in TABLE I.

The lambs were slaughtered in a commercial slaughterhouse (Certified Food Standard, Grade A, Certification Body LSQA S.A. for exportation by BRC Global Standard). At 72 hours *post mortem* the *longissimus thoracis* muscle (between 9th and 12th vertebrate) was withdraw, vacuum packaged and stored at -20°C, until analysed.

TABLE I
Lipid content (% of dry matter) and fatty acids composition of glycerolipids and glycerophospholipids (g·100 g^{-1} fatty acids) of pastures grazed by lambs

	Pasture P1	Pasture P2		
	Oat (*Avena sativa*)	Legumes (*Trifolium repens*)	Gramineae (*Dactylis glomerata*)	Undefined Pasture
Lipids	3.38±0.03	2.10±0.02	3.65±0.03	3.25±0.03
Glycerolipids fatty acids				
C14:0	1.47±0.71	1.26±0.79	0.61±0.06	0.65±0.13
C16:0	16.9±0.69	13.8±0.57	15.00±0.42	18.70±0.63
C16:1	1.49±0.06	1.51±0.06	2.64±0.24	2.49±0.05
C18:0	1.92±0.02	2.07±0.08	1.18±0.11	2.08±0.03
C18:1	2.92±0.10	3.35±0.56	1.79±0.04	2.83±0.09
C18:2n6	8.88±0.36	22.40±0.68	10.3±0.10	13.40±0.16
C18:3n3	49.20±1.41	45.60±1.06	60.5±0.65	50.00±1.18
C20:0	0.58±0.13	1.80±0.10	0.30±0.01	0.95±0.37
C22:0	2.51±0.19	2.47±0.78	0.81±0.03	1.17±0.07
C24:0	3.74±0.20	0.78±0.12	1.29±0.07	0.98±0.07
Unidentified fatty acids	10.40±1.38	4.91±0.66	5.67±1.22	6.68±0.34
Glycerophospholipids fatty acids				
C14:0	0.12±0.02	0.17±0.03	0.08±0.01	0.12±0.02
C16:0	16.40±0.48	12.40±1.40	13.20±0.54	17.20±0.55
C16:1	1.97±0.25	1.94±0.14	1.72±0.22	3.18±0.19
C18:0	1.10±0.07	1.48±0.12	0.88±0.05	1.19±0.01
C18:1	2.11±0.04	2.42±0.46	1.33±0.06	2.36±0.09
C18:2n6	7.06±0.34	13.4±1.05	7.28±0.09	12.00±0.04
C18:3n3	51.00±0.80	54.50±4.31	66.70±1.15	54.40±2.18
C20:0	0.48 ±0.05	1.67±0.71	0.31±0.08	1.06±0.45
C22:0	3.52±0.24	4.56±0.76	1.13±0.15	1.59±0.12
C24:0	4.52±0.56	1.52±0.11	1.65±0.32	1.27±0.19
Unidentified fatty acids	11.60±1.41	5.94±0.89	5.61±1.25	5.60±1.89

Data are mean ± SEM of three samples of pasture. Animals have been concomitantly transferred between P1 and P2 and inversely, depending of the availability of forage

Analytical determination

The plant lipids were determined on a dry ground sample, dried at 105°C for 6 hours in a forced air dryer. Lipids of three replicates of 10 g were extracted by Soxhlet method (AOAC Method 945.16), using hexane (Carlo Erba, France, HPLC grade) as extraction solvent. The intramuscular lipids were extracted according to Folch *et al.* [6]. Briefly, a sample of 4 grams of meat of *longissimus thoracis* muscle (free of dissectible visible fat) was homogenized at 25.000 rpm with an IKA T25 homogenizer (IKA Brandt, Sweden) during 1 min with 80 mL of chloroform: methanol 2:1, (Baker brand HPLC grade, USA). Afterward, the homogenate was filtered on fritted funnel (Fisher brand, graduation M, USA), transferred to a separating funnel, mixed by shaking and inverting for one minute and decanted overnight. The lower phase (chloroform containing lipids) was recuperated in a glass balloon (Fisher Brand, USA), evaporated at 45°C with a light vacuum in a Rotavapor (IKA basic, Sweden). The balloon was dried in an oven at 35°C for 60 min and cooled at ambient temperature overnight in a vacuum desiccator protected from light. The balloon was weighted at 0.0001 g. to determine the percentage of lipids of each sample. The methylation of fatty acids from glycerolipids fraction followed the procedure described by Ichihara *et al.* [7]. That procedure target the fatty acids from triacylglycerols as well as those from phospholipids, both associated to a glycerol backbone. For the selective methylation of fatty acids from glycerophospholipids (polar glycerolipids), the procedure described by Ichihara *et al.* [8] has been used. The determination of fatty acids by gas chromatography followed a procedure using fused-silica capillary column CPSIL-88 of 100 m installed in a split/split less chromatograph Clarus 500 (Perkin Elmer Instruments, USA) with a FID detector. The samples injection was done using an autosampler from the same manufacturer. One µl of each methylated sample was injected with a split ratio of 50%. Hydrogen (Brand Linden, Uruguay, purity of 99.9995%) was used as carrier gas having a ratio air/H_2 of 350 mL/35 mL. Filtered air was proven by compressor GAST model 3HBB-11T-M300AX (USA). The thermal

conditions were: Injector/detector temperatures 250°C/250°C, oven held at 90°C for one minute after the injection of the sample. The split valve was open 30 seconds after injection. Afterward the oven temperature was increased to 225°C at 15°C·min^{-1}. Fatty acids methylated esters (FAMEs) were determined comparing the retention time of authentic standards and the 37 component FAME standard mixture (Sigma–Aldrich, USA). Individuals FAME were quantified as a percentage of total detected FAMEs. The integration of signal has been conducted on Total Chrome software from Perkin Elmer (USA).

Calculus of health indices

The calculus of health indices was performed from the data of fatty acid composition of glycerolipids. The following indices were calculated:

- Indice of atherogenicity (AI): Compute the relationship between the sum of the main saturated fatty acids (pro–atherogenic) and the unsaturated (anti–atherogenic) fatty acids [9]. It was calculated as follows:

  $$AI = (4 \times C14{:}0 + C16{:}0) / [\sum MUFA + \sum (n{-}6) + \sum (n{-}3)]$$

- Indices of thrombogenicity (TI): Estimate the potential to form clots in the blood vessels [9], determined by the relationship between the pro–thrombogenic and the anti–thrombogenic fatty acids (Sum of MUFA and PUFA). It was calculated as follows:

  $$IT = (C14{:}0 + C16{:}0 + C18{:}0) / [0.5 \times \sum MUFA + 0.5 \times \sum (n{-}6) + 3 \times \sum (n{-}3) + \sum (n{-}3) / \sum (n{-}6)]$$

- Hypocholesterolemic/Hypercholesterolemic ratio (h/H): Compute the relation between unsaturated fatty acids (MUFA and PUFA) and the saturated fatty acids 14:0 and 16:0. The h/H ratio was calculated according to Fernández *et al.* [10] as follows:

  $$h/H = (C14{:}1 + C16{:}1 + C18{:}1 + C20{:}1 + C22{:}1 + C18{:}2 + C18{:}3 + C20{:}3 + C20{:}4 + C20{:}5 + C22{:}4 + C22{:}5 + C22{:}6) / (C14{:}0 + C16{:}0)$$

Enzyme activity indices

The enzyme activity of desaturases, elongase and thioesterase was estimated by relating the amount of the specific substrate to the corresponding product of the respective enzyme. The calculus of those indices was performed from the data of fatty acid composition of glycerolipids. The calculated ratios were 16:1n–7 to 16:0 and 18:1n9 to 18:0, and their sum, for the activity of stearoyl–CoA desaturase (Δ–9–desaturase). The Δ–5 desaturase and Δ–6 desaturase were used as an index for the estimation of catalyzing the formation of long chain n–6 and n–3 starting from the corresponding precursor C18:2n6 and C18:3n3, respectively. Also, the ratio 18:0 to 16:0 was calculated to estimate the elongase activity. The thioesterase was estimated as the ratio of C16:0 to C14:0.These indices can be used as surrogates of the measure of the true enzyme activities [11].

Statistical analysis

Data are presented as mean ± SEM. Results were analysed by ANOVA one way to compare six genotypes, and *post hoc* Tukey–Kramer Test (P<0.05), using the NCSS 12 Statistical Software (2018, NCSS, LLC. Kaysville, Utah, USA, https://www.ncss.com).

In addition, a principal component analysis (PCA) on the standardized variables at unit scale, associated to human health as intramuscular fat content (lipids), and C14:0, C16:0, C17:0ai,C18:3n3, CLA, BCFAai and BCFAi of the total fatty acids were conducted to evaluate the relative differences of the meat samples in these lipid profile among breeds.

Another principal component analysis (PCA) on the variables associated to structure of membrane as C16:0, C16:1, C18:1, C18:2n6, C18:3n3 C20:4n6, EPA, DPA, DHA in glycerophospholipids fraction to evaluate if the breeds present differences. Variables were graphed in a biplot with different colour related their contribution and the distribution of observations were graphed in a biplot using ellipses with 95% confidence interval. Statistical analysis was conducted using the PCA function of the package FactoMineR for the principal components analysis in the R software version 4.2.2 (R Core Team, 2022). To visualize de PCA results, the factoextra package in the R software was used.

RESULTS AND DISCUSSION

Lipids

The intramuscular fats content is perhaps one of the most relevant parameters, principally thought their fatty acids composition, when the nutritional quality has to be considered to characterize ruminant meat. Thus, depending of their specific fatty acids composition, lipids in meat could affect positively or negatively the health of consumers. Indeed, they could respond positively to the nutritional requirement for growth and metabolism at all ages of consumers, but they could influence negatively the human health thought the occurrence of cardiovascular and cancer diseases [3, 12]. However, for most consumers, the content of lipids expressed as g of total lipids by 100 g of meat is perceived as a key indicator to classify the meat products, as well as other foods, in regard to their incidence on health.

In the present work, the comparison between the different breeds showed lipids contents ranged between 2.39–4.49 g of lipids by 100 g of meat. Significant differences have been observed only between C×AM and H (TABLE II). Limited information was available in the scientific literature, for comparison, about the fat content of C×AM and H. However, Jalloul *et al.* [13] report low lipid content in H lamb ranged between 1.07–1.18 g of lipids by 100 g of meat from *longissimus thoracis*.

Nevertheless, lambs were housed in small pens and fed corn, citrus pulp, rice bran or soybean hulls. Thus, the difference of weight at slaughtering (30 kg versus 54 kg in our experiment) or the kind of feed offered to animals, or both, could explain the different fat content of meat. Regarding the other breeds studied in the present investigation, for C, lipids content reported in meat obtained in similar conditions in Uruguay showed levels around 3.65 g of lipids by 100 g of meat [14]. In the case of the present experiment, meat was from *longissimus thoracis* while in the work of Lucas [14], meat was from *longissimus lumborum*. In both experiments, animals were of similar age and fed pasture in extensive production system. Another experiment in extensive condition of Uruguay compared C lamb produced on pasture, but at two different ages. Results showed that there are difference in lipids content of meat of 3.05 versus 5.92 g by 100 g of meat from *longissimus thoracis*, at 3–4 months and 12–13 months, respectively [15]. The higher content of lipids, compared to the reported in our experiment, could be explained by the nature of pasture, probably more than by the difference of ages between the two works, that is, 12–13 months versus 11–12 months in our work. However, Diaz *et al* [15] do not reported, the composition of pastures offered to the animals.

TABLE II
Lipids content (% of wet tissue) and fatty acids composition (g·100 g^{-1} fatty acids) of glycerolipids present in *Longissimus thoracis* muscle from lambs of different breeds produced on pasture

	Breeds						
	H (n=15)	**MD (n=11)**	**C (n=11)**	**CPR (n=15)**	**C×AM (n=15)**	**RM (n=4)**	***P***
Lipids	2.39^{b} ± 0.18	3.21^{ab} ± 0.44	3.46^{ab} ± 0.30	4.17^{a} ± 0.40	4.25^{a} ± 0.33	4.49^{ab} ± 1.01	0.009
Saturated Fatty acids (SAT)							
C14:0	2.52 ± 0.23	3.46 ± 0.36	2.43 ± 0.20	3.04 ± 0.34	3.10 ± 0.28	2.45 ± 0.47	NS
C15:0i	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	NS
C15:0ai	0.16 ± 0.01	0.17 ± 0.02	0.14 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.03	NS
C15:0	0.51 ± 0.04	0.59 ± 0.04	0.50 ± 0.03	0.55 ± 0.03	0.51 ± 0.04	0.46 ± 0.08	NS
C16:0i	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.18 ± 0.03	NS
C16:0	24.0^{ab} ± 1.03	26.10^{a} ± 1.43	21.20^{b} ± 0.78	23.00^{ab} ± 0.89	24.40^{ab} ± 0.92	21.10^{ab} ± 0.62	0.02
C17:0i	0.48 ± 0.04	0.39 ± 0.05	0.49 ± 0.04	0.52 ± 0.03	0.48 ± 0.03	0.55 ± 0.05	NS
C17:0ai	0.47^{a} ± 0.03	0.35^{b} ± 0.06	0.51^{a} ± 0.04	0.50^{a} ± 0.03	0.52^{a} ± 0.02	0.58^{a} ± 0.02	0.01
C17:0	1.50^{ab} ± 0.06	1.35^{b} ± 0.08	1.67^{a} ± 0.06	1.59^{ab} ± 0.04	1.47^{ab} ± 0.05	1.57^{ab} ± 0.12	0.03
C18:0	20.4^{ab} ± 0.72	18.9^{ab} ± 0.33	21.10^{a} ± 0.50	18.90^{ab} ± 0.62	18.3^{b} ± 0.62	20.8^{ab} ± 0.97	0.01
C20:0	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.12 ± 0.02	NS
C22:0	0.02 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	NS
Σ SAT	49.30 ± 1.08	50.90 ± 1.58	47.30 ± 0.92	47.40 ± 0.77	48.10 ± 1.03	47.10 ± 0.87	NS
Monounsaturated Fatty acids (MUFA)							
C16:1	1.77^{ab} ± 0.11	1.57^{b} ± 0.10	1.80^{ab} ± 0.09	2.04^{a} ± 0.07	2.10^{a} ± 0.11	1.87^{ab} ± 0.11	0.004
C17:1	0.83 ± 0.04	0.72 ± 0.04	0.81 ± 0.03	0.80 ± 0.03	0.78 ± 0.03	0.78 ± 0.07	NS
C18:1	40.20 ± 0.62	38.40 ± 0.56	40.00 ± 0.60	39.90 ± 0.49	39.60 ± 0.65	40.00 ± 0.82	NS
Σ MUFA	42.80 ± 0.68	40.80 ± 0.63	42.60 ± 0.61	42.80 ± 0.56	42.50 ± 0.70	42.70 ± 0.91	NS
Polyunsaturated fatty acids (PUFA)							
C18:2n6	3.14 ± 0.24	3.52 ± 0.42	3.87 ± 0.23	3.62 ± 0.27	3.48 ± 0.18	3.81 ± 0.24	NS
C18:3n3	0.77^{b} ± 0.09	0.96^{ab} ± 0.12	1.21^{a} ± 0.12	1.04^{ab} ± 0.11	1.04^{ab} ± 0.07	1.14^{ab} ± 0.11	0.05
CLA	0.61^{b} ± 0.12	0.87^{ab} ± 0.16	0.99^{ab} ± 0.11	1.15^{a} ± 0.13	1.15^{a} ± 0.09	1.24^{ab} ± 0.19	0.008
C20:3n6	0.09^{b} ± 0.02	0.14^{ab} ± 0.03	0.21^{a} ± 0.03	0.14^{ab} ± 0.02	0.17^{ab} ± 0.02	0.18^{ab} ± 0.05	0.01
C20:3n3	0.21 ± 0.06	0.37 ± 0.11	0.43 ± 0.07	0.34 ± 0.07	0.32 ± 0.05	0.33 ± 0.08	NS
C20:4n6	0.12 ± 0.04	0.19 ± 0.05	0.26 ± 0.04	0.19 ± 0.04	0.19 ± 0.03	0.22 ± 0.06	NS
EPA	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	NS
DPA	0.46 ± 0.07	0.33 ± 0.05	0.36 ± 0.04	0.41 ± 0.06	0.29 ± 0.03	0.26 ± 0.04	NS
DHA	0.08 ± 0.05	0.04 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.11 ± 0.08	NS
Σ PUFA	4.89 ± 0.43	5.56 ± 0.74	6.41 ± 0.52	5.76 ± 0.51	5.53 ± 0.35	6.06 ± 0.45	NS
Unidentified Fatty Acids							
–	1.26 ± 0.17	0.98 ± 0.19	1.45 ± 0.11	1.63 ± 0.16	1.48 ± 0.19	1.78 ± 0.21	–

Data are mean ± SEM. H=Highlander, MD=Merino Dohne, C=Corriedale, CPRO=Corriedale PRO, C×AM=Corriedale × Australian Merino, RM=Romney Marsh. For each fatty acid, mean values bearing different low case letters are significantly different. *P* = Significance level. NS = non–significant. i = iso, ai = anteiso, EPA=C20:5n3, DPA=C22:5n3, DHA=C22:6n3

Fatty acids of glycerolipids

Regarding the fatty acids composition of glycerolipids, in the case of SAT there is differences between the breeds for C16:0, C17:0 anteiso, C17:0 and C18:0 (TABLE II). C16:0 showed a higher content for MD compared to C. The fatty acid C16:0 (palmitic acid), among all SAT, is considered an atherogenic fatty acid and promotes inflammation [16]. Thus its consumption is advised to be reduced but not avoided because of its important physiological role in lipids metabolism, particularly in neonate and infants [17]. As stated by those authors, both deficiency and excess of palmitic acid in diet are detrimental for health. Probably this concept could be valid at all age. In the breeds studied in the present work, the level of C16:0 in meat ranged 21.1–26.1 g·100 g^{-1} fatty acids. Those levels are of the same order detected in

meat from *longissimus thoracis* of lamb from different breeds produced in other countries. Indeed, Cadavez *et al.* [18] working on various local Iberian breeds produced in different rearing systems, that is extensive, semi-extensive and intensive, showed for C16:0 a level in g·100 g^{-1} fatty acids ranged between 19.9–24.7. Diaz *et al.* [15] compared meat from *longissimus dorsi*, in its *thoracis* part, of different lamb breeds produced in typical production system of Spain, United Kingdom, Germany and Uruguay. In that investigation, the composition for C16:0 expressed as g·100 g^{-1} fatty acids showed levels ranged 22.5–24.7. In the same investigation the breed evaluated in Uruguay was C produced in two systems. One of them, called heavy lamb, consisted in animals aged of 12–13 months and the other, called light lamb; the animals were aged of 3–4 months. However, the levels of palmitic acids were of 24.66% and 24.73% in heavy lamb and light lambs, respectively. This apparent stability of contents of palmitic acid in meat, is probably due to the fact that the intake in this fatty acid and the lipogenesis *de novo* for the same, act together to maintain a stable level of palmitic acid in tissues, even when the animals are of different ages as reported by Diaz *et al.* [15], for C lamb produced on pasture in Uruguay.

Another fatty acid, the anteiso C17:0ai is present in the meat of the six breeds (C17:0ai, TABLE II), showed a level, expressed as g/100g fatty acids of all detected fatty acids, ranged 0.35–0.58. However, only meat from MD presented a significant lower level for this fatty acid, in comparison to the other breeds. This fatty acid is mainly synthesized during the microbial fermentation processes in the rumen, and consequently is typical of ruminant milk and meat products. Also, its amount in meat seems to be influenced by the level of fattening of lamb when fed pasture [19]. However, in our work, although there is difference between the breeds regarding their fattening, there is not a clear relationship between lipids content in meat and level of anteiso C17:0 (TABLE II). It could be explained by the differences in the experimental conditions, that is kind of muscles, breeds and pastures. There is limited information about the nutritional importance in human health of anteiso C17:0. However, in the report of Vahmani *et al.* [20], the C17ai fails to present anti-carcinogenic properties in cultured MCF 7 cells, a mammary human cancer established cell line. In that investigation it is rather the iso C17:0 (C17:0i) which presents a significant anti-carcinogenic effect in the same cell line MCF 7. Those promissory fatty acids, and their potential effects on human health, warrant future investigations to refine their action against some diseases [21]. In the same way, the odd fatty acids C17:0 presented values ranged 1.35–1.67 when expressed as g·100 g^{-1} fatty acids (TABLE II). The breed C showed more C17:0 than MD breed (TABLE I). These contents were higher than those reported by Diaz *et al.* [15] using lambs from C breed produced on pasture in Uruguay, but slaughtered at different ages. However, the rearing conditions were very close to those followed in our experiment.

The combined effect on consumer's health of odd and branched fatty acids detected in our experiment will be presented in Monomethyl branched and odd fatty acids point of the present discussion.

Another fatty acid, the C18:0, showed a higher content for C than C×AM. The level for C18:0 observed in our experiment ranged 18.3–21.1, being quantitatively the second saturated fatty acid present in meat of lambs (TABLE II). The value of C18:0 reported in our work are of the same order of those reported by Lucas *et al.* [14] and Ramos *et al.* [22], even when crossbreed animals were used in those experiments. However, in the work of Cadavez *et al.* [18], working on Iberian local breed of lambs aged around 4–4.5 months and produced in different typical extensive, semi-extensive and intensive systems of Spain and Portugal, reported a level expressed in g·100 g^{-1} fatty acids ranged 12.3–15.4. This lower level of C18:0 reported in that investigation could be due to the age of animals. But in another work comparing C lamb of 3–4 months with others aged of 11–13 months, showed levels of C18:0, in one part close of those observed in our work, and for other part no difference has been detected between the animals of the two ages [15]. Therefore, those differences could be attributable to the breeds used in those experiments.

In relation to the human health effect when C18:0 is consumed, it has been defined that these fatty acids have a neutral fatty acid regarding the cardiovascular diseases. However, there are some recent reports that point a potential negative effect for human health. This controversial situation must be clarified in future investigation [16, 23].

In the case of MUFA, the C16:1 presented a significant difference between CPRO and C×AM versus MD. The levels of this fatty acid, expressed as g·100 g^{-1} fatty acids of all detected fatty acids, ranged 1.57–2.10 (TABLE II). Those levels are of the same order as those reported in the experiment of Diaz *et al.* [15], Ramos *et al.* [22], Lucas *et al.* [14] and close or slightly lower to the levels observed by Cadavez *et al.* [18], even when different productive systems and breeds were used.

The fatty acid C16:1 has been proposed as lipokine, principally when it is endogenously synthesized. For other part, the positive health effect for humans, when foods enriched with C16:1 were consumed, is not so clearly demonstrated and the information remains controversial [24].

Regarding the PUFA, the level of C18:3n3 expressed as g·100 g^{-1} fatty acids ranged 0.77–1.21, and the C have more C18:3n3 than H (TABLE II). In comparison with results from other reports, this fatty acid is present with levels of the same order than those reported by Ramos *et al.* [22], and slightly lower than values reported by Lucas *et al.* [14]. However, the levels of C18:3n3 in our work were lower to those reported by Diaz *et al.* [15], particularly when C is highlighted. Indeed, in that investigation, C aged of 3–4 months and others of 11–14 months present levels of C18:3n3 approximately almost three times those detected in the present work. It could be due to the pasture quality offered to the animals, since the productive system was extensive in both experiments. As expected, in the work of Cadavez *et al.* [18] the animals reared in extensive system present a higher content of C18:3n3 in comparison to those reared in a semi-extensive or an intensive system.

The fatty acid C18:3n3 (α-linolenic acid) is an essential fatty acid precursor of other valuables fatty acids of n-3 family. It is known to have favourable effect on health of consumers, directly or after its conversion to C20:5n3 (EPA) and C22:6n3 (DHA). Those three fatty acids have protective effects against cardiovascular diseases, cancer and probably some neurodegenerative diseases [16]. Thus, meat of lamb of our experiment could be considered as a good source of C18:3n3. Unfortunately, the level of EPA and DHA detected in meat of the lamb of our experiment is not so high to consider it as relevant (TABLE II). The cause of the low level of those fatty acids will be considered in our future investigations.

Another fatty acid, CLA (Conjugated linoleic acid), showed levels expressed as g·100 g^{-1} of fatty acids ranged 0.61–1.24, and CPRO and C×AM presented levels higher than H (TABLE II). Those levels in CLA were of the same order than in the work of Diaz *et al.* [15] working with different breeds produced in Europe and Uruguay. In that experiment,

only lamb from Spain showed a lower content of CLA compared to the other breeds used in the experiment. The levels of CLA observed in our work were also in accord with those reported by Ramos *et al.* [22], even when the animals were dietary supplemented with different levels of protein. In other part, the levels of CLA observed in the present work were also in accord with those reported by Lucas *et al.* [14].

The CLA was a typical fatty acids present in relevant amount in ruminant meat and milk. The health effect of intake of CLA in human has been associated with antitumor action, anti-obesity, and other beneficial effects such as effects against cardiovascular diseases [25].

Fatty acids of glycerophospholipids

For SAT, the fatty acid C17:0 showed levels expressed as g·100 g^{-1} fatty acids ranged 0.95–1.31. The breed H presented a higher content than RM for this fatty acid (TABLE III).

There are no differences for total SAT for the breeds used in our work (TABLE III). For other part, the comparison of our results to other reports showed that the level of C16:0 was in the same order to the results reported by Aurousseau *et al.* [26] and slightly higher compared to the results reported by Popova [27], in both experiments animals were fed pasture. However, our results for this fatty acid were higher compared to the results reported by Garcia *et al.* [28]. In this last work, the use of animals from Merino breed typical for wool production, could explain that differences. Furthermore, the lambs were fed shrub grass steppes which could also explain this result. For the C18:0, the levels expressed as g·100 g^{-1} fatty acids reported by Popova [27] were of the same order that those observed in our work. However, the level of C18:0 reported by Aurousseau *et al.* [26] and Garcia *et al.* [28] were lower when compared to our own results for this fatty acid (TABLE III). As expressed before, differences due to the breed or pasturage, or both, could explain those results.

For MUFA, C17:1 presented level expressed as g·100 g^{-1} fatty acids ranged 0.71–1.30. RM presented a higher level compared to CPRO and C×AM (TABLE III). There are scarse results for the content of this fatty acid in lamb meat in the scientific literature. However, Garcia *et al.* [28] reported approximately three times more C17:1, expressed in g·100 g^{-1} fatty acids, in comparison to our results.

In the case of C18:1, the levels expressed as g·100 g^{-1} fatty acids ranged 31.7–37.2 and H presented a higher level than CPRO and C×AM (TABLE III). Those levels are of the same order to those reported by Aurousseau *et al.* [26] and Popova [27], by much higher compared to the results of Garcia *et al.* [28]. The same explanation proposed before, to explain the differences between our results and those of Garcia *et al.* [28], could be proposed again here. That is, lambs were Merino, typical breed for wool production, fed shrub grass steppes.

For PUFA, the fatty acid C18:2n6 presented a level expressed as g·100 g^{-1} of fatty acids ranged 8.92–13.5 (TABLE III). The C exhibit more C18:3n3 than H (TABLE III). This range of levels was of the same order than the levels reported by Aurousseau *et al.* [26] and Garcia *et al.* [28], but lower than those reported by Popova [27]. In our work C×AM exhibit more C18:2n6 in comparison to H. This fatty acid is the most represented in tissue glycerophospholipids, and is the essential precursor of other fatty acids of the n-6 family, as for example the C20:4n6 (arachidonic acid). At the same time this last is a prevalent precursor of many pro-inflammatory eicosanoids, leukotrienes, thromboxanes, among others biomarkers of inflammation [16].

In the case of the C18:3n3, the levels observed in our work ranged 2.29–4.71 when expressed as g·100 g^{-1} of fatty acids. This range of levels was of the same order or even slightly higher than the levels reported in other investigations [26, 27, 28]. Naturally, in those experiments the animals were fed pasture or shrub grass steppes [28]. The favourable effect on health of consumers for these fatty acids has been presented in point "Fatty acids of glycerolipids" above. Another fatty acid that showed differences between the breeds used in our work was the 20:3n6 (TABLE III). The same exhibited levels expressed as g·100 g^{-1} of fatty acids ranged 0.36–0.85, and C showed more 20:3n6 than H (TABLE III). The values detected in our work were of the same order than those reported by Popova [27] and Garcia *et al.* [28] with lamb fed pasture. This fatty acid has been implicated, not alone but concomitantly with other n-6 fatty acids, in the prevalence of higher severity of depressive and anxiety symptoms in patients with depression [3]. Furthermore, other pathologies linked to the inflammatory process could have relation with the metabolism of this fatty acid [29]. Taking into account the levels of these fatty acids in lamb meat and its effect on human health, as described above, it could be interesting and important to consider it as relevant fatty acids to be considered in future investigations on lamb meat.

Finally, although there is a not difference between the breeds for glycerophospholipids for C20:4n6, EPA, DPA and DHA, the Authors of the present work think justified to compare the levels of those fatty acids obtained in our work, to levels reported elsewhere. Indeed, taking into account the nutritional importance of these fatty acids for human health [30, 31], it will be interesting to evaluate the contents of those fatty acids in meat of lamb produced on pasture in Uruguay. For C20:4n6 fatty acid, the levels recorded in the work expressed as g/100 g fatty acids ranged 1.29–3.15 (TABLE III). Those values were lower than the reported by Aurousseau *et al.* [26] and Garcia *et al.* [28]. In the case of work of Popova [27], the value reported were substantially more elevated compared to our work and those cited before, that is 8.23% and 7.21% for muscles *longissimus lumborum* and *semimembranosus*, respectively. Consumption by human of elevated amount of C20:4n6 is not advised, because this fatty acid is a precursor of prostanoids of series 2, leukotrienes of series 4, and many other eicosanoids, all of them promoting inflammation and causing vasodilatation. This fatty acid also could elevate the risk of hypertension and arteriosclerosis [16, 32].

For EPA, the levels detected in our work ranged 0.22–0.45 g·100 g^{-1} (TABLE III), while the values reported by Aurousseau *et al.* [26] were of 4.1, and 8.23 for Popova [27], both results expressed as g/100 g fatty acids. However, in the work of Garcia *et al.* [28] the level of EPA was of 1.60 expressed as g·100 g^{-1} fatty acids. For DPA, Aurousseau *et al.* [26] and Garcia *et al.* [28] reported level of 1.10 and 1.35 as g·100 g^{-1} of fatty acids, respectively. In the case of Popova [27], the reported content in DPA was of 3.28 for *longissimus lumborum* and 2.76 for semitendinosus, both expressed as g·100 g^{-1} of fatty acids. For the DHA, in the current work the observed levels were ranged 0.26–0.35 expressed as g·100 g^{-1} of fatty acids. In comparison with the report of Popova [27], the levels were 0.74 and 0.57 as g·100 g^{-1} of fatty acids, for *longissimus lumborum* and *semitendinosus*, respectively. In the case of Garcia *et al.* [28], the reported levels were 0.56. In the work of Aurousseau *et al.* [26] the amount of DHA in meat of lamb was not reported.

The levels of those valuables n-3 PUFA in glycerophospholipids, that is EPA, DPA and DHA detected in our work clearly present a lower content in comparison to other reports previously cited. Those differences could be explained, on one hand, by the environmental

TABLE III
Fatty acids composition (g·100 g^{-1} fatty acids) of glycerophospholipids present in *Longissimus thoracis* muscle from lambs of different breeds produced on pasture

	Breeds						
	H (n=15)	**MD (n=11)**	**C (n=11)**	**CPRO (n=15)**	**C×AM (n=15)**	**RM (n=4)**	***P***
Saturated Fatty Acids (SAT)							
C16:0	22.50±1.38	20.10±1.51	18.80±1.45	19.50±1.49	18.60±1.09	17.40±1.61	NS
C17:0	1.31^{a}±0.08	1.19^{ab}±0.10	0.98^{ab}±0.09	1.08^{ab}±0.09	1.00^{ab}±0.06	0.95^{b}±0.24	0.05
C18:0	18.80±0.57	17.7±0.76	18.00±0.47	17.60±0.47	17.10±0.43	18.00±2.27	NS
C22:0	0.18±0.04	0.31±0.08	0.33±0.05	0.33±0.06	0.33±0.04	0.40±0.05	NS
Σ SAT	42.80±1.72	39.3±2.09	38.10±1.77	38.60±1.82	37.00±1.46	39.30±2.09	NS
Monounsaturated Fatty Acids (MUFA)							
C16:1	1.21±0.06	1.17±0.14	0.82±0.08	1.06±0.14	1.03±0.06	0.87±0.14	NS
C17:1	0.89^{ab}±0.08	0.81^{ab}±0.07	0.74^{ab}±0.06	0.71^{b}±0.06	0.92^{b}±0.08	1.30^{a}±0.20	0.005
C18:1	37.20^{a}±0.97	34.50^{ab}±1.35	31.70^{ab}±1.19	32.30^{b}±1.28	32.40^{b}±0.96	31.90^{ab}±1.30	0.008
Σ MUFA	39.30^{a}±1.00	36.50^{ab}±1.25	33.30^{b}±1.25	34.10^{ab}±1.41	34.60^{ab}±0.96	36.50^{ab}±1.26	0.029
Polyunsaturated Fatty Acids (PUFA)							
C18:2n6	8.92^{b}±0.87	12.20^{ab}±1.35	13.30^{ab}±1.01	12.80^{ab}±1.32	13.30^{a}±0.78	13.50^{ab}±1.13	0.02
C18:3n3	2.29^{b}±0.45	3.48^{ab}±0.56	4.71^{a}±0.59	3.92^{ab}±0.56	4.12^{ab}±0.41	4.46^{ab}±0.66	0.02
C20:3n6	0.36^{b}±0.07	0.68^{ab}±0.14	0.85^{a}±0.11	0.74^{ab}±0.11	0.70^{ab}±0.08	0.76^{ab}±0.16	0.01
C20:3n3	1.44±0.41	2.40±0.51	3.11±0.47	2.87±0.51	2.83±0.37	3.17±0.67	NS
C20:4n6	1.29±0.48	1.64±0.41	2.66±0.55	2.34±0.54	2.54±0.46	3.15±0.91	NS
EPA	0.30±0.11	0.22±0.09	0.34±0.10	0.29±0.08	0.35±0.09	0.45±0.20	NS
DPA	0.67±0.21	1.01±0.25	1.57±0.26	1.43±0.26	1.58±0.32	1.64±0.40	NS
DHA	0.31±0.04	0.27±0.04	0.35±0.05	0.26±0.02	0.26±0.02	0.31±0.06	NS
Σ PUFA	15.60±2.48	21.9±3.16	26.90±2.88	24.60±3.20	25.7±2.27	21.90±3.16	NS
Unidentified Fatty Acids							
–	2.40±0.26	2.34±0.32	1.78±0.45	2.66±0.33	2.92±0.27	1.66±0.80	–

Data are mean ± SEM. H=Highlander, MD=Merino Dohne, C=Corriedale, CPRO=Corriedale PRO, C×AM = Corriedale × Australian Merino, RM=Romney Marsh. For each fatty acid, mean values bearing different low case letters are significantly different. *P* = Significance level. NS = non–significant. EPA= C20:5n3, DPA=C22:5n3, DHA=C22:6n3

condition of the rearing and the global managing of the animals which include the kind and the quality of offered pastures [33]. On other part, the breed used in our work could have a reduced capacity to convert efficiently the C18:3n3 to EPA, DPA and DHA as stated by Sinclair *et al.* [34]. In this sense, it could be noted that the levels of C18:3n3, the precursor of EPA, DPA and DHA, present in our work a clear higher level in meat in comparison to those levels reported by Aurousseau *et al.* [26], Popova [27] and Garcia *et al.* [28]. That means that, perhaps, there was more storage of this fatty acid in the muscle rather than being converted into other n–3 fatty acids [34]. This hypothesis needs to be verified in future investigation in lamb.

Monomethyl branched and odd fatty acids

In TABLE IV were grouped the total of monomethyl branched chain fatty acids (BCFA) detected in our study.

The levels of total BCFA were ranged 1.23–1.57 g·100 g^{-1}. There are not differences between the breeds studied in our work. That range of values was slightly higher than those reported by Gomez–Cortes *et al.* [35] and Pena–Bermudez *et al.* [36], but slightly lower than those presented (control group) by Mele *et al.* [37]. In the three mentioned experiments the animals were fed concentrate and the expression of results was as g·100 g^{-1} fatty acids. Obviously the differences of breed, ages, feeding system and the kind of management between the works probably determined differences of the reported levels in BCFA. The diversity regarding the levels of BCFA in ovine meat can be clearly visualized in the review by Vahmani *et al.* [38]. However, probably the offered food, that is the composition of concentrate or the botanical composition of pasture could be the most important factor which could explain the differences in BCFA in lamb meat. For example, C in the present experiment presented a level of BCFA of 1.43 g·100 g^{-1} fatty acids which included C15:0 iso and anteiso, C16:0 iso and C17:0 iso and anteiso (TABLE II).

In another experiment by our laboratory, using also C, meat presented a level of BCFA of 0.21 g·100 g^{-1} fatty acids and only C15:0 iso and anteiso were detected. No other BCFA were detected above the threshold of 0.01 g·100 g^{-1} fatty acids [14]. In both experiments, the animals are of the same age (11–12 months), reared extensively

TABLE IV
Branched and odd fatty acids (g·100 g^{-1}) of meat from *Longissimus thoracis* muscle of lambs of different breeds produced on pasture

	Breeds						
	H (n=15)	**MD (n=11)**	**C (n=11)**	**CPRO (n=15)**	**C×AM (n=15)**	**RM (n=4)**	***P***
BCFA	1.39±0.07	1.23±0.09	1.43±0.08	1.46±0.06	1.42±0.06	1.57±0.14	NS
BCFA i	0.76±0.05	0.71±0.05	0.78±0.04	0.80±0.03	0.76±0.04	0.85±0.10	NS
BCFA ai	0.63^{ab}±0.03	0.52^{b}±0.05	0.65^{ab}±0.04	0.66^{ab}±0.03	0.67^{ab}±0.02	0.73^{a}±0.05	0.03
Odd Fatty Acids	4.23±0.15	3.89±0.18	4.41±0.17	4.39±0.11	4.18±0.13	4.38±0.38	NS

Data are mean ± SEM. H=Highlander, MD=Merino Dohne, C=Corriedale, CPRO=Corriedale PRO, C×AM=Corriedale x Australian Merino, RM=Romney Marsh. Within lines, mean values bearing different low case letters are significantly different. *P* = Significance level. NS = non–significant. BCFA = sum of total branched fatty acids, i = iso, ai = anteiso, BCFAi = sum C15:0i + C16:0i + C17:0i, BCFAai = sum C15:0ai + C17:0ai, Odd Fatty acids=C15:0 + C15:0i + C15:ai + C17:0 + C17:0i + C17:0ai + C17:1. All calculations were performed on base of results of *TABLE II*

and fed pasture, slaughtered in similar commercial conditions and all the procedures for extraction and detection of the fatty acids were identical to those described in the present work. There are only two differences between the two works. One of them was the muscle evaluated, *longissimus thoracis* in the present work versus the *longissimus lumborum* in the work of Lucas *et al.* [14]. The other was the type of offered pastures regarding their botanical composition, mainly oat and legumes in the present work (see TABLE I), versus grasses in the work of Lucas *et al.* [14].

There are reports that support the concept that the kind and composition and type of pasture, could influence the level and type of fatty acids, including BCFA, in lamb meat [39]. Indeed, in the rumen the interrelation between the microbial populations [40], the specific fatty acids synthetases and the composition of pasture regarding fatty acids and amino acids, will lead to different kind of fatty acids present in ruminant meat [41, 42]. In the case of amino acids, the content of leucine, isoleucine and valine in the pasture seems to have a particular influence on the kind of final BCFA present in ruminant meat [41]. This effect of amino acids in the composition of meat regarding the BCFA, could explain partially the differences observed between the present experiment and that of Lucas *et al.* [14] Indeed, the differences between the two investigations could account for the richness of legumes and oat in those three amino acids, in comparison to the work of Lucas *et al.* [14] where the animal were fed quantitatively mainly graminae [43, 44]. This interesting point needs more exploration and investigation to better understand how BCFA, particularly those linked to positive effect on health, could be incorporated in lamb meat, thanks to use of different kind of offered pastures.

Regarding the BCFA iso (BCFAi) and anteiso (BCFAai), the level of the former was ranged 0.71–0.85 expressed as g·100 g^{-1} fatty acids (TABLE IV). No differences between the breeds studied in this work were detected. The range of BCFAi observed in our work was of the same order that those reported by Gomez–Cortes *et al.* [35] and Mele *et al.* [37], but higher to the values reported by Pena–Bermudez *et al.* [36]. Note that in those three reports the lambs were fed concentrate. For BCFAai the range observed was between 0.52–0.73 g·100 g^{-1} of fatty acids, and RM presented a higher content than MD (TABLE IV). The range of BCFAai observed here is of the same order than those reported by Mele *et al.* [37], but higher than those reported by Gomez–Cortes *et al.* [35] and Pena–Bermudez *et al.* [36]. Certainly, the explanation presented before that in the rumen the interrelation between the microbial populations, the specific fatty acids synthetases and the composition of pasture regarding fatty acids and amino acids leading to the different kind of BCFA in meat could be introduced newly here. This is particularly true for BCFAi and BCFAai, regarding the amino acids composition of pasture [41].

In the case of the Odd fatty acids detected in our work, the range of values was 3.89–4.41. There were not differences between the breeds studied in our work (TABLE IV). That range was in the same order of levels reported by Mele *et al.* [37], slightly higher to values of Garcia *et al.* [28], but markedly higher in comparison to values reported by Gomez–Cortes *et al.* [35] and Pena–Bermudez *et al.* [36]. As indicated before, the lambs used in the works of Mele *et al.* [37], Gomez–Cortes *et al.* [35] and Pena–Bermudez *et al.* [36] were fed concentrate, while the lambs in the work of Garcia *et al.* [28] were fed shrub grass steppes. As for BCFA, the Odd fatty acids are influenced by the rumen metabolism which is, in turn, influenced by its microbial population, the primers present for lipogenesis that is the balance of acetyl–CoA versus propionyl–CoA, and of course the kind of food consumed by the animals [41]. This could explain the observed differences between the different studies highlighted here. In the same direction, Lucas *et al.* [14] reported level of Odd fatty acids of 2.40 g·100 g^{-1} fatty acids for C, a lower level in comparison to the value observed in the present work (4.41 g·100 g^{-1} fatty acids) for the same breed C (TABLE IV). As stated before, the main difference between the two studies was the botanical type of pasture offered to the lamb, if the muscle difference that is *longissimus thoracis* here versus *longissimus lumborum* in Lucas *et al.* [14], is ruled–out as main factor, to explain the differences.

As stated before in the text for individual BCFA and Odd fatty acids, it seems to have some beneficial effect on health of consumers related to those fatty particular acids [21]. More investigation must be undertaken in the future to improve the knowledge about the effect on human health of this kind of component present in meat and milk of ruminants.

Lipids health indices

In TABLE V were grouped some indices that help to known the nutritional characteristics associated to the health of consumers of this kind of lamb meat. The sum of n–6 fatty acids presented a range between 3.35–4.34 g·100 g^{-1} of fatty acids.

These levels are of the same order that those reported by Lucas *et al.* [14] working on C and Ramos *et al.* [22] using crossing between C and MD, both investigations were conducted in condition of Uruguay. However, another investigation using also C [15], reported a slightly

higher values in n–6 fatty acids, that is 5.14 versus 4.34 g·100 g^{-1} fatty acids / 100 g, as reported in this work (TABLE V). Note that in those experiments, the animals were of similar age, reared extensively and fed pasture. Outside of Uruguay, using local breed reared extensively on pasture, Elaffifi *et al.* [45], reported 3.59 g of n–6 / 100 g of fatty acids. A value within the range observed in our work. However, other trials using other breeds fed pastures showed higher levels of n–6 fatty acid, such as in the work of Faria *et al.* [46] and Garcia *et al.* [28], 10.56 and 9.23 g·100 g^{-1} fatty acids, respectively. This last work used Merino lamb fed shrub grass steppes.

Regarding the n–3 fatty acids, the same pattern as reported for the n–6 has been noted. Indeed, the works of Lucas *et al.* [14], Ramos *et al.* [22] and Elaffifi *et al.* [45] reported values within the range observed in the present work, while the reports of Diaz *et al.* [15], Garcia *et al.* [28] and Faria *et al.* [46] reported a much higher level of n–3 fatty acids in meat.

In the case of the n–6:n–3 ratio, the range observed in our work were between 2,24–2.31. These values were in the same order that those reported by, Cadavez *et al.* [18], Ramos *et al.* [22], Garcia *et al.* [28] and Elaffifi *et al.* [45]. In contrast, the values observed in our work were slightly lower compared to the report of Faria *et al.* [46], or clearly lower particularly for C, when reared in similar productive conditions in Uruguay [15]. In practice, the content of n–6, n–3 and their ratio in meat of the animals evaluated in the current work, seems to have values in accord with other results reported in lamb. Albeit there are not specific advices about the adequate intake of n–6 and n–3 fatty acids regarding human health, the ratio between those two classes of fatty acids has been recommended to be near of 4–5 [47], or even between 1:1 and 2:1 [48]. The meat of the animals evaluated in the present work, present all a ratio n–6:n–3 in accord to the recommendation of 2:1. However, nowadays that ratio becomes open to question about its usefulness regarding the human health [49]. Future investigation should improve the knowledge to establish better parameters related to the human consumption of lipids and their fatty acids present in lamb meat.

Another index established as parameters related to the human health, was the ratio between PUFA and SAT (P/S). In our experiment, the P/S ratio observed varied between 0.10 and 0.14 (TABLE V). Those values were of the same order that those reported by Ramos *et al.* [22], but largely below to those reported by Diaz *et al.* [15], Cadavez *et al.* [18], Garcia *et al.* [28], and Faria *et al.* [46]. The recommended levels of P/S in different kind of meat to ensure an adequate health in human in regard to the cardiovascular diseases must be between 0.4 and 1 [50]. Thus, meat of the animals used in our work is below that recommended level, as reported in TABLE V. Therefore, this point justifies a particular attention in the future investigations.

The other indices used in our work to assess the potential protection against cardiovascular disease were AI, TI and h/H [51]. For AI, the value observed in our work ranged 0.63–0.89 (TABLE V). The advised level of AI must be as low as possible. The range observed was of the same order or slightly higher than those reported by Cadavez *et al.* [18], Belhadj *et al.* [52] and the compilation work by Procisur–IICA [53]. For TI, the values observed in our work ranged 1.51–1.80 (TABLE V). Those levels are higher than the reported by Cadavez *et al.* [18], Belhadj *et al.* [52] and the compilation of Procisur–IICA [53]. The recommended value of TI in meat must be as low as possible, to reduce the thrombogenic effects in human [54].

In the case of h/H indices, the values observed in the current work ranged 1.64–2.12 and the C showed a higher indice than MD (TABLE V). For that index, the recommended value in meat must be as high as possible to minimize the risk of hypercholesterolemia leading to cardiovascular diseases [55]. The range of levels observed in our work was of the same order or slightly lower than those reported by Belhadj *et al.* [52] working with four local breeds fed pasture in Morocco, and Murariu *et al.* [56] in Romania using Karakul lamb fed pasture and supplemented with hay and cereals in winter season.

Some of the presented indices such as P/S and TI were not within the recommended value, thereby more investigation must be undertaken to try to improve those parameters. This could be done through the modification of feeding system of the animals using different kind of pasture. This point is an important challenge for the ovine production in Uruguay, as a way to help farmers to promote their products in base to the health of consumers.

Enzyme activity indices

Enzyme indices for desaturases Δ–9, Δ–5 and Δ–6, elongase, and thioesterase have been calculated in an attempt to detect differences

TABLE V
Lipids health indices, of meat from *Longissimus thoracis* muscle of lambs of different breeds produced on pasture

	Breeds						
	H (n=15)	**MD (n=11)**	**C (n=11)**	**CPRO (n=15)**	**C×AM (n=15)**	**RM (n=4)**	**P**
Σn–6	3.35±0.29	3.85±0.49	4.34±0.29	3.94±0.32	3.83±0.23	4.21±0.27	NS
Σn–3	1.53±0.14	1.72±0.25	2.08±0.23	1.82±0.19	1.70±0.13	1.85±0.19	NS
n–6/n–3	2.25±0.12	2.32±0.08	2.24±0.14	2.25±0.09	2.31±0.06	2.31±0.13	NS
P/S	0.10±0.01	0.11±0.02	0.14±0.01	0.12±0.01	0.12±0.01	0.13±0.01	NS
AI	0.73±0.06	0.89±0.09	0.64±0.04	0.73±0.06	0.78±0.05	0.63±0.05	NS
TI	1.70±0.08	1.80±0.14	1.51±0.07	1.55±0.07	1.62±0.07	1.51±0.05	NS
h/H	1.87^{ab}±0.11	1.64^{b}±0.13	2.12^{a}±0.11	1.93^{ab}±0.09	1.80^{ab}±0.09	2.08^{ab}±0.09	0.05

Data are mean ± SEM. H=Highlander, MD=Merino Dohne, C=Corriedale, CPRO=Corriedale PRO, C×AM = Corriedale × Australian Merino, RM=Romney Marsh. Within lines, mean values bearing different low case letters are significantly different. *P* = Significance level. NS = non–significant. Σn–6 = total n–6 fatty acids, Σn–3 = total n–3 fatty acids, EPA = C20:5n3, DHA = C22:6n3, P/S = PUFA/SAT ratio, AI = atherogenic indices, TI=thrombogenic indices, h/H=hypocholesterolemiant indices, BCFA = total branched fatty acids, i = iso, ai = anteiso, Odd FA = odd fatty acids

in the lipids metabolism between the breeds studied in the present work. These indices are generally used as surrogates the measure of the true enzyme activities. This procedure has been used also in medical field as simple way to evaluate the activities of enzymes such as desaturases, elongases and thioesterases in some human's pathologies [57]. The enzyme activities were presented in

TABLE VI and it can see that C, CPRO and C×AM have a much more active Δ–9–C16 than MD. This result could maybe explain the differences for C16:1 content, reported for glycerolipids in TABLE II, for CPRO and CxAM, but not for C.

In the case of the enzyme Δ–9–C18, as well as the sum of the activities of both enzymes Δ–9–C16+C18, there are no differences between the animals studied in the present work (TABLE VI). Δ–9 enzyme introduce a cis– double bond in the D9 position between carbons 9 and 10, and the preferred substrates are palmitoyl–CoA for Δ–9–C16 and stearoyl–CoA for Δ–9–C18, which lead to their conversion into palmitoleoyl–CoA and oleoyl–CoA, respectively [58]. MUFA, and particularly C18:1, are of great importance for membrane structure and function based on phosphoglycerides [59]. However, globally there is not a clear difference between the breeds regarding the *de novo* synthesis and the deposition of MUFA, in glycerolipids as well as in glycerophoapholipids, in *longissimus thoracis* muscle. Probably the fact that the animals have been reared in identical conditions, that is fed same pasture and management, could have minimised the possible enzymes expression differences between the breeds.

The enzyme indices for Δ–5 do not show either differences between the animals, while for Δ–6, C have a higher activity than H (TABLE VI). This result probably explains the higher content in C18:3n3 for C compared to H, as well as for glycerolipids than for glycerophospholipids (TABLES II and III). Indeed, Δ–5 and Δ–6 desaturases are crucial for the synthesis of PUFA [46, 57].

In the case of the elongase activities, C showed higher activity compared to MD (TABLE VI). The enzymes elongase add two carbon atoms to the fatty acid C16:0 obtaining the fatty acid C18:0, but also elongate other fatty acids from the two essential fatty acids C18:2n6 and C18:3n3 [60, 61]. However, the comparison between C and MD regarding the content of C18:0 and PUFA; do not show differences neither for glycerolipids nor for glycerophospholipids (TABLES II and III). Probably, the differential activity observed for elongases between C and MD was too small to affect significantly the content of C18:0 and PUFA in meat of both breeds. As stated before for desaturases, the fact that the animals have been reared in identical conditions, fed same pasture and conducted within a same extensively system, possibly could have minimised the differences between breeds for elongase activities.

For the thioesterase indice, there is not differences between the six breeds studied here (TABLE VI). The thioesterase is part of the fatty acid synthase enzyme complex encoded by the FASN gene in mammals, and regulating principally the formation of C16:0 as final product and C14:0 as a minor one [62]. Taking into account, as reported above, the implication of C16:0 in cardiovascular diseases in human, hence it could be interesting to investigate how the thioesterase activity in lamb meat can be modulated. That focus could help to improve the nutritional and health quality of meat regarding the content of C16:0 in meat. Future research could open the way in that direction.

Interrelations among fatty acids of glycerolipids and glycerophospholipids

A principal component analysis carried out on total lipids and selected fatty acids of glycerolipids of lambs meat (FIG. 1a), shows that the two first principal components accounted for 66.7% of the data variability. The first component (43.2%) was positively correlated with C17:0ai (r=0.870), 18:3n3 (r=0.769), CLA (r = 0.798) and BCFAai (r= 0.771), and associated negatively to C14:0 (r=0.544) and to C16:0 (r=0.820).

The second component that explains 23.5% of the data variability was positively associated mainly to intramuscular fat content (r=0.562), C14:0 (r=0.731) and BCFAi (r=0.648). The principal component analysis shows that lamb meat with a higher content of linolenic acid tend to have lower content of saturated fatty acids, C14:0 and C16:0. When the individual observations for glycerolipids are projected in the two dimensional space (FIG. 1b), there is an evidence that H and MD genotypes are differentiated from others by CLA, BCFAai, C17:0ai, and 18:n3.

By other side the variability of RM is associated to component two, BCFAi, intramuscular fat content and C14:0. In terms of lipid attributes

TABLE VI
Enzymes indexes of fatty acid metabolism estimated on the basis of fatty acid composition of *Longissims thoracis* muscle of lambs of different breeds produced on pasture

	Breed					
	H (n=15)	**MD (n=11)**	**C (n=11)**	**C×AM (n=15)**	**RM (n=4)**	***P***
Δ–9 – C16	$6.98^{ab} \pm 0.46$	$5.88^{b} \pm 0.56$	$7.91^{a} \pm 0.44$	$7.97^{a} \pm 0.40$	$8.11^{ab} \pm 0.47$	0.02
Δ–9 – C18	66.40 ± 0.89	67.00 ± 0.48	65.50 ± 0.77	68.50 ± 0.90	65.80 ± 1.38	NS
Δ–9 – C16+C18	48.60 ± 0.87	47.10 ± 1.02	49.70 ± 0.75	49.40 ± 0.86	50.00 ± 1.00	NS
Δ–5	45.00 ± 5.10	49.70 ± 6.60	51.60 ± 3.92	50.40 ± 2.98	53.50 ± 5.03	NS
Δ–6	$2.51^{b} \pm 0.35$	$3.49^{ab} \pm 0.47$	$4.83^{a} \pm 0.51$	$4.33^{ab} \pm 0.42$	$4.62^{ab} \pm 1.05$	0.005
Elongase	$0.88^{ab} \pm 0.06$	$0.75^{b} \pm 0.05$	$1.01^{a} \pm 0.05$	$0.77^{b} \pm 0.04$	$0.99^{ab} \pm 0.07$	0.008
Thioesterase	10.10 ± 0.55	7.99 ± 0.51	9.08 ± 0.47	8.46 ± 0.52	9.38 ± 1.35	NS

Data are mean ± SEM. H=Highlander, MD=Merino Dohne, C=Corriedale, CPRO=Corriedale PRO, C×AM=Corriedale x Australian Merino, RM= Romney Marsh. Within lines, mean values bearing different low case letters are significantly different. *P* = Significance level, NS = non–significant, C16= palmitic acid, C18= stearic acid. Δ–9= Δ–9–desaturase, Δ–5= Δ–5–desaturase, Δ–6= Δ–6–desaturase, indexes of Δ–9, Δ–5 and Δ–6 desaturases, and elongase (ratio C18:0/C16:6) and thioesterase (ratio C16:0/C14:0) indexes were calculated according to del Puerto *et al.* [11]

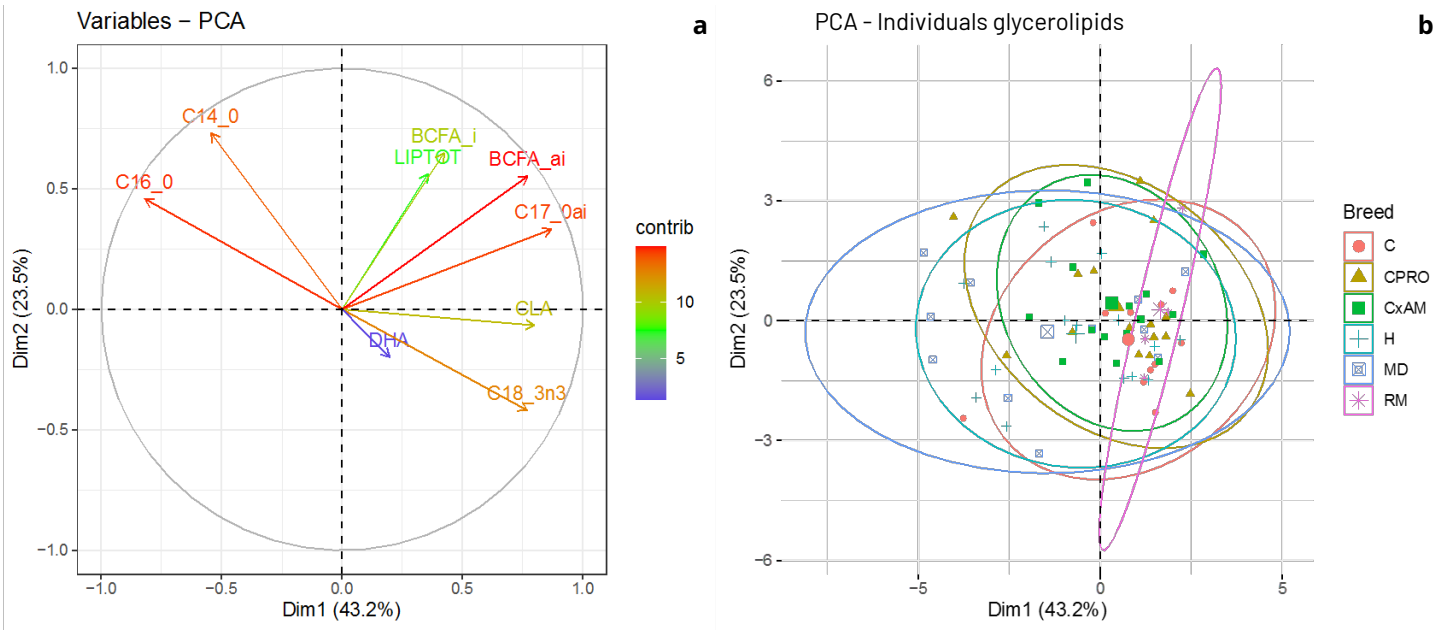

FIGURE 1. a) Variables factor map of compositional lipid metabolism of glycerolipids related to human health of lamb meat from six breeds. Liptot= total lipids; C14:0= myristic acid; C16:0= palmitic acid; C17:0ai=margaric acid anteiso ; BCFAai= anteiso branched chain fatty acid; BCFAi=iso branched chain fatty acid; C18:3n–3=linolenic acid; CLA= conjugated linoleic acid ; DHA= C22:6n3. b) Individuals factor map of compositional lipid metabolism of glycerolipids in lamb meat grouped by breed, with ellipses superimposed at α = 0.95. C=Corriedale; CPRO=Corriedale Pro; C×AM=Corriedale x Australian Merino; H=Highlander; MD=Merino Dohne; RM= Romney Marsh

selected, lamb meat of C, CPRO, and C×AM overlap in quality attributes, associated with higher CLA and 18:3n3 and H with the lower content and also lower content of lipids. RM shows higher level of BCFAai possibly associated to intramuscular fat content. When a principal component analysis was carried out for glycerophospholipids, a clearer patron of association among variables is observed (FIGS. 2A and 2B). Two components explain the 77.9% of the total variability.

First component that explains the 66.2% of the variability is positively and hardly associated with C18:2n6 (r=0.922), C18:3n3 (r=0.951), C20:4n6 (r=0.903) and DPA (r=0.922) and negatively with C16:0 (r=0.948) and C18:1

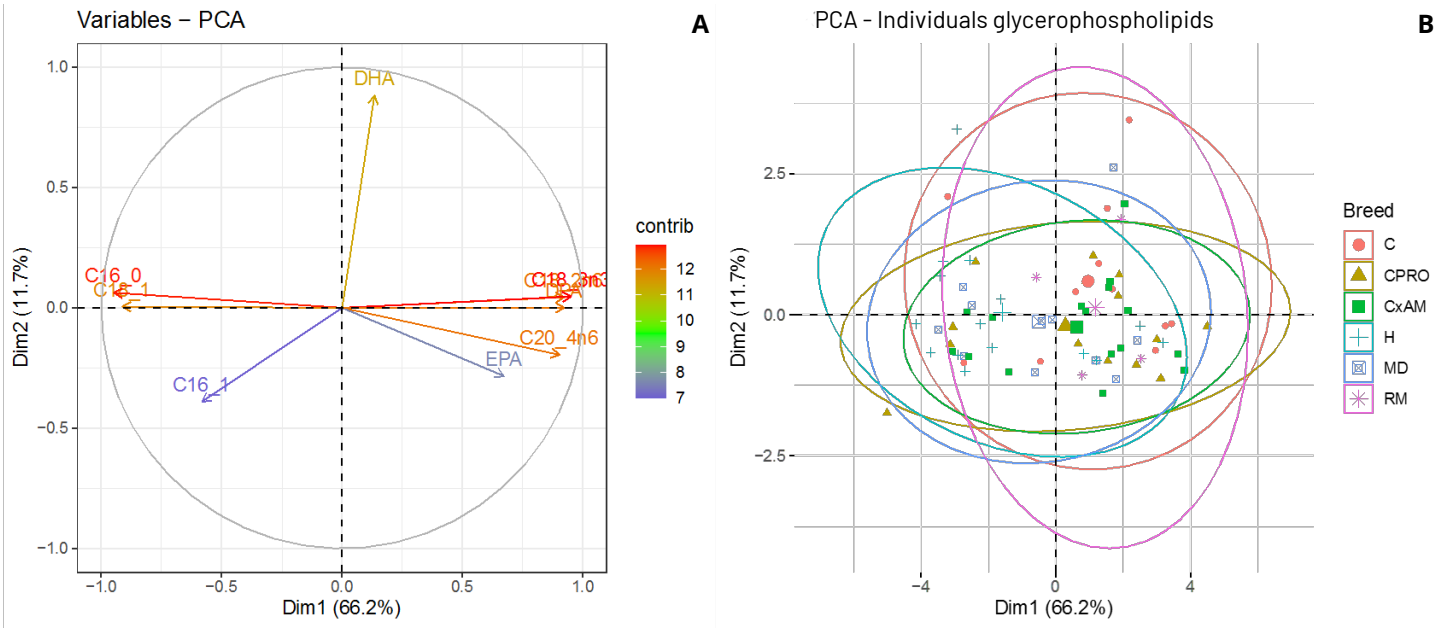

FIGURE 2. A) Variables factor map of compositional lipid metabolism of glycerophospholipids fraction related to membrane structure of lamb meat from six breeds. C16:0= palmitic acid; C16:1= palmitoleic acid; C18:1= oleic acid; C18:2n6= linoleic acid; C18:3n–3=linolenic acid; C20:4n6= arachidonic acid; EPA= C20:5n3; DPA=C22:5n3; DHA= C22:6n3. B) Individuals factor map of compositional lipid metabolism associated to glycerophospholipids fraction of lamb meat grouped by breed, with ellipses superimposed at α = 0.95. C=Corriedale; CPRO=Corriedale Pro; CxAM=Corriedale x Australian Merino; H=Highlander; MD=Merino Dohne; RM= Romney Marsh

(r=0.913). The component two that explains 11.7% of the variability is mainly associated positively to DHA (r=0.882). Concerning individual observations for glycerophospholipids only CPRO is associated with the variables of the first component, while for individuals of C, a relation is evidenced for the variables that affect the component two, the DHA.

CONCLUSIONS

The results of the study show overlapping among breeds related of compositional lipid metabolism, except for few relevant fatty acids such as C16:0, C18:3n3 and CLA for glycerolipids, and C18:1, C18:2n6 and C18:3n3 for glycerophospholipids. Likewise, other differences were outlined such as for BCFAai, h/H and enzymes activity of Δ-9-C16, Δ-6 and elongase. But actually the differences are just between two or three breeds of the six studied in the present investigation, and not for all those relevant fatty acids.

Thus, it can be said that overall the studied breeds present good lipid nutritional indicators in comparison with the results of other research in lambs, except for EPA and DHA fatty acids, as those breeds present a relatively low content in comparison to the values indicated in some reports from the scientific literature. This last point will be taken into account in our future studies, in order to improve the final composition of meat of those breeds, with the most relevant n-3 fatty acids regarding the health of consumers.

As mentioned throughout the text, the animals were fed and managed in identical conditions. This could explain why there are not more substantial differences between the breeds, regarding the fatty acid composition of meat. Maybe other conditions, such as the ages of the animals, different kind of pasture with or without supplementation, could well affect differently each of the breeds used here. This hypothesis should be considered in future experiments. Anyway, the results of the present investigation established indicators, based on typical productive conditions of Uruguay, about the lipids and fatty acids content of lamb meat for the breeds studied here. Those lipids parameters, not determined before, could be used as a baseline for future study directed to the nutritional quality of lamb meat produced on pasture in Uruguay.

Conflict of interest

The Authors declare that there is no conflict of interest.

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