Arch. Anim. Breed., 59, 1–8, 2016
www.arch-anim-breed.net/59/1/2016/
doi:10.5194/aab-59-1-2016

Open Access
Archives Animal Breeding

Meta-analysis of intramuscular fatty acid composition of Mediterranean lambs

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Received: 23 June 2015 – Revised: 11 December 2015 – Accepted: 17 December 2015 – Published: 15 January 2016

Abstract. The aim of this work was to study the intramuscular fatty acid profile of Mediterranean lambs using a meta-analysis. The database was created from five journal articles and one doctoral dissertation, and included lambs of the Talaverana, Comisana, Churra Tensina, Merino Branco and Pramenka breeds. All analyses were performed using Comprehensive Meta-Analysis and R statistical software. According to the overall pooled result, lambs of Mediterranean breeds included in our meta-analysis had 22.85 % of palmitic (C16:0), 14.78 % of stearic (C18:0) and 5.32 % of myristic acid (C14:0). The overall pooled result for oleic acid (C18:1) was 32.53 %, and it was 5.60 % for linoleic acid (C18:2 n-6) and 1.95 % for α-linolenic acid (C18:3 n-3). Regardless of the proportions of the main fatty acids, Mediterranean lambs had 47.85 % of SFA (saturated fatty acid), 37.87 % of MUFA (monounsaturated fatty acid) and 11.50 % of PUFA (polyunsaturated fatty acid). The overall pooled result for the PUFA / SFA ratio was 0.23, and it was 2.22 for the n-6 / n-3 ratio. The results of our meta-analysis enabled us to gain an important scientific insight into the lipid profile of intramuscular fat in Mediterranean lambs. A systematic combination of quantitative data from several carefully selected studies provided estimates of greater statistical power and enabled a more truthful comparison of results originating from different independent studies than any previously published research on this issue.

1 Introduction

Lamb meat has a commercial value, both on the national and international market, due to consumer habits that are largely influenced by economical and social characteristics (Cruz et al., 2011). Although the commercial value of the carcasses is usually only slightly influenced by chemical and physical properties of fat, it is reported that the type of fat and fatty acid composition affects eating habits and maintains the quality of meat (Schmid, 2011). Previous studies have shown that a low intake of saturated fatty acids (SFAs) and an increased polyunsaturated to saturated fatty acid ratio (PUFA / SFA) are associated with a low risk of human cardiovascular diseases (Prates and Bessa, 2009; Hoenselaar, 2012). Being aware of these findings, consumers have become more diet conscious and their perception regarding meat healthiness is primarily related to its fat content and fatty acid composition (Saatchi et al., 2013).

It is generally known that ruminant meats have a high content of SFA and a low PUFA / SFA ratio due to the hydrogenating action of the rumen microorganisms. On the other hand, ruminant meat is characterized by a low n-6 / n-3 ratio, which has been shown to be beneficial in the prevention and treatment of human cardiovascular diseases (Wood et al., 2003). Many factors in the production, such as breed, gender, diet, production system, weight, age and cut of the meat, can be involved in the lipid composition of lamb meat (Júarez et al., 2008; Marino et al., 2008; Borys et al., 2012). To enable comparability of results originating from several studies, a meta-analytical approach was used in this paper. This analytical approach, i.e. an inferential statistical method, enables all published data to be combined and analysed to draw

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quantitative conclusions which cannot be obtained from experimental studies or bibliographic reviews (Sauvant et al., 2005). To our knowledge, there are no reports on the use of meta-analysis in the evaluation of lipid composition of lamb meat in the literature. Therefore, the aim of this work was to study the intramuscular fatty acid profile of Mediterranean lambs using a meta-analysis.

2 Materials and methods

2.1 Data collection

Data were extracted systematically from all relevant and available publications i.e. peer-reviewed journals, conference proceedings, reports, original scientific papers and doctoral dissertations. A database was created to compile the data of lipid composition in lamb meat according to the following inclusion criteria: (1) meat came from male lambs of dual-purpose Mediterranean sheep breeds; (2) lambs were slaughtered at a live weight of 23 to 26 kg; (3) lambs were reared on pasture. Furthermore, the lipid composition of lamb meat had to be (4) analysed in the longissimus dorsi muscle and (5) expressed as a percentage of individual fatty acids. Key word searches were performed using the terms "lamb" and "fatty acids" in combination with "meat quality", "lipid composition" and "carcass quality". The required inclusion criteria for a data set were met by 15 studies. The inability to obtain additional information from the authors (rearing system, feeding regime, gender, slaughter weight of the animals, presented data) and inconsistency in analytics were the reasons for the non-inclusion of many candidate studies. Finally, a database was created from five published journal articles (Díaz et al., 2002; Belo et al., 2005; Joy et al., 2008; Chiofalo et al., 2010; Panea et al., 2011) and one doctoral dissertation (Kaić, 2013).

2.2 Data analysis

Guidelines for conducting the meta-analysis were based on meta-analytic techniques described by Borenstein et al. (2009). All analyses were performed using the Comprehensive Meta-Analysis (Borenstein et al., 2005) software package. Standardized effect sizes were expressed using a method of arithmetic means (Lipsey and Wilson, 2001). According to this method, effect size statistics were calculated as follows:

$$\mathrm{ES} = \frac{\sum x_i}{n}, \tag{1a}$$

$$\mathrm{SE} = \frac{\mathrm{SD}}{\sqrt{n}}, \tag{1b}$$

$$w = \frac{1}{\mathrm{SE}^2} = \frac{n}{\mathrm{SD}^2}, \tag{1c}$$

where ES is the effect size of the study, x_i is an individual score for the subject i, n is the total sample size, SE is the standard error of the study, SD is the standard deviation of x, and w is the weight of the study. To identify the presence of true heterogeneity among studies, Cohran's Q chi-square test (Hedges and Olkin, 1985) was applied. Q statistics were computed as

$$Q = \sum_{i=1}^{k} w_i y_i^2 - \frac{\left(\sum_{i=1}^{k} w_i y_i\right)^2}{\sum_{i=1}^{k} w_i}, \tag{2}$$

where w_i is the weight of the study, y_i is the effect size of the study, and k is the number of studies. Under the null hypothesis that all studies share a common effect size, the Q value followed a central chi-squared distribution with degrees of freedom (df) equal to $k-1$. The test for heterogeneity of the included studies indicated that there was no significant difference between the means of the lipid composition of lamb meat and the null hypothesis was rejected. Because of a relatively low power of the test with a small number of studies (Huedo-Medina et al., 2006), heterogeneity was additionally quantified with the I^2 statistics (Higgins et al., 2003). The I^2 statistics described the proportion of the total study variance that was due to between-study variation and was calculated as

$$I^2 = \left(\frac{Q - \mathrm{df}}{Q}\right) \times 100. \tag{3}$$

Hence, I^2 is the percentage of the chi-squared statistics which is not explained by the variation within the studies. Values of I^2 in our research were, in accordance with the distribution of Higgins et al. (2003), described as low and moderate so they were not investigated further by subgroup analysis or meta-regression. Variance of the true effect size across the population of studies (between-studies variance) was calculated by the method of moments or the DerSimonian and Laird method (Borenstein et al., 2009):

$$T^2 = \frac{Q - \mathrm{df}}{C}, \quad \text{where} \tag{4a}$$

$$C = \sum w_i - \frac{\sum w_i^2}{\sum w_i}. \tag{4b}$$

Further analyses were performed using the following general random-effects model:

$$y_i = \mu + \zeta_i + \varepsilon_i, \tag{5}$$

where y_i is the observed effect, μ is the mean of all true effects, ζ_i is the true variation in effect sizes, ε_i is the sampling error. A random-effects model includes between-study variability (differences in the true effect sizes) and within-study variability (sampling error) and estimates the mean of a distribution of effects (Borenstein et al., 2009). The summary statistics were computed using the random-effects analysis

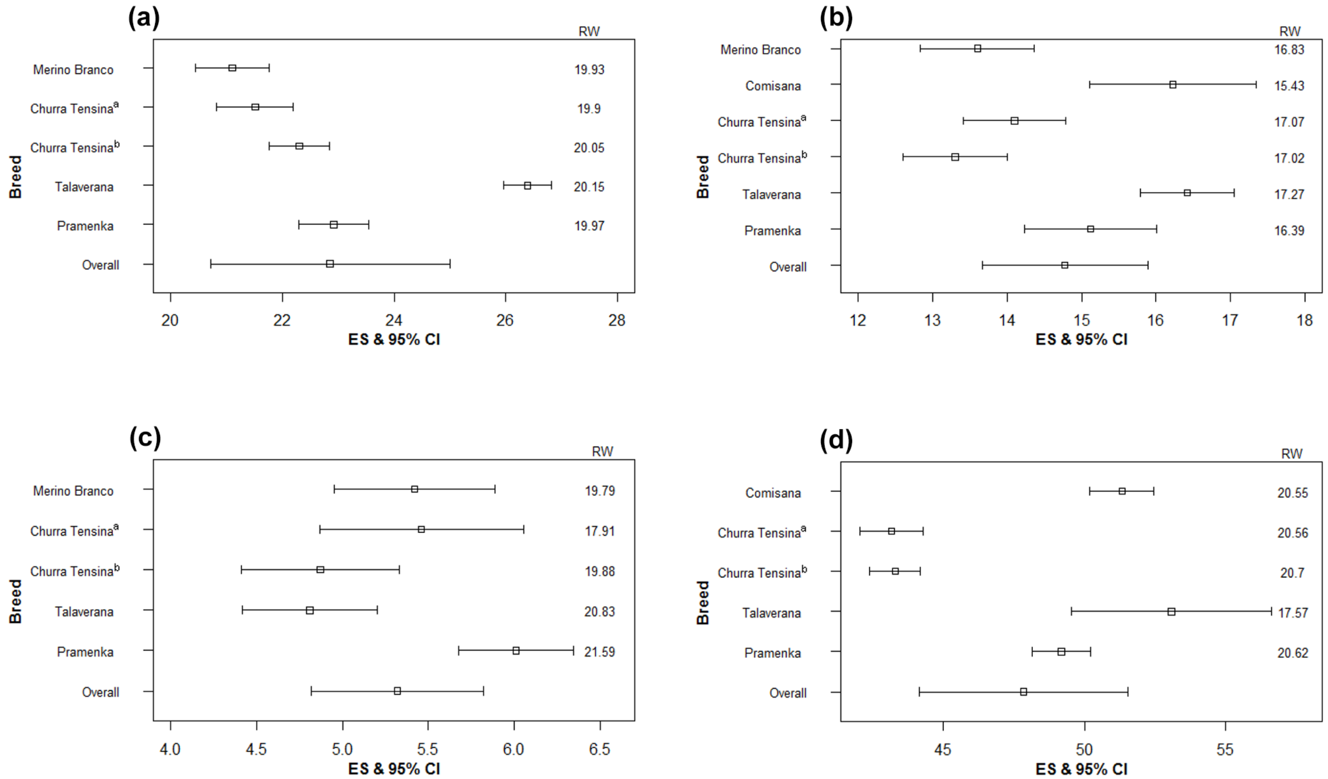

Figure 1. Meta-analysis of C16:0 **(a)**, C18:0 **(b)**, C14:0 **(c)** and SFA **(d)**. Different superscript letters ([a,b]) indicate lambs of the same breed included in different studies; CI: confidence interval; RW: relative weight of the study; Overall: overall pooled result.

as follows:

$$w_i = \frac{1}{V y_i}, \tag{6a}$$

$$M = \frac{\sum_{i=1}^{k} w_i y_i}{\sum_{i=1}^{k} w_i}, \tag{6b}$$

$$V_M = \frac{1}{\sum_{i=1}^{k} w_i}, \tag{6c}$$

$$\mathrm{SE}_M = \sqrt{V}_M, \tag{6d}$$

$$\mathrm{CI_L} = M - 1.96 \times \mathrm{SE}, \tag{6e}$$

$$\mathrm{CI_U} = M + 1.96 \times \mathrm{SE}, \tag{6f}$$

where w_i is the weight assigned to each study, $V y_i$ is within-study variance for study i, M is the weighted mean, V_M is the variance of the summary effect, SE_M is the standard error of the summary effect, $\mathrm{CI_L}$ is the lower limit of the 95 % confidence interval for the summary effect, and $\mathrm{CI_U}$ is the upper limit of the 95 % confidence interval for the summary effect. The main results (effect sizes and confidence intervals of each study and overall effect estimate with confidence interval) for the lipid composition of lamb meat are visually displayed in forest plots that were created using the R statistical software (R Core Team, 2013).

3 Results and discussion

The total lipid content of ruminant tissues is comprised of about 40 % SFA, 40 % MUFA (monounsaturated fatty acids) and 2–25 % PUFA (Wood et al., 2008). Although ruminant meat is an important source of nutrients, its relatively high content of SFA has been criticized and linked with an increase in total cholesterol; consequently, it has been related to cardiovascular diseases (Ribeiro et al., 2011). Nevertheless, it has been evidenced that individual fatty acids in this family have considerably different effects. For instance, lauric acid (C12:0) and myristic acid (C14:0) have a greater total cholesterol raising effect than palmitic acid (C16:0), whereas stearic acid (C18:0) has a neutral effect on the concentration of total serum cholesterol, including no apparent impact on either low-density lipoprotein or high-density lipoprotein (Daley et al., 2010). Within the SFA, the predominant ones in ruminant meat are palmitic (C16:0), stearic (C18:0) and myristic (C14:0) fatty acids (Cruz et al., 2011). Forest plots illustrating C16:0, C18:0 and C14:0 of Mediterranean lambs are presented in Fig. 1a, b and c, respectively.

Data used for a meta-analysis of myristic acid (C14:0) were compiled from five studies and altogether included 82 lambs. Lambs of the Merino Branco, Churra Tensina,[a,b1] the Talaverana and Pramenka breeds were used for the analysis. The overall pooled result for C14:0 was 5.32 %, with a 95 % CI of 4.81–5.82 %. All studies included in the analysis were within the range of the overall estimate.

The meta-analysis of palmitic acid (C16:0) was compiled from five individual studies and altogether included 82 lambs of the Merino Branco, Churra Tensina[a,b], Talaverana and Pramenka breeds. The overall pooled result (estimate) for C16:0 was 22.85 %, with a 95 % confidence interval (CI) from 20.71 to 24.99. This result gave us an estimate of the mean effect size and its precision. The study that included Talaverana lambs was not within the range of the overall pooled result. Talaverana lambs had 26.39 % of C16:0, with a 95 % confidence interval from 25.96 to 26.82. This result could be due to the fact that Talaverana lambs were weaned, while all other breeds included in meta-analysis were suckled by their dams until slaughter. According to Bas and Morand-Fehr (2000), this effect could be due to the presence of some fatty acids laid down in adipose tissue before weaning , *de novo* endogenous synthesis of fatty acids, the length of the post-weaning period, the fattening level, and the age and weight at weaning.

Stearic (C18:0) fatty acid was analysed using a data from six studies. Altogether, it included 92 lambs of the Merino Branco, Comisana, Churra Tensina[a,b], Talaverana and Pramenka breeds. The overall pooled result for C18:0 was 14.78 %, with a 95 % CI from 13.66 to 15.89. All studies included in the analysis were within the range of the overall estimate. Although not significantly different, the proportion of C18:0 in Talaverana lambs (16.42) was slightly higher than in the other breeds included in the study. Compared to the Talaverana lambs, which were weaned and had a short starter diet at pasture, the feeding management of all other breeds included in the study was based only on suckling and grazing. This feeding management of Talaverana lambs could be followed by higher rumen biohydrogenation of dietary PUFA, and therefore we assumed that they would have a significantly higher proportion of C18:0 as linoleic (LA, C18:2) and α-linolenic acid (ALA, C18:3) are usually available in grains and forages. They are extensively biohydrogenated in the rumen, resulting in a higher outflow of C18:0 to the duodenum than the actual intake of C18:0 as well fatty acids intermediates of biohydrogenation (*trans*-11 C18:1 vaccenic acid and *cis*-9, *trans*-11 conjugated linoleic acid). The proportion of C18:0 in lambs of the Comisana breed (16.23) was also close to Talaverana (16.42) but with a lower weight of the study (15.43 vs. 17.27) and a larger 95 % CI (95 % CI from 15.11 to 17.35 in Comisana vs. 95 % CI from 15.79 to 17.04 in Talaverana). With smaller CI and higher weight assigned to the more precise studies, it should be mentioned that the data of Talaverana lambs in our analysis were more precise. The neutral effect of C18:0 on the concentration of total serum cholesterol implies that this SFA may not increase the risk of cardiovascular disease (Daley et al., 2010). Therefore, a slightly higher proportion of C18:0 in Talaverana lambs could be denoted as the most desirable among the breeds included in our study. Nevertheless, the proportion of all other fatty acids and their influence on a human health must be taken into consideration.

Beside the criteria included in these analyses, it must be considered that C16:0, C18:0 and C14:0 are strongly associated with the overall fat levels in breeds. This trait was not analysed in the present study because data for overall fat levels of all included breeds were not available.

The analysis of SFA was performed using data from five individual studies and included 84 lambs. The breeds included in the analysis were Comisana, Churra Tensina[a,b], Talaverana and Pramenka. The overall pooled result of SFA was 47.85, while a 95 % CI ranged from 44.16 to 51.55. All included studies were within the range of the overall estimate (Fig. 1d). Although not significantly different, proportions of SFA in Churra Tensina[a] lambs were slightly lower than the others. Taking into account that a lower proportion of SFA is desirable from the perspective of the human health (Prates and Bessa, 2009), the SFA proportion of the Churra Tensina[a] lambs can be considered as the most desirable among the breeds included in this study.

In contrast to SFA, it has been proven that MUFA and PUFA are beneficial for human health (Prates and Bessa, 2009; Hoenselaar, 2012). The beneficial effects of PUFA have been associated with its ability to protect against cardiovascular diseases (Harris et al., 2007), whereas MUFAs have been associated with a hypocholesterolemic effect and with a beneficial effect on insulin sensitivity (Ros, 2003; López-Huertas, 2010). Approximately 50 % of the intramuscular fat of the lamb is made up of unsaturated fatty acids; MUFAs are made up predominantly from oleic acid (C18:1) and PUFAs from linoleic (LA, C18:2) and α-linolenic (ALA, C18:3) fatty acids, respectively (McAfee et al., 2010). Oleic acid (C18:1) originates either from dietary sources or endogenous synthesis of C18:0 by the enzyme complex Δ9-desaturase (stearoyl-CoA desaturase). It has been considered to be hypolipidemic, reducing plasma cholesterol as well as triglycerides (Mills et al., 1992), and can be considered as a desirable component of the human diet. A forest plot illustrating C18:1 of Mediterranean lambs is presented in Fig. 2a. A meta-analysis was performed using data from six studies and included the Comisana, Merino Branco, Talaverana, Churra Tensina[a,b] and Pramenka breeds. Altogether, 92 lambs were included in the analysis. The overall pooled result for C18:1 was 32.53 %, with a 95 % CI from 29.96 to 35.11. Studies that included Churra Tensina[a,b] lambs and Comisana lambs were not within the range of the overall pooled result. Churra Tensina[a] lambs had 36.64 % of C18:1, with a 95 % confidence interval from 35.16 to 38.11, while Churra Tensina[b] lambs had 37.7 % of C18:1, with a 95 % confidence inter-

[1]Different superscript letters ([a,b]) indicate lambs of the same breed included in different studies

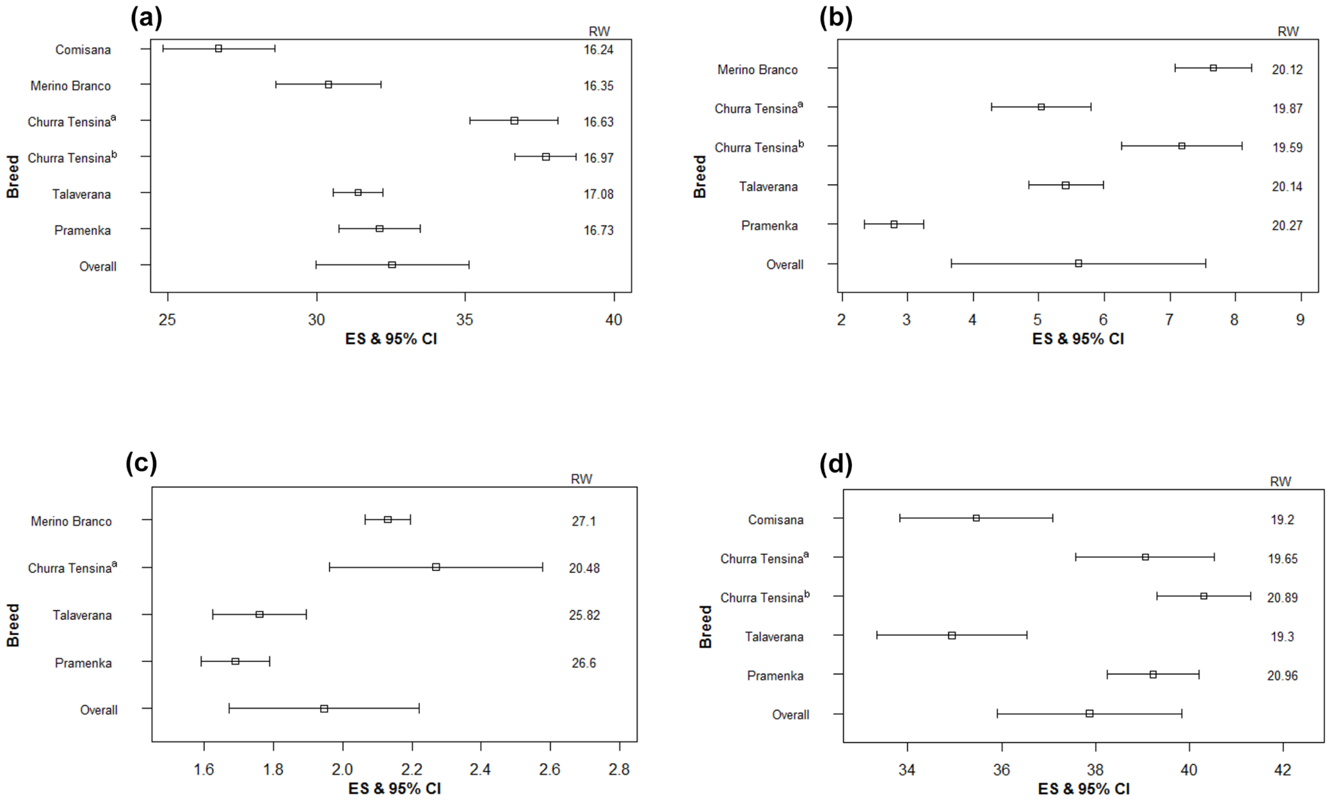

Figure 2. Meta-analysis of C18:1 **(a)**, C18:2 n-6 **(b)**, C18:3 n-3 **(c)** and MUFA **(d)**. Different superscript letters (^{a,b}) indicate lambs of the same breed included in different studies; ES: effect size; CI: confidence interval; RW: relative weight of the study; Overall: overall pooled result.

val from 36.67 to 38.72. By contrast, Comisana lambs had 26.7 % of C18:1, with a 95 % confidence interval from 24.82 to 28.58. According to Dervishi et al. (2010), the lipid profile of ruminants can be manipulated by diet, and grazed lambs have a lower proportion of C18:1 compared to all other feeding systems. Because of this and the feeding management of Churra Tensina and Comisana lambs, which were similar to other breeds included in the study (based on suckling and grazing), we expected that their proportions of C18:1 would be in the range of our overall estimate. Nevertheless, it must be considered that C18:1 can also originate from endogenous synthesis of C18:0 and could also be a major factor that had an impact on our results. Mills et al. (1992) and Wood et al. (2003) reported that diets with a higher proportion of C18:1 are regarded as beneficial to human health. In accordance with this, Churra Tensina^{a,b} lambs had significantly higher and consequently more favourable proportions of C18:1 than the other breeds included in our study.

Linoleic (LA; C18:2 n-6) and α-linolenic (ALA; C18:3 n-3) acids are essential n-6 and n-3 PUFAs in humans, i.e. they are not synthesized by the human body and should be taken up through the diet. They are precursors of potent lipid mediators, termed eicosanoids, which have an important role in the regulation of inflammation. Eicosanoids derived from n-6 PUFAs such as arachidonic acid (C20:4 n-6) have proinflammatory and immunoactive functions, whereas eicosanoids derived from n-3 PUFAs such as eicosapentaenoic acid (C20:5 n-3) and docosahexaenoic acid (C22:6 n-3) have anti-inflammatory properties, attributed to their ability to inhibit the formation of n-6-PUFA-derived eicosanoids (Wall et al., 2010). However, it must be considered that in ruminants, LA and ALA are degraded to MUFA and SFA in the rumen by microbial biohydrogenation (70–95 and 85–100 %, respectively) and only a small proportion, around 10 % of dietary consumption, is available for incorporation into tissue lipids (Nieto and Ros, 2012). A forest plot illustrating the LA of Mediterranean lambs is presented in Fig. 2b. The analysis of LA was performed using a data from five studies. The analysis altogether included 82 lambs of the Merino Branco, Churra Tensina^{a,b}, Talaverana and Pramenka breeds. The overall pooled result was 5.60 %, with a 95 % CI from 3.67 to 7.54. The analysis showed that the proportion of LA in Pramenka lambs was significantly lower (2.79 % of LA, with a 95 % confidence interval from 2.34 to 3.24) than the overall pooled result and therefore than in the other breeds included in our study. The main dietary sources of LA (such as sunflower, safflower or corn oils) that could influence its proportion in lamb meat were excluded from the analysis (ex-

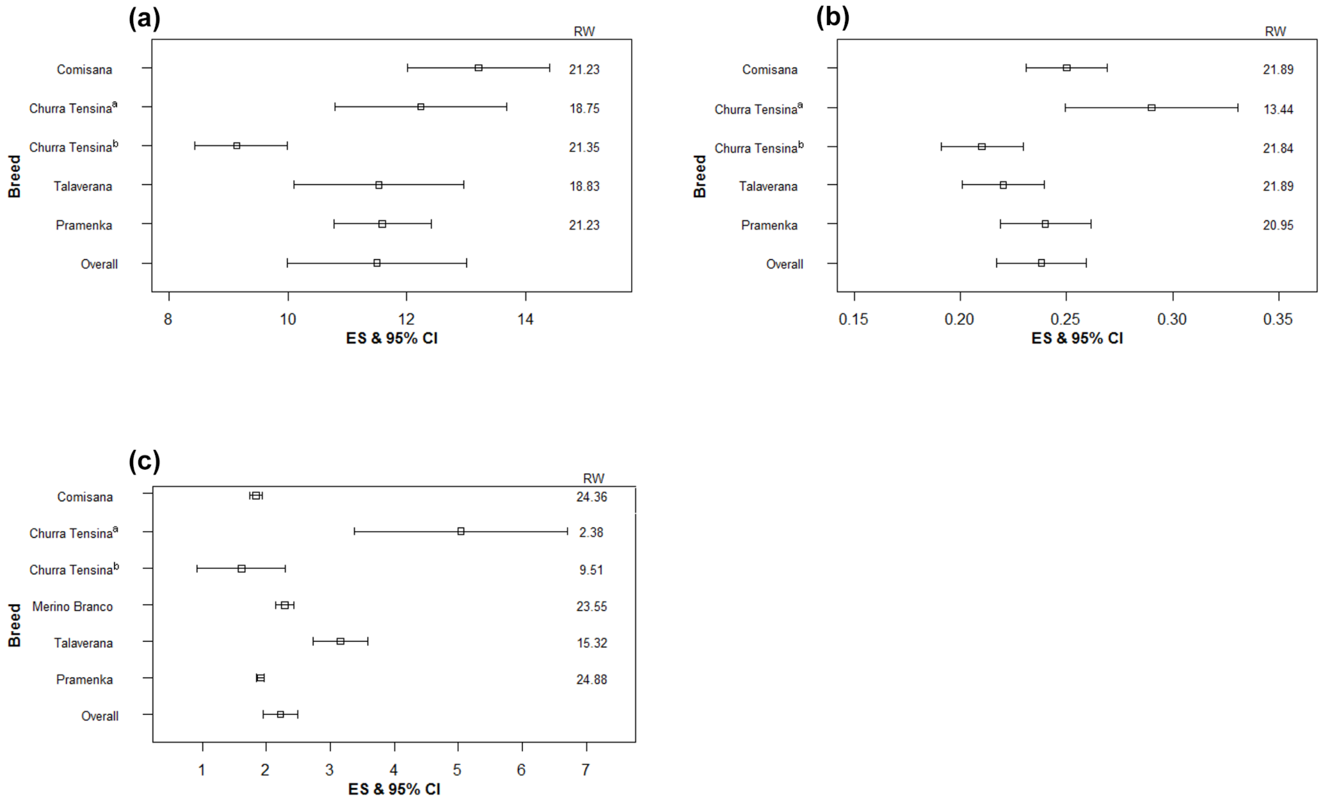

Figure 3. Meta-analysis of PUFA **(a)**, PUFA / SFA ratio **(b)** and n-6 / n-3 ratio **(c)**. Different superscript letters ([a,b]) indicate lambs of the same breed included in different studies; ES: effect size; CI: confidence interval; RW: relative weight of the study; Overall: overall pooled result.

cept for a short starter diet in Talaverana lambs). Thus, it was expected that their proportions would be similar within the overall pooled estimate.

A forest plot illustrating the α-linolenic (ALA, C18:3 n-3) acid of Mediterranean lambs is presented in Fig. 2c. A meta-analysis of ALA was compiled from four individual studies and altogether included 63 lambs of the Merino Branco, Churra Tensina[a], Talaverana and Pramenka breeds. The overall pooled result for ALA was 1.95 %, with a 95 % CI from 1.67 to 2.22. All studies included in the analysis were within the range of the overall estimate. The main feeding and rearing systems (herbage and milk of pasture-fed ewes) related to a higher content of ALA and its proportion in lamb meat were included in the analysis (except for a short starter diet in Talaverana lambs). Therefore, non-significant differences between lambs included in our survey may be a consequence of similarity in feeding and rearing systems.

Meta-analyses of MUFA and PUFA were compiled from five individual studies and altogether included 84 lambs of Comisana, Churra Tensina[a,b], Talaverana and Pramenka breeds. The overall pooled result for MUFA was 37.87 %, with a 95 % CI from 35.90 to 39.83. In this case all studies were within the range of the overall estimate (Fig. 2d). The overall pooled result for PUFA was 11.50 %, with a 95 % CI from 9.98 to 13.01. All studies were within the range of the overall estimate (Fig. 3a). According to these results it can be reported that the proportions of MUFA and PUFA between the meat of the lambs included are very similar. Since it has been proven that MUFA and PUFA are beneficial to human health (Prates and Bessa, 2009; Hoenselaar, 2012), their proportions will have a similar beneficial impact on human health.

Nutritional value of meat is determined by the PUFA / SFA ratio and the balance between fatty acids of the n-6 and n-3 series (Warren et al., 2008). From a human health viewpoint the recommended values of the PUFA / SFA ratio are higher than 0.45 (World Health Organization, 2003). Nevertheless, it must be considered that meat from ruminants has a lower PUFA / SFA ratio than that of non-ruminants because of the hydrogenating action of the rumen microorganisms. In the present study, the analysis of PUFA / SFA ratio was compiled from five studies and included 84 lambs of the Comisana, Churra Tensina[a,b], Talaverana and Pramenka breeds. The overall pooled result for the PUFA / SFA ratio was 0.23, with a 95 % CI from 0.21 to 0.25. All studies were within the range of the overall estimate (Fig. 3b). The proportions of the PUFA / SFA ratio between the meat of the lambs included are low but very similar. Diets with a lower PUFA / SFA ratio of 0.45 are less healthy (Warren et al., 2008) and therefore lower PUFA / SFA ratios of the meat of the lambs included will have similar negative influence on human health.

In contrast with the situation for the PUFA / SFA ratio, ruminant meat has advantages from a human health viewpoint because it is generally characterized by a low n-6 / n-3 ratio. From a human health viewpoint, the n-6 / n-3 ratio below 4.0 is required in the diet (World Health Organization, 2003). According to Simopoulos (2008), a very high n-6 / n-3 ratio promotes the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas a lower n-6 / n-3 ratio exerts suppressive effects. The analysis of the n-6 / n-3 ratio was compiled from six studies and altogether included 92 lambs of the Comisana, Churra Tensina[a,b], Merino Branco, Talaverana and Pramenka breeds. The overall pooled result for the n-6 / n-3 ratio was 2.22, with a 95 % CI from 1.95 to 2.49. From the studies included, the ones with Talaverana and Churra Tensina[a] lambs were not within the range of the overall estimate (Fig. 3c). Talaverana lambs had a significantly lower n-6 / n-3 ratio (3.16, with a confidence interval from 2.73 to 3.59), while Churra Tensina[a] lambs had a significantly higher n-6 / n-3 ratio (5.04, with a 95 % confidence interval from 3.37 to 6.70). Although Talaverana lambs had a short starter diet that could influence their n-6 / n-3 ratio, we think that the main reason for this result is, as in the case of Churra Tensina[a] lambs, the calculation of their ratios. In the studies that included Talaverana and Churra Tensina[a] lambs, n-6 / n-3 ratios were calculated only from linoleic (C18:2) and α-linolenic (C18:3) fatty acids, whereas in the other studies these ratios were calculated from a larger number of acids that belong to the n-6 and n-3 series. This calculation must be treated with caution because the main problem in the papers arises from the data presented. Cividini et al. (2014) also reported that some of the authors calculate the main fatty acid proportions and ratios only from the fatty acids presented in the study, while the others calculate it from the all fatty acids obtained by the analysis. Therefore, the conclusions drawn from this analysis must certainly take into account calculations of n-6 / n-3 ratios. Regardless of this and bearing in mind recommendations from the World Health Organisation (2003), in our analysis only Churra Tensina[a] lambs had an n-6 / n-3 ratio that was a little higher (5.04) and that could be negatively related to human consummation and thus human health.

4 Conclusions

The results of our meta-analysis allowed an important scientific insight into the lipid profile of intramuscular fat in Mediterranean lambs. A systematic combination of quantitative data from several carefully selected studies provided estimates of greater statistical power and enabled a more truthful comparison of results originating from different independent studies than any previously published research on this issue. Moreover, individual profiles of the breeds included in the study have also been presented, which is particularly important in the conditions of the free market when the market shelves are supplied with meat originating from countries all over the world. We believe that the future will offer more published studies that could contribute to more accurate insights into fatty acid profiles in Mediterranean lambs.

Edited by: S. Maak
Reviewed by: M. Brka and two anonymous referees

The Supplement related to this article is available online at doi:10.5194/aab-2-1-2016-supplement.

Edited by: S. Maak
Reviewed by: M. Brka and two anonymous referees

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