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Original article

Effect of using a flax seed enriched concentrate on the fatty acid composition of omental fat in Pramenka lambs reared indoors

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Abstract

This study was intended to determine the effect of adding flax seeds to a concentrate for lamb fattening on the fatty acid composition of the omental fat depot in Pramenka lambs reared indoors. A total of 63 lambs (10±3 kg of live body weight, 30±7 days of age, 30 males and 33 females) were used. They were divided into two groups: a control (CON) fed on hay, ewe's milk, and a 300-g daily ration of a commercial concentrate, and an experimental group (FS) fed on hay, ewe's milk and 300 g/day of the concentrate enriched with 5% of flax seeds. After a 60-day fattening period for each group, 10 lambs (5 males and 5 females) were selected and omental fat samples were analysed for fatty acid composition. Highly significant differences ($p<0.001$) were found between CON and FS in α-linolenic acid, the sum of n-3 fatty acids, and the ratio n-6/n-3 fatty acids. The effect of sex on the fatty acid content in the fat depot was only significant for C20:0 fatty acid ($p<0.05$).

Key words: flax seed, fat depot, lamb nutrition, n-3 fatty acid, lamb quality

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Introduction

There is a rising trend towards the consumption of food abundant in n-3 polyunsaturated fatty acids (PUFA) (European Commission 2010). Lambs fed on large quantities of concentrate tend to present fat with a low n-6/n-3 PUFA ratio, lower than the recommended values (Wood, 2003). In this context, many studies have been focused on finding ways to modify the fatty acid composition of indoor reared lambs in order to both increase the n-3 PUFA content and to reduce the concentration of SFA. The fatty acid (FA) profile of ruminant meat can be intentionally modified to some extent by feeding animals on diets containing a high proportion of PUFA (Demeyer and Doreau 1999).

One of the most promising feeds for improving the FA profile in meat is flax (*Linum usitatissimum*) seeds because its oil contains around 50% of α-linolenic acid (n-3 C18:3) (Rubilar et al. 2010) in addition to a relatively low linoleic acid (n-6 C18:2) content, and thus there is a very favourable ratio of n-6/n-3 PUFA (Azain et al. 2004). Researchers have reported that dietary supplementation with flax seed oil or flax seeds in fattening lambs can increase the concentration of n-3 PUFA, i.e. n-3 C18:3 and long-chain n-3 PUFA, and decrease the ratio of n-6/n-3 PUFA in lamb meat and fat depots (Wachira et al. 2002, Cooper et al. 2004, Demirel et al. 2004, Bas et al. 2007, Berthelot et al. 2010, Urrutia et al. 2015, Ponnampalam et al. 2016, Bhatt et al. 2020, Li F et al. 2020). In their study, Delmotte et al. (2005) stated that the practice of adding flax seed to the concentrates for feeding ewes and lambs is a natural and healthy mode of modifying the fatty acid content of their products (milk and meat).

As Wachira et al. (2002), Bas et al. (2007) and Berthelot et al. (2010) stated that the concentrations of α-linolenic acid (n-3 C18:3) and long-chain n-3 PUFA, (EPA, DPA, DHA) in the meat and fatty tissues of lamb can be increased by supplementing lambs with flax seed. Also, an increase in the level of EPA and DPA n-3 PUFA in the subcutaneous fatty tissue of lamb carcasses was recorded by Demirel et al. (2004) by using flax seeds in the lamb diet. In their study, Wachira et al. (2002) recorded differences in EPA (n-3 C20:5) and DHA n-3 PUFA, in a shorter period of time, between the control and the experimental group of lambs fed meal with the addition of flax seeds. Similar to these results, Urrutia et al. (2015) indicate that the addition of flax and chia seeds to the meal of lambs can be a useful nutritional strategy for increasing total n-3 polyunsaturated fatty acids (PUFA) and α-linolenic acid (n-3 C18:3), potentially beneficial for human health, as well as for decreasing the values of the ratios of n-6/n-3 PUFA in the meat of lambs. Flax seeds in the nutrition of lambs can efficiently affect the modification of fatty acid content and increase the quantity of nutritively active fatty acids in lamb meat (Trabalza-Marinucci et al. 2016). Feeding with extruded flax during the weaning period leads to an increase in ALA and long-chain n-3 PUFA in the muscle and fatty tissue of lambs from intensive breeding (Berthelot et al. 2012). Also, Gómez-Cortés et al. (2014) confirmed that there was an increase in vaccenic, rumenic, and docosahexaenoic/DHA acids as well as a considerably lower ratio of n-6/n-3 PUFA in intramuscular and subcutaneous fatty tissue in the carcasses of suckling lambs whose mothers had been fed with extruded flax. Wachira et al. (2002), Cooper et al. (2004), Demirel et al. (2004), and Nute et al. (2007) determined that alternative diet systems that included flax seeds and flax seed oil, as sources rich in α-linolenic acid (n-3 C18:3), improve the content of n-3 PUFA in the meat of lambs. In contrast this, meal with concentrate based on common cereals and protein supplements are abundant in linoleic acid (n-6 C18:2), precursor n-6 PUFA. By experimenting with different percentages of flax seeds in meal for lambs, Mele at al. (2014) found in their study that the addition of flax seeds to the concentrates causes a modification of the fatty acid content of the intramuscular tissue of lambs, thus increasing the degree of unsaturation of membrane and neutral lipids as well as decreasing the ratio of n-6/n-3 PUFA to a value below 3. Ponnampalam et al. (2016) reported a significantly more favourable ratio of PUFA/SFA and the ratio of n-6/n-3 PUFA of fatty acids in muscular tissue of lamb carcasses fed meal with added flax seed and meal with the addition of flax seeds and algae, when compared to the control groups of lambs fed with elementary meal consisting of ryegrass hay and pelleted clover hay. Feeding lambs with meal rich in α-linolenic acid (n-3 C18:3) leads to an increase of this fatty acid and LCPUFA in the intramuscular fatty tissue of lambs (Kitessa et al. 2010).

Lamb production is relevant in Bosnia and Herzegovina, where the prevailing local breed is Pramenka and their cross breeds (Nenadović et al. 2020). Most of the sheep farms in this country are small and medium sized (up to 100 ewes) with breeding fattening flocks (flocks with ewes). Lambs are commonly reared under extensive systems (Sinanović et al. 2011). However, a rearing system is emerging that aims to improve lamb growth rates and production efficiency. This system consists of rearing lambs indoors with their ewes and supplementing the lambs' diet with a restricted amount of concentrate. The aim of this study was to determine the effect on the fatty acid composition of lamb fat tissue of adding flax seeds into a concentrate offered at a restricted amount to lambs reared indoors with their mothers.

Table 1. Fatty acid composition, expressed as percentage of total fatty acids, in commercial concentrate (CON) and commercial concentrate enriched with 5% flax seed (FS)

	CON	FS
C 10:0	0.10	0.06
C 12:0	0.06	0.04
C 14:0	0.21	0.14
C 15:0	0.11	0.08
C 16:0	15.59	11.44
C 17:0	0.11	0.09
C 18:0	2.26	2.80
C 20:0	0.26	0.20
C 22:0	0.25	0.19
C 23:0	0.21	0.17
C 24:0	0.10	0.06
C 16:1*n-7*	0.33	0.24
C 18:1*n-9*	23.76	20.97
C 20:1	0.42	0.31
C 22:1	0.09	0.07
C 18:2n-6	50.01	35.40
C 20:2n-6	0.09	0.06
C 18:3*n-3*	6.17	27.75
Sums and ratios		
SFA	19.23	15.28
MUFA	24.51	21.52
PUFA	56.27	63.21
PUFA/SFA	2.93	4.14
n-6/n-3	8.12	1.28

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

Materials and Methods

Animals and diets

For this study, a sheep farm in the settlement of Gata, Bihac, Bosnia and Herzegovina (latitude: 44°55′42.3768″ N, Longitude: 15°49′29.6832″ E; 289 m above the sea level) was used. The farm is oriented mainly towards milk production, for making sheep cheese, and the production of lamb meat. The study was carried out during the winter period and lasted 60 days. A total of 63 newly born lambs of the Pramenka breed or cross-breeds, out of which 30 were male (47.6%) and 33 female (52.4%), were selected for the study. After 1 month, lambs were blocked according to live weight (10±3 kg), age (30±7 days), and sex and then randomly divided into two experimental groups: the control concentrate group (CON) and the flax-seed concentrate group (FS). Both groups of lambs stayed together with their ewes in the barn during the day. Every evening, the lambs of CON and FS groups were segregated into separate pens and offered 300 g of the corresponding experimental concentrate. By using separating pens, the intermixing of lambs of the monitored groups as well as intermixing with ewes while feeding was eliminated. The composition of the commercial concentrate (CON), which contained 18% protein and 1.3% crude fat, was as follows: barley (15%), soy flour (15%), corn (13%), wheat (10%), oat (8%), wheat flour (8%), sunflower scone (5%), dehydrated lucerne (7%), sugar beet shreds (10%), fodder yeast (3%), limestone (1.8%), common salt (1.5%), sugar beet molasses (2%), and other additives (1.7%). The FS concentrate was the same commercial concentrate enriched with 5% of ground flax seeds. The fatty acid composition of both CON and FS concentrates, which was analysed by gas chromatography (GC) following Joseph and Ackman (1990), is shown in Table 1.

Lambs and ewes from both groups had hay, water, and salt *ad libitum*. The ewes were additionally fed on maize grain separately from the lambs. At the end of the experimental period, on the 60th day of the trial, the fattened lambs were weighed. 10 lambs from each group were then selected (5 male and 5 female) such

Table 2. Effects of diet, commercial concentrate (CON) or 5%-flax seed enriched commercial concentrate (FS), and sex on fatty acid composition, expressed as percentage of total fatty acids, in omental fat depot from 3-month old lambs.

	Diet			Sex			*P*-value	
	CON (n=10)	FS (n=10)	SED	Male (n=10)	Female (n=10)	SED	Feeding	Sex
C10:0	0.21	0.38	0.039	0.32	0.28	0.055	0.001	0.429
C12:0	0.23	0.58	0.068	0.43	0.38	0.104	<0.001	0.513
C14:0	3.44	6.24	0.497	4.89	4.79	0.825	<0.001	0.854
C15:0	0.49	0.69	0.044	0.62	0.56	0.063	<0.001	0.200
C16:0	19.08	21.26	0.774	19.90	20.45	0.921	0.015	0.505
C17:0	1.56	1.41	0.054	1.45	1.52	0.063	0.013	0.222
C18:0	29.61	20.16	1.658	25.16	24.62	2.773	<0.001	0.760
C19:0	0.19	0.19	0.011	0.20	0.18	0.010	0.056	0.812
C20:0	0.25	0.21	0.020	0.25	0.21	0.018	0.053	0.018
C22:0	0.05	0.05	0.006	0.06	0.05	0.006	0.468	0.094
C12:1	0.07	0.09	0.014	0.09	0.07	0.015	0.089	0.369
C14:1	0.08	0.15	0.012	0.12	0.11	0.020	<0.001	0.371
C16:1	1.05	1.42	0.077	1.23	1.24	0.117	<0.001	0.886
C18:1	37.93	41.52	1.810	39.40	40.05	1.993	0.076	0.764
C20:1	0.16	0.16	0.012	0.17	0.16	0.012	0.880	0.278
C18:2n-6	4.43	3.89	0.299	4.34	3.97	0.313	0.086	0.227
C20:4n-6	0.09	0.05	0.008	0.08	0.07	0.012	<0.001	0.143
C18:3n-3	0.77	1.29	0.067	1.02	1.04	0.140	<0.001	0.762
C20:5n-3	0.04	0.05	0.009	0.04	0.05	0.009	0.744	0.744
C22:5n-3	0.14	0.13	0.021	0.14	0.13	0.021	0.604	0.900
Sums and ratios								
SFA	55.09	51.14	1.970	53.24	52.99	2.177	0.074	0.902
MUFA	39.26	43.35	1.869	40.99	41.62	2.098	0.053	0.751
PUFA	5.45	5.34	0.322	5.60	5.20	0.309	0.743	0.219
PUFA/SFA	0.10	0.10	0.008	0.11	0.10	0.008	0.640	0.413
n-6	4.53	3.92	0.308	4.42	4.02	0.326	0.059	0.199
n-3	0.92	1.43	0.066	1.17	1.18	0.136	<0.001	0.983
n-6/n-3	5.06	2.75	0.427	4.09	3.72	0.686	<0.001	0.416

SED = standard error of difference; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

that their live weights were the closest to the average of their respective groups. The mean values (± standard deviation), expressed as kg, were 31.8±3.4 and 24.9±2.6 for the FS and CON groups, respectively. The average daily gain during the experimental period (60 days) was 223±3 g/d for the CON group lambs and 284±4 g/d for the FS group.

The selected lambs were then transported to an authorized slaughterhouse where they were offered feed and water. The animals were then fasted for 24 hours before being slaughtered. All handling practices involving the animals prior to slaughter followed the Law on the Protection and Welfare of Animals of Bosnia and Herzegovina (Official Gazette of BiH, No. 25/09) and the recommendations of Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

Sampling of omental fat depot and fatty acid analysis

Sampling of the omental fat depot from the carcasses was carried out at 24 hours post-mortem. A 200-g fat tissue sample was taken from each carcass. Samples were immediately labelled, packed in plastic bags, and transported at 4 ºC to the laboratory of the Institute of Emona Razvojni Center za Prehrano (Ljubljana, Slovenia), where they were kept at -20 ºC until FA analysis. Sampling this omental fat depot does not result in a loss of the carcass economic value, and omental

FA composition responds to dietary changes (Adeyemi et al. 2015); thus, it can be used as a marker to test the FA dietary-related changes in fat tissues. Furthermore, omental fat (caul fat) is typically consumed in the region.

The esterification of FAs was carried out in duplicate using the "*in situ transesterification*" technique (Park and Goins 1994). FA analysis was carried out using a 6890N Network GC System (Agilent Technologies, Santa Clara, CA, USA) gas chromatograph equipped with a flame ionization detector and an OMEGAWAX 320 (30 m x 0.320 mm x 0.25 µm) capillary column (Supelco, Bellefonte, PA, USA). The operation conditions were those described by Joseph and Ackman (1990). Identification of FA methyl esters (FAMEs) was carried out by comparing the sample retention peak times with those of FAME standards (Nu-Check Prep, Inc, Elysian, USA). Quantification was carried out according to the theoretical conversion factors as laid down in the AOAC procedure (1990, 1996).

Statistical analysis

The mean FA percentages of the replicates for each fat sample were subjected to a general linear model analysis of variance (ANOVA). Feeding treatment and sex were considered as fixed factors and the interaction between the two fixed factors was also tested in the model used. The ANOVA was declared significant at $p<0.05$. The standard errors of the difference between the means (SED) for feeding treatment and sex were also calculated. The analysis was carried out using the SPSS 24.0 statistical package (IBM, Somers, NY, USA).

Results

Table 2 shows the fatty acid profile in the omental fat depot of the lambs from CON and FS groups and in male and female lambs. For brevity, those FAs showing percentages lower than 0.05% and the feeding x sex interaction, due to no significance being found, were not included in the table.

No differences were found in the sum of SFA between dietary treatments. However, the enrichment of concentrate with flax seeds resulted in higher levels of C10:0-C16:0 SFA and lower levels of C17:0 and C18:0 ($p<0.05$). Regarding the effect of sex on SFA, only the content of C20:0 was significantly affected.

The percentage of the sum of monounsaturated fatty acids (MUFA) in the omental fat depot did not differ between feeding regimes or sex. In contrast, PUFA contents were affected by the feeding regimen. On one hand, and in agreement with the FA profile of the concentrates (Table 1), the addition of 5% of flax seeds to the concentrate of lambs fattened for 60 days had a significant effect ($p<0.001$) in increasing the percentage of C18:3 n-3 in the omental fat depot; the amount was almost twice that in CON. On the other hand, no differences were found for the major n-6 fatty acid, i.e., linoleic acid (C18:2 n-6), in spite of this being more abundant in the non-enriched concentrate; however, the percentages of minor n-6 fatty acids (C20:4 n-6) were affected (Table 2). In this study, the n-6/n-3 ratio in FS omental fat was almost half of that in the CON fat.

Discussion

As expected for a ruminant fat depot, saturated FAs (SFA) were the most abundant FAs. In partial agreement with the present results, Urrutia et al. (2015), Bhatt et al. (2020), and Mujic et al. (2018) found that the sum of SFA in a lamb fat depot was not affected by dietary supplementation of flax seed (concentrate enriched with flaxseed). However, those authors found that none of the individual SFAs were affected. Regarding the effect of sex, our results are in contrast with those of Díaz et al. (2003). These authors found the subcutaneous fat of male lambs contained a higher amount of SFA compared to female lambs.

The dietary effect found for the C10:0-C16:0 individual SFAs was not in concordance with the SFA profile of the concentrates used in this study (Table 1), i.e., the flax-seed enriched concentrate showed similar or higher C10:0-C17:0 FAs and lower C18:0 percentages than the non-enriched commercial feed. This apparent discrepancy could be related to differences in the intake of ewes' milk between the lambs from each group, i.e., the results might suggest a higher milk intake in the FS group since ewes' milk is particularly rich in C10:0-C14:0 FAs (Balthazar et al. 2017) which were more abundant in the fat depot from this group. Furthermore, this might be also attributed to concomitant effects of flax seed on the ruminal microbial population (Andrés et al. 2016, Li et al. 2020) or in *de novo* synthesis in adipocytes (Urrutia et al. 2015), with both affecting the fatty acid profile. Furthermore, regarding MUFA content, our results agree with Urrutia et al. (2015) who did not find an effect of concentrate enrichment with flax seed on the MUFA percentages of the subcutaneous fat of lambs. However, in the present study, differences in the percentages of C12:1, C14:1 and C16:1 were detected between feeding treatments ($p<0.01$). These were lower in CON samples which, as seen before, presented lower percentages of the saturated counterparts, i.e., C12:0, C14:0 and C16:0. On the other hand, no differences were found for the larger chain MUFAs, C18:1 or C20:1. Urrutia et al. (2015)

have described an effect of dietary flax seed on the amounts of different C18:1 isomers, i.e., the levels of trans-vaccenic acid (*t*11-C18:1) were increased, and those of *c*9- and *c*11-C18:1 fatty acids decreased. In the present study, due to the methodology used, separation between isomers was not achieved, and thus the percentage of C18:1 would include the sum of those isomers.

Increased percentages of C18:3 n-3 in the fat depots of lambs fed on flax-seed enriched concentrates were also reported by Wachira et al. (2002), Berthelot et al. (2010), and Urrutia et al. (2015). These authors found two to four-fold increases in C18:3 n-3 percentages in lamb fat depots due to the use of 7-10% flax seed enriched concentrates for lamb fattening. Moreover, the increase of this FA in meat (intramuscular fat) observed by those authors was comparable to that of the fat depots, i.e., two-five fold.

As a result of the changes in the n-3 and n-6 fatty acid contents, the most favourable n-6/n-3 ratio was found in omental fat from the FS feeding system. This was near the recommended ratio 1-2/1 for a healthy diet (Simopoulos 2002). The reduction in the n-6/n-3 ratio of the FS omental fat was in the range reported for other fat depots in other studies (Wachira et al. 2002, Berthelot et al. 2010, Urrutia et al. 2015) where lambs were also reared indoors, although without the presence of their dams (early weaned lambs). They found that the n-6/n-3 ratio was reduced by two or three fold not only in fat depots but also in the meat of lambs fattened with flax-seed enriched concentrates, when compared to controls (lambs fed on non- enriched concentrate). This suggests that in the lamb meat from the present study, the n-6/n-3 ratio in FS lambs compared to CON might also have been reduced by half.

Conclusions

Including flax seeds at 50g/kg of concentrate offered in a restricted amount, i.e. 300g/day, to fattening lambs from one to three months of age, reared indoors with *ad libitum* availability of dams' milk and hay, significantly increased the C18:3 n-3 in the lamb omental fat depot. This increase, which was accompanied with a non-significant change in the level of C18:2 n-6 linoleic and total n-6 FA, reduced by half the n-6/n-3 PUFA ratio in the fat depot and presumably in the lamb muscle. The proposed feeding regime allows lamb meat with a more favourable ratio of n-6/n-3 PUFA to be obtained. For omental fat, this is very near to the recommended values for healthy diets. Moreover, the enrichment resulted in higher percentages of those FAs with chain lengths <18 carbons. This was not directly related to the FA differences between non-enriched and enriched concentrate.

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References

Adeyemi KD, Ebrahimi M, Samsudin AA, Sabow AB, Sazili AQ (**2015**) Carcass traits, meat yield and fatty acid composition of adipose tissues and Supraspinatus muscle in goats fed blend of canola oil and palm oil. J Anim Sci Technol 57: 1-14.

Andrés S, Bodas R, Tejido ML, Giráldez FJ, Valdés C, López S (**2016**) Effects of the inclusion of flaxseed and quercetin in the diet of fattening lambs on ruminal microbiota, in vitro fermentation and biohydrogenation of fatty acids. J Agric Sci 154: 542-552.

AOAC (**1990**) Official Method 976.21 Fat (Crude) in Meat, Rapid Specific Gravity Method. 15th ed., Assoc Offic Anal Chem, Washington DC, USA. http://www.aoacofficialmethod.org/

AOAC (**1996**) Official Method 996.01 Fat (total, saturated, unsaturated and monounsaturated) in Cereal Products. Official Methods of Analysis of the AOAC. 17th ed., AOAC Int. Gaithersburg MD, USA. http://www.aoacofficialmethod.org/

Azain MJ (**2004**) Role of fatty acids in adipocyte growth and development. J Anim Sci 82: 916-924.

Balthazar CF, Pimentel TC, Ferrao LL, Almada CN, Santillo A, Albenzio M, Mollakhalili N, Mortazavian AM, Nascimento JS, Silva MC, Freitas MQ, Sant'Ana AS, Granato D, Cruz AG (**2017**) Sheep milk physicochemical characteristics and relevance for functional food development. Compr Rev Food Sci Food Saf 16: 247-262.

Bas P, Berthelot V, Pottier E, Normand J (**2007**) Effect of level of linseed on fatty acid composition of muscles and adipose tissues of lambs with emphasis on trans fatty acids. Meat Sci 77: 678-688.

Berthelot V, Bas P, Pottier E, Normand J (**2012**) The effect of maternal linseed supplementation and/or lamb linseed supplementation on muscle and subcutaneous adipose tissue fatty acid composition of indoor lambs. Meat Sci 90: 548-557.

Berthelot V, Bas P, Schmidely P (**2010**) Utilization of extruded linseed to modify fatty composition of intensively-reared lamb meat: Effect of associated cereals (wheat vs. corn) and linoleic acid content of the diet. Meat Sci 84: 114-124.

Bhatt RS, Soni LK, Sahoo A, Gadekar YP, Sarkar S (**2020**) Dietary supplementation of extruded linseed and calcium soap for augmenting meat attributes and fatty acid profile of longissimus thoracis muscle and adipose tissue in finisher Malpura lambs. Small Rumin Res 184: 106062.

Cooper SL, Sinclair LA, Wilkinson RG, Hallett KG, Enser M, Wood JD (**2004**) Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. J Anim Sci 82: 1461-1470.

Delmotte C, Rondia P, Raes K, Dehareng F, Decruyenaere V (**2005**) Omega 3 and CLA naturally enhanced levels of animal products: Effects of grass and linseed supplementation on the fatty acid composition of lamb meat and sheep milk. In: Priolo A, Biondi L, Ben Salem H, Morand-Fehr P (ed.) Advanced nutrition and feeding strategies to improve sheep and goat: Proceedings of the 11 Seminar of the FAO-CIHEAM Sub-network on Sheep and Goat Nutrition, Sep 08 10, Catania (Italy). Zaragoza: CIHEAM, 2007. pp 41-48. (Options Méditerranéennes: Série A. Séminaires Méditerranéens; no. 74).

Demeyer D, Doreau M (**1999**) Targets and procedures for altering ruminant meat and milk lipids. Proc Nutr Soc 58: 593-607.

Demirel G, Wachira AM, Sinclair LA, Wilkinson RG, Wood JD, Enser M (**2004**) Effects of dietary n-3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. Br J Nutr 91: 551-565.

Díaz MT, Velasco S, Pérez C, Lauzurica S, Huidobro F, Cañeque V (**2003**) Physico-chemical characteristics of carcass and meat Manchego-breed suckling lambs slaughtered at different weights. Meat Sci 65: 1085-1093.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Communities 2010, L276/33.

European Commission (**2010**). Functional foods. Publications Office of the European Union, Luxembourg: pp 1-21.

Gómez-Cortés P, Gallardo B, Mantecón AR, Juárez M, De La Fuente MA, Manso T (**2014**) Effects of different sources of fat (calcium soap of palm oil vs. extruded linseed) in lactating ewes' diet on the fatty acid profile of their suckling lambs. Meat Sci 96: 1304-1312.

Joseph JD, Ackman RG (**1992**) Capillary column gas chromatogrphic method for analysis of encapsulated fish oils and fish oil ethyl esters: collaborative study. J AOAC Int. 75: 488-506.

Nenadović K, Karać P, Vučinić M, Teodorović R, Živanov D, Trailović R, Beckei Z, Janković Lj (**2020**) Assessment of the welfare of extensively managed autochtonous sheep breed Vlasicka Zackel using animal-based measurements. Acta Vet 70: 207-218.

Kitessa S, Liu S, Briegel J, Pethick D, Gardner G, Ferguson M, Allingham P, Nattrass G, McDonagh M, Ponnampalam E, Hopkins D (**2010**) Effects of intensive or pasture finishing in spring and linseed supplementation in autumn on the omega-3 content of lamb meat and its carcass distribution. Anim Prod Sci 50: 130-137.

Law on Protection and Welfare of Animals (**2009**) No. 25/09, Sarajevo: Official Gazette of the Bosnia and Herzegovina. https://www.ecolex.org/details/legislation/law-on-the-protection-and-welfare-of-animals-lex-faoc148359/

Li F, Zhang Z, Li X, Zhu B, Guo L, Li F, Weng X (**2020**) Effect of duration of linseed diet supplementation before slaughter on the performances, meat fatty acid composition and rumen bacterial community of fattening lambs. Anim Feed Sci Technol 263: 114457.

Mele M, Serra A, Pauselli M, Luciano G, Lanza M, Pennisi P, Conte G, Taticchi A, Esposto S, Morbidini L (**2014**) The use of stoned olive cake and rolled linseed in the diet of intensively reared lambs: effect on the intramuscular fatty-acid composition. Animal 8: 152-162.

Mujić E, Omanović H, Mateo J, Pračić N, Vilić H, Šahinović R, Nedić D, Červek M (**2018**) Effect of flax-Seed enriched concentrate supplementation in grazing Pramenka breed lamb's diet on omental fat fatty acids. Acta Vet 68: 502-511.

Nute GR, Richardson RI, Wood JD, Hughes SI, Wilkinson RG, Cooper SL, Sinclair LA (**2007**) Effect of dietary oil source on the flavour and the colour and lipid stability of lamb meat. Meat Sci 77: 547-555.

Park PW, Goins RE (**1994**) In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J Food Sci 59: 1262-1266.

Ponnampalam EN, Burnett VF, Norng S, Hopkins DL, Plozza T, Jacobs JL (**2016**) Muscle antioxidant (vitamin E) and major fatty acid groups, lipid oxidation and retail colour of meat from lambs fed a roughage based diet with flaxseed or algae. Meat Sci 111: 154-160.

Rubilar M, Gutiérrez C, Verdugo M, Shene C, Sineiro J (**2010**) Flaxseed as a source of functional ingredients. J Soil Sci Plant Nutr 10: 373-377.

Simopoulos AP (**2002**) The importance of the ratio of omega-6/ /omega-3 essential fatty acids. Biomed Pharmacother 56: 365-379.

Sinanović N, Šakić V, Katica V, Varatanović M, Ališah A (**2011**) Possibilities of enrichment of Pramenka with Awassi sheep in Bosnia and Herzegovina. Veterinaria 60: 245-250.

Trabalza-Marinucci M, Mughetti L, Ranucci D, Acuti G, Olivieri O, Miraglia D, Branciari R (**2016**) Influence of maternal and postweaning linseed dietary supplementation on growth rate, lipid profile, and meat quality traits of light Sarda lambs. Sci World J 2016: 5391682

Urrutia O, Soret B, Insausti K, Mendizabal JA, Purroy A, Arana A (**2015**) The effects of linseed or chia seed dietary supplementation on adipose tissue development, fatty acid composition, and lipogenic gene expression in lambs. Small Rumin Res 123: 204-211.

Wachira AM, Sinclair LA, Wilkinson RG, Enser M, Wood JD, Fisher AV (**2002**) Effects of dietary fat source and breed on the carcass composition, n-3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. Br J Nutr 88: 697-709.

Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Enser M (**2004**) Effects of fatty acids on meat quality: A review. Meat Sci 66: 21-32.