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Short communication

EFFECT OF FLAX-SEED ENRICHED CONCENTRATE SUPPLEMENTATION IN GRAZING PRAMENKA BREED LAMB'S DIET ON OMENTAL FAT FATTY ACIDS

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This research is aimed at determining the impact of supplementing extensively reared lambs with a limited amount of flax seed enriched-concentrate on the fatty acid profile of the lamb meat (omental fat depot). A total of 96 60-day old Pramenka breed lambs (23.85±3.98 kg live body weight; 60±10 days of age; 42 males and 54 females) raised on pasture with their dams were randomly divided in three experimental groups: control (CON) or extensive rearing group, which did not receive any supplementary feed; concentrate (CC) group, receiving 300 g of a conventional commercial concentrate feed per animal daily, and concentrate-flax seed (CC-FS) group, receiving 300 g/day of the concentrate supplemented with 5% of flax seed. After 60 days of experimental period, 10 lambs were selected (5 males and 5 females) from each group and slaughtered. Omental fat samples were taken and analyzed to determine their fatty acid profile. Significant (P>0.05) differences in the fatty acid profile between CON and CC groups were small. However, enrichment of the concentrate with 5% flax seed increased the percentages of PUFA and n-3 PUFA when compared to both CON and CC groups and thus slightly improved the nutritional characteristics of the omental fat. The effect of sex on the fatty acid profile was only observed for C20:4 *n*-6 and C20:5 *n*-3 (P < 0.05).

Key words: flax seed, fat depot, fatty acid, sheep nutrition.

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INTRODUCTION

Young lambs are traditionally produced in Mediterranean countries, usually linked to systems of available natural resources and local breeds, providing a range of typical dairy products and fresh and processed meat [1]. Bosnia and Herzegovina has great potential for sheep herding. Breeding is mostly carried out in mountainous regions, with abundant meadows and grassland, and is directed to meat production [2]. The extensive system of lamb rearing, which is normally carried out in small farms (1 to 3 hectares; thirteen sheep in average) is in Bosnia and Herzegovina the most predominant [3]. The most prevailing breed is the domestic Pramenka, and its various crossbreeds, with a 50% share in the overall sheep population, and the rest goes mainly to Merino crossbreeds [2]. Extensive systems and local breeds have the advantages of low production inputs, high respect to the environment and animal welfare, and geographical typicality [4]. Furthermore, from the nutritional point of view, extensive grass-based feeding provides the lamb meat with a higher (and favorable) percentage of *n*-3 polyunsaturated fatty acids (PUFA) and a lower percentage of *n*-6 PUFA as compared to lambs fed concentrate, leading to a more favorable *n-6/n-3* ratio [5,6]. On the other hand, raising lambs in extensive systems presents the problem of slow growth rates affecting the efficiency of production [7]. This can be compensated, however, providing lambs with concentrate feeds to better satisfy their nutritional requirements for a faster growth.

The fatty acid profile of ruminant meat may be intentionally modified to some extend by feeding animals diets containing specific fatty acid profiles [8]. Thus, alternative concentrate-based feeding using rich sources of linolenic acid (*n-3* C18:3), such as flax-seed or flax seed oil, as rich sources of *n-3* C18:3, has been suggested to improve the content of *n-3* PUFA in ruminant fat [9-12].

To our knowledge, the existing research studying the effect of flax-seed dietary supplementation on the fatty acid profile of lamb meat has been mainly focused on intensively reared lambs and scarcely on grazing lambs. The supplementation of free range grazing lambs with restricted concentrate would improve their growth rates but at the same time it could modify the meat fatty acid composition and thus the meat nutritional value and edible quality. Furthermore, the eventual response to supplementation on the fatty acid profile could be affected by sex. Therefore, the aim of the study was to evaluate the effect of supplementing a limited level of concentrate with or without flax-seed on the fatty acid profile of fat from lambs, males and females, reared under a typically regional extensive rearing system.

MATERIALS AND METHODS

Animals and diets

A total of 96 Pramenka breed and cross-breeds of Merinolandschaf x Pramenka lambs (42 males and 54 females) reared under a grazing system, on pastures in Dubovsko, Bihać, Bosnia and Herzegovina (latitude: 44° 43' 0" N, longitude: 16° 2' 0" E; 642 m above sea level), were used in this research. The climate of this area is moderate-continental and moderate-mountainous with average 1519.6 mm of annual rainfall, and temperature of 10.8 °C.

At approximately two months of age the lambs were weighed, ear-tagged and stratified on the basis of body weight (23.85±3.98 kg), age (60±10 days) and sex and finally randomly assigned to one of three experimental groups (32 animals per group): control group (extensive rearing; CON), which did not receive any supplementary feed; concentrate group (CC), receiving 300 g of concentrate per animal daily, and flax-seed added concentrate CC-FS group, supplemented with 300 g/day of the concentrate added with 5% of ground flax seed.

The trial was carried out during the summer season and lasted 60 days. Lambs were maintained on pasture (60% of *Poaceae*, 15% of *Fabaceae*, 12 % of *Ranunculaceae*, 10% of *Plantaginaceae* (10) and 3% of other plant families) with their dams until slaughter, with free access to fresh water and salt *ad libitum*. Every evening, the lambs from the CC and CC-FS groups were separated from the ewes and brought into small pens and fed with the experimental concentrates.

The composition of the commercial concentrate, which contained 18% of protein and 1.3% of 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%). Composition of fatty acids from fodder mixtures, which was analyzed by gas chromatography (GC) according to Joseph and Ackman (1990) [13], is shown in Table 1.

At the end of the experimental period, animals were weighed and 10 lambs per each experimental group, those with the body weight closest to the mean weight value of their respective group (5 males and 5 females) were selected and transported to a commercial abattoir for slaughter. The average daily gain during the experimental period was of 68 g for the control lambs and almost twice for both groups of lambs fed with concentrate. All handling practices involving the animals previous to slaughter followed the recommendations of Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

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

	CC	**CC+FS**
C 10:0	0.39	0.24
C 12:0	0.16	0.11
C 14:0	0.35	0.26
C 15:0	0.14	0.12
C 16:0	22.20	18.84
C 16:1*n-7*	0.78	0.58
C 17:0	0.14	0.14
C 18:0	3.40	4.66
C 18:1*n-9*	22.27	23.41
C 18:2*n-6*	41.95	30.15
C 18:3*n-3*	5.78	19.40
C 20:0	0.36	0.32
C 20:1*n-11*	0.72	0.47
C 20:2n-6	0.12	0.06
C 22:0	0.44	0.39
C 22:1	0.33	0.39
C 23:0	0.14	0.16
C 24:0	0.31	0.31
Sums and ratios		
SFA	28.05	25.55
MUFA	24.10	24.84
PUFA	47.84	49.61
PUFA/SFA	1.71	1.94
n-6/n-3	7.28	1.56

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

Sampling of omental fat depot and fatty acid analysis

The study analyzed the fatty acids of the omental fat depot based on sampling of this depot does not result in a loss of carcass economic value and omental fatty acid composition responds to dietary changes [14], so it can be used as a marker to test the fatty acid dietary-related changes. Furthermore, this depot (caul fat) is typically consumed in the region. At 24 hours post-mortem a 200-g fat tissue sample was taken from each carcass. Immediately samples were labeled, packed in plastic bags and transported at 4ºC to the laboratory of the Institute Emona Razvojni Center za Prehrano (Ljubljana, Slovenia) where they were kept at -20ºC until analysis.

The esterification of fatty acids was carried out in duplicate by the *"in situ transesterification"* technique [15]. Fatty acid analysis was carried out by gas chromatography (GC) using a 6890N Network GC System (Agilent Technologies, Santa Clara, CA, USA), equipped with a flame ionization detector (FID) and a OMEGAWAX 320 (30 m x 0320 mm x 0.25 µm) capillary column (Supelco, Bellefonte, PA, USA). The operation conditions were those described by Joseph and Ackman (1990) [13]. Identification of 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 AOAC procedure (1996) [16].

Statistical analysis

The mean fatty acid percentages of the replicates for each fat sample were subjected to general linear model analysis of variance. In this analysis, feeding treatment and sex were considered as fixed factors. The model also tested the interaction between the two fixed factors. The levels of statistical significance were set at $P<0.05$, $P<0.01$ and $P<0.001$, and a near to significance level ($P<0.1$) was also considered. Significant differences for each fatty acid between the different feeding treatments were determined at a significance level $P\leq0.05$ using the Tuckey post *hoc test*. The analyses were carried using the SPSS 24.0 statistical package (IBM, Somers, NY, USA).

RESULTS AND DISCUSSION

Table 2 shows the fatty acid profile of the omental fat depot from the lambs fed extensively and those fed semi-extensively, these either supplemented with a commercial concentrate (CC) or a commercial concentrate enriched with 5% flax seeds (CC+FS). SFA was the predominant group of fatty acids in the omental fat depot regardless the dietary treatment, PUFA accounted for up to 5% of total fatty acids and the *n-6*/*n-3* ratio was near to 2. These results are in agreement with those reported in previous studies on pasture-fed 3- to 9-month lambs for omental, perirenal or subcutaneous fat [5,17]. In those studies, there was a higher proportion of neutral lipids in the adipose depots, omental fat included, whereas intramuscular fat contained a higher amount of phospholipids [18]. Actually, for the intramuscular fat the above mentioned studies reported PUFA values of 10-15 percent while SFA levels decrease approximately to 50%.

Range-grasses are characterized by having 1-2 g of fatty acids per 100 g of grass of which the main ones tend to be C18:3 (35-65%), C18:2 (12-20%) and C16:0 (12-16%), while C18:0 and C18:1 appear in amounts lower than 5% [19,20]. The percentages of C18:3 and C18:2 in range grasses, and thus of PUFA, widely differs from those found in the commercial concentrate offered to the lambs in this study, which contains a higher percentage of C18:2 and C18:1 and a considerably lower percentage of C18:3 (Table 1). In spite of these dietary differences, no significant differences were found in the fatty acid profile between the omental fat of CON and CC lambs. In contrast to our results, studies where the meat of carcasses of grazing vs concentrated-fed lambs was compared have reported for the latter, besides a decrease in the muscle/total fat ratio of the carcass, a decrease in *n-3* and an increase in *n-6* PUFA percentages in the fat, and in some cases a decrease in SFA percentage [21,22,7]. Santos-Silva et al. (2002) also reported a significantly higher percentage of *n-6* (LA) in the intramuscular fat from lambs fed pastures supplemented with commercial concentrate than in that from exclusively grass-fed lambs [23].

Table 2 The effect of diet and sex on the fatty acid composition (expressed as percentage of total fatty acids) in the omental fat depot of 4-month old lambs feed with different feeding systems

	Feeding system			Sex			Significance	
	CON (n=10)	CC (n=10)	CC+FS (n=10)	Male (n=15)	Female (n=15)	RMSE	Feeding	Sex
C10:0	0.35	0.34	0.24	0.29	0.32	0.167	NS	NS
C12:0	0.62	0.60	0.38	0.54	0.52	0.296	NS	NS
C13:0	0.05	0.05	0.05	0.06	0.02	0.039	NS	NS
C14:0	5.68^{a}	5.46^{a}	3.70^{b}	5.17	4.78	1.201	*	NS
C14:1*n-5*	0.10	0.11	0.06	0.10	0.09	0.039	NS	NS
C15:0	1.13	1.06	1.01	1.08	1.06	0.122	NS	NS
C16:0	20.76	21.47	19.80	20.38	20.91	1.290	NS	NS
C16:1*n-7*	1.16	1.38	1.08	1.14	1.26	0.351	NS	NS
C17:0	1.48	1.46	1.48	1.47	1.47	0.166	NS	NS
C18:0	31.34	29.68	34.92	32.53	31.56	6.727	NS	NS
C18:1*n-9*	32.33	33.30	31.51	31.82	32.81	4.000	NS	NS
C18:2*n-6*	2.50^{b}	2.73^{b}	3.04^{a}	2.86	2.68	0.077	**	NS
C19:0	0.30	0.26	0.31	0.29	0.28	0.039	NS	NS
C18:3*n-3*	1.06	1.00	1.22	1.13	1.06	0.109	#	NS
C20:0	0.36	0.31	0.32	0.34	0.32	0.022	#	NS
C20:1*n-11*	0.30	0.31	0.35	0.33	0.31	0.100	NS	NS
C21:0	0.08	0.07	0.07	0.07	0.07	0.100	NS	NS
C20:4*n-6*	0.04^{b}	0.06^{a}	0.06^{a}	0.05	0.06	0.005	**	*
C20:5*n-3*	0.07	0.07	0.07	0.06	0.08	0.045	NS	*
C22:0	0.11^{a}	0.08^{ab}	0.09^{b}	0.1	0.09	0.006	*	NS
C23:0	0.05	0.04	0.04	0.05	0.04	0.022	NS	NS
C22:5*n-3*	0.14	0.14	0.20	0.16	0.16	0.039	NS	NS
C22:6*n-3*	0.01	0.04	0.02	0.01	0.03	0.004	NS	NS
Sums and ratios								
SFA	62.25	60.82	62.36	62.31	61.43	4.437	NS	NS
MUFA	33.90	35.11	33.00	33.38	34.47	4.410	NS	NS
PUFA	3.76^{b}	3.99^{b}	4.56^{a}	4.21	4.02	0.107	**	NS
PUFA/SFA	0.06	0.07	0.07	0.07	0.07	0.006	#	NS
Total *n-6*	2.54^{b}	2.78^{ab}	3.10^{a}	2.88	2.71	0.063	**	NS
Total *n*-3	1.27^{b}	1.20^{b}	1.48^{a}	1.33	1.31	0.166	*	NS
n-6/n-3	2.01	2.32	2.09	2.22	2.08	0.404	NS	NS

CON = 4 month-aged lambs grazing with their mothers until slaughtering, extensive system. CC = 4 month-aged lambs grazing with their mothers and supplemented daily with 300 g of commercial concentrate for the last two months of age. CC+FS = 4 month-aged lambs grazing with their mothers and supplemented daily with 300 g of commercial concentrate enriched with 5% flax seed for the last two months of age. NS, not significant; #, $P < 0.01$; *, $P < 0.05$; **, $P < 0.01$. [a,b] Means of the feeding treatments with a same superscript were not significantly different as assessed by the Tuckey test, $P>0.05$

In this study, the limited amount of the dietary lipids ingested by the CC lambs from the concentrate (c.a., 4 g per day) could account for the lack of differences in the fatty acid profile between CON and CC lambs. Both groups of lambs (CON and CC) were allowed to graze range grasses together with their dams during the trial and most of the dietary lipids would come from the grass grazed and the ewe milk suckled [24].

However, supplementing the diet of the grazing lambs with the 5%-flax seed-enriched commercial concentrate (CC+FS), which represents in average a 7-g daily intake of lipids per animal in the CC+FS group, increased the total PUFA, *n-3* and *n-6* fatty acid percentages in the omental fat depot with regard to extensively reared lambs (CON), and the PUFA and *n-3* percentages with regard the lambs offered not-enriched commercial concentrate (CC). The increase of *n-3* fatty acid can be explained by an increase in the C18:3 *n-3* intake by the lambs due to the high percentage of this fatty acid in the flax seed-enriched concentrate (Table 1). Such results of our analyses are completely in line with the argument that inclusion of flax seed in the concentrates for lamb fattening leads to a higher concentration of *n-3* in muscle and different fat depots of lambs [9,12,25-27].

Furthermore, the above-mentioned increase of *n-6* fatty acids when comparing CC+FS vs CC could be explained by both, the higher percentage of *n-6* fatty acid in the lipids of flax seeds with regard to the other dietary sources of lipids (ewe milk and grasses), and the high content of total lipids in flax seed, c.a., 45% [28].

Finally, the effect of sex on the fatty acid percentage was only observed for C20:4 *n-6*: 0.05 % for males and 0.06 % for females, and for C20:5 *n-3*, 0.06 % for males and 0.08 % for females (data not included in the Table for brevity). Accordingly, other authors have found very slight effects of sex on the fatty acid percentages in fat depots from lambs reared under different feeding systems [29-31].

CONCLUSIONS

The supplementation of the diet of Pramenka breed and cross-breeds grazing lambs, which were maintained on pasture with their mothers between the second and the fourth month of age, with an average of 300 g of a commercial concentrate (based on barley, soy flour, corn, wheat and oat) per animal and day would not affect the fatty acid profile in the lambs' omental fat depot with regards to that of lambs fed only by grazing. However, the enrichment of the concentrate with 5% of flax-seed would increase the PUFA and *n*-3-PUFA percentage, thus improving the nutritional characteristics of the omental fat of lambs, and presumably of other carcass fat depots. However, changes are slight and probably would not significantly change the typical characteristics of this traditional grazing-reared light lamb.

Authors' contributions:

ME has designed and conducted practical researches. He participated in the analysis of fatty acid composition of the lamb meat, statistical analysis and interpretation of the results as well as writing the manuscript. OH has designed the research, took a part in practical research as well as in analysis and interpretation of the obtained data. MJ took a part in interpretation of the results, designed and carried out statistical analysis of the obtained data. PN participated in practical research as well as in writing the manuscript. VH assisted in interpretation of the results and critique review of the paper/manuscript. SR participated in harmonising and critique review of the manuscript. ND participated in the design and coordination of the research. GFJ participated in interpretation of the results and critique review of the manuscript. LJM participated in the statistical data processing and interpretation of the research results. ČM carried out sampling and the analysis of fatty acid composition of the lamb meat. All authors read and approved the final manuscript.

Declaration of conflicting interests:

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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UTICAJ DODAVANJA KONCENTRATA OBOGAĆENOG SEMENKAMA LANA U ISHRANU JAGNJADI RASE PRAMENKA NA OMENTALNE MASNE KISELINE

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Ovo istraživanje imalo je za cilj da se utvrdi uticaj prihranjivanja jagnjadi sa ograničenom količinom koncentrata obogaćenog semenom lana na masno-kiselinski sastav jagnjećeg mesa (omentalnog depoa masti). Ukupno 96 jagnjadi pramenke (23,85±3,98 kg žive telesne težine, 60 ± 10 dana starosti, 42 mužjaka i 54 ženke) iz pašnog sistema držanja nasumično su podeljeni u tri eksperimentalne grupe: kontrolna (CON) ili ekstenzivna uzgojna grupa, koja nije primila nikakvu dodatnu hranu; koncentrat grupa (CC), koja je dnevno po životinji primala 300 g komercijalnog koncentrata, i grupa koncentrat-lan (CC-FS), koja je dnevno primala 300 g koncentrata obogaćenog sa 5% lanenog semena. Nakon 60 dana eksperimentalnog perioda iz svake grupe je odabrano 10 jagnjadi (5 mužjaka i 5 ženki). Za analize masnokiselinskog sastava jagnjećeg mesa uzimani su uzorci trbušnog masnog tkiva (omentalno masno tkivo). Značajne (P> 0,05) razlike u profilu masnih kiselina između CON i CC grupa bile su male. Međutim, obogaćivanje koncentrata sa 5% semena lana povećalo je procente PUFA i n-3 PUFA u poređenju sa CON i CC grupom, a samim tim poboljšalo je i nutritivne karakteristike omentalne masti. Uticaj pola na profil masnih kiselina bio je značajan samo za C 20:4 *n-6* i C 20:5 *n-3* (P <0,05).