Bulgarian Journal of Agricultural Science, 14 (No 1) 2008, 100-107
National Centre for Agrarian Sciences

CONTENT AND FATTY ACID COMPOSITION OF DIFFERENT FAT DEPOTS OF LAMBS RECEIVING FISH OIL SUPPLEMENTED DIET

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Abstract

POPOVA, T., P. MARINOVA, V. BANSKALIEVA and V. VASILEVA, 2008. Content and fatty acid composition of different fat depots of lambs receiving fish oil supplemented diet. *Bulg. J. Agric. Sci.,* 14: 100-107

Two groups of 7 animals (age 75 days) each of local Zapadnostaroplaninska sheep breed were fed for 28 days controlled iso-nitrogenous diets, containing either no added fat (control) or fish oil (experimental) added at 2.5 % as feed of concentrate. The content of subcutaneous fat in the carcass of fish oil treated animals did not change, whereas the intermuscular fat increased by 16%, compared to the control animals. The effect of fish oil differed among the carcass cuts. Besides the leg and abdomen, where the subcutaneous fat slightly increased, in the rest carcass cuts (loin, shoulder, neck) the opposite changes were observed. Fish oil stimulated deposition of more intermuscular fat in shoulder ($P<0.05$), leg and abdomen , but did not change the fat proportion in the neck. In loin both – subcutaneous and intermuscular fat were reduced ($P<0.05$). No significant changes in the weights of sweetbread and perirenal fat were observed. After fish oil supplementation, the relative part of C16:0 did not change in the triacylglycerol fraction of the most of the studied fat depots, except of the sweetbread where it was significantly elevated ($P<0.01$). The contents of C16:1 and C18:1 were increased, more substantially ($P<0.01$) in the internal fat depots and in the subcutaneous fat over *m. Longissimus dorsi* and at the base of the tail, accompanied with a reduction of C18:0. As a result of the changes of the individual fatty acids, the sum of the saturated fatty acids decreased, and the sum of the monounsaturated fatty acid was enhanced in the fat depots of the experimental animals.

The changed deposition and distribution of the carcass fat, and the fatty acid composition of the different fat depots, suggest that fish oil (rich in PUFA) could have a favorable effect on the carcass fatness and quality.

Key words: lambs, fish oil, fat depots, fatty acids

Introduction

A shift of the nutrients toward deposition of more inter- and intramuscular fat, than subcutaneous and internal in meat, with more desirable fatty acid composition from health point of view, should influence production efficiency, carcass fatness and consumers acceptance. In recent years interesting discoveries of the role fish oil, added to animal diets had been found. In experiments with sheep and beef cattle it was established that fish oils, rich in very long-chain polyunsaturated fatty acids (PUFA) influenced the back fat thickness, the percentage of carcass fat, as well as the fatty acids of meat lipids by increasing the levels

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of n-3 PUFA and lowering the n-6/n-3 ratio to values considered more optimal for human diet (Mandell et al., 1997; Ponnampalam et al., 2001; Scollan et al., 2001; Wachira et al., 2002). The potential of the fish oil for upgrading the lipid metabolism and affecting the deposition of body fat could influence the nutritional qualities of lamb meat more closely to the recommended for the human diet.

However, so far, there is not a common conception for the amount of added fat and duration of treatment, breed, age and state of development of ruminants, where the type of diet and the type of fish oil is of importance as well.

The objective of this study was to study the effect of fish oil supplemented diet, offered in controlled amounts, on the deposition of the carcass fat and fatty acid composition of fat depots of different anatomical locations in growing lambs.

Material and Methods

The experiment was carried out with weaned (75 days old, with average live weight 16.53±1.35 kg) male lambs of local Zapadnostaroplaninska sheep breed, gradually switched (over 21 days period) to a commercial diet with an ingredient and fatty acid composition as presented in Table 1. The lambs were separated into two groups, control and experimental, 7 animals each. The concentrate of daily diet of the experimental animals was gradually supplemented with fish oil, starting with a level of 0.5% (of concentrate as feed weight) to a level of 2.5% at the end of the first week. After the adjustment period, the animals of the experimental group received 2.5% fish oil supplemented diet (prepared fresh every day) for 28 days. Representative concentrates (no added oil) and hay samples were subjected to routine proximate analysis (AOAC, 1996).

Lambs from both groups were offered their total ration of concentrate and hay in half two times a day. The average daily intake of concentrate was 500g and 485 g/day/animal and that of hay - 900 g and 915g/day/animal, respectively for the control and experimental groups. The daily dry matter intake was 1.26 kg/ animal for both groups. The daily fat intake (g/animal) and the daily ME intake (MJ/ animal), were 32.08 and 41.86, 12.36 and 12.75, respectively for the control and experimental groups. Water was available *ad libitum*. The animals were weighed at the beginning and at the end of the treatment period.

The lambs were slaughtered at the age of 105 days. Immediately after slaughter the internal fats (caul, perirenal and sweetbread) were taken and weighed. The carcasses were stored at 4°C for 24 hours. After storage, samples of subcutaneous fat from 3 different locations: over *m. Longissimus dorsi* (at the 11th rib),

Table 1
Ingredient and fatty acid composition of diet for lambs

Ingredients		%
Corn		26.30
Wheat		30.00
Wheat bran		15.00
Sunflower meal		14.00
Soya meal		12.00
Mineral-vitamin mix		0.10
Limestone		1.10
Dicalcium phosphate		1.00
Salt		0.50
Fatty acid composition, %		
Fatty acids	Concentrate	Hay
C14:0	0.49	1.99
C16:0	27.48	32.83
C16:1	0.38	6.13
C17:0	0.23	0.71
C18:0	5.48	6.09
C18:1	31.77	15.67
C18:2	32.90	20.95
C18:3	1.27	11.96
C20:0	-	3.67

at the breast plate and at the base of the tail were taken. The left half of each carcass was separated into five prime cuts: neck, shoulder, abdomen, loin and leg. After dissection of each cut into components of lean meat, bone and fat (subcutaneous and intermuscular), the weight of the fat was measured and calculated as percentage of the weight of the whole cut.

Total lipids from the fat depots were extracted according to the method of Bligh and Dyer (1959). The triacylglycerols (TG) were isolated by preparative thin layer chromatography on silicagel G in a system of solvents: hexane: diethyl ether: acetic acid, 80:20:1; v/v. Methyl esters of fatty acids were obtained using 0.01% sulfuric acid in dry methanol at 47°C for 14 h as described by Cristie (1973). The fatty acid composition of TG was determined using a Carlo Erba gas chromatograph, with a capillary column (DB-WAX, length 30 m, internal diameter 0.32 mm, film thickness 0.25μm) and H_2 as a gas vector. The temperature program was as follows: the oven temperature was first set at 150°C for 1 minute and then programmed to 170 °C at the rate of 8°C/min. The temperature was kept constant at 170°C for 1 minute and increased to 205°C for 3 min at a rate of 10°C/min - after that set at 207°C for 1 minute at the same rate. The injector and detector temperatures were maintained at 250°C and 260°C respectively. The output from the flame ionization detector was quantified using a computing integrator (Spectra Physics 4100). Fatty acids methyl esters were identified by comparing retention times of the standards and were expressed as percentages of the total fatty acid methyl esters.

The data is presented as mean ± standard deviation for each animal group. For significance evaluation *t*-criterion of Student was used with P>0.05-NS; P<0.05-*; P<0.01-**; P<0.001-***.

Results and Discussion

The fish oil did not induce significant changes in the live weight of the experimental animals -22.71±1.48 kg vs. 22.50±2.17 kg for the control group, as it is reported by Marinova et al. (2007). No changes were found in the live weight of kids, receiving the same fish oil supplemented diet for 33 days (Marinova et al., 2005). Wachira et al. (2002) showed breed dependent decrease of daily gain in sheep, fed fish oil. In our experiment the fish oil supplementation was 2.5% (in concentrate), corresponding to an amount of 0.9% to the whole daily diet offered to the lambs. It could be suggested that the applied fat have been too low to substantially affect the animal performance.

However, the results of the current experiment show that the fish oil supplementation for 28 days affected to some extent the deposition of the fat in the carcass and the internal fat depots, and the fatty acid composition of the TG fraction in the different fat depots.

The relative content of the subcutaneous fat (Table 3) in the whole left side of the carcass of the experimental animals did not change, whereas the intermuscular fat increased by 16 %, compared to the control

Table 2
Fatty acid composition (% of total fatty acids) of fish oil

Fatty acids	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2 n-6	C18:3 n-3	C20:1 n-6	C20:2 n-6	C20:3 n-6	C20:4 n-6	C20:5 n-3	C22:4 n-6	C22:5 n-3	C22:6 n-3
Fish oil	5.94	0.32	16.14	7.08	0.62	2.80	21.50	7.37	1.07	7.93	0.63	0.60	1.76	8.32	6.97	1.95	8.98

Table 3
Content of subcutaneous and intermuscular fat in half carcass and different carcass cuts of lambs

Fat depots	Half carcass [a]		Carcass cuts [b]									
			Leg		Loin		Shoulder		Neck		Abdomen	
	Groups [c]											
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Subcutaneous	10.5± 1.32	10.09± 1.47	6.33± 3.13	7.49± 1.15	12.67± 1.91	7.64± 4.58*	10.66± 0.92	8.97± 2.86	9.94± 5.74	7.16± 7.56	20.63± 8.48	21.86± 2.44
Intermuscular	6.55± 1.29	7.79± 0.76	3.93± 2.64	4.38± 1.68	11.22± 4.97	9.18± 3.63	6.46± 1.43	9.24± 2.22*	10.93± 4.33	11.07± 7.27	8.48± 2.53	10.32± 1.86

[a] Percentage of the half carcass weight.
[b] Percentage of the carcass cut weight.
[c] Control: no added fish oil; experimental: fish oil supplemented diet (2.5% of concentrate wet weight).
* $P < 0.05$.

animals. It could be suggested that fish oil favors deposition of more internal fat, at expense of the subcutaneous fat.

Data for the half carcass reflects changes in the five carcass cuts, where the effect of the dietary fat differed among the cuts (Table 3). Besides, the leg and abdomen, where the subcutaneous fat tended to increase, in the loin, shoulder and neck of the fish oil fed animals, the subcutaneous fat decreased by 40 % ($P<0.05$), 16% and 28% respectively as compared to the control animals. Opposite changes were observed for the deposition of intermuscular fat. The fish oil stimulated deposition of more intermuscular fat in the shoulder - by 30 % ($P<0.05$), leg - 10% and abdomen - 18%, but did not change the amount of intermuscular fat in the neck. It is interesting to mention that in the loin, not only the subcutaneous, but also the intermuscular fat was decreased. The lower amount of deposited fats in the loin corresponds to the significant increase ($P<0.05$) of intramuscular fat in *m.Longissimus dorsi* of lambs (Popova et al., 2007), which could have a positive effect on the marbling of lamb meat. These results are in agreement with those for kids fed fish oil (Marinova et al., 2005). Wachira et al. (2002) also found a decrease of subcutaneous fat over the loin of sheep after fish oil supplementation. In both groups the highest fat deposition was observed in the sweetbread (Table 4). Fish oil did not change the weights of the sweetbread and perirenal fat, whereas the weight of the caul tended to increase. Fish oil supplemented diet had similar effect in an experiment with kids (Marinova et al., 2005). Because of the big individual variations, not only in the caul, but also in some of the carcass fat depots, the observed changes were not significant. The results for

Table 4
Content (g) of internal fat depots of lambs

Groups [a]	Fat depots		
	Caul	Perirenal	Sweetbread
Control	122.00 ± 50.20	73.00 ± 10.37	176.00± 77.33
Experimental	147.50 ± 52.32	72.50 ± 32.98	183.33± 33.86

[a] Control: no added fish oil; experimental: fish oil supplemented diet (2.5% of concentrate).

the fatty acid composition of TG in the subcutaneous fat from the three different locations (at the breast plate, over *m. Longissimus dorsi*, at the base of the tail*),* in the three internal fat depots (caul, sweetbread, perirenal fat) and in the intermuscular fat (under *m. Semimembranosus*) are presented in Tables 5 and 6.

Despite of the different anatomical locations of the fat depots, the major fatty acids of TG of both groups of animals, consist of C16:0, C18:0 and C18:1, and the most saturated were the TG of the internal fat depots. Palmitic acid constituted between 23% and 27% of the total fatty acids. After fish supplementation, except in the sweetbread, where palmitic acid was elevated ($P<0.01$), no changes in its relative part in the rest six fat depots were observed although the fish oil contained approximately 16% palmitic acid (Table 2). Cooper et al. (2004), however, reported an elevation of C16:0 in the subcutaneous fat of lamb. Stearic acid decreased significantly in the sweetbread ($P<0.05$), in perirenal fat ($P<0.05$), and a tendency for a lower relative part in the TG of the caul, tail, above *m. Longissimus dorsi* and of the intermuscular fat was observed as well.

The proportions of C18:2 and C18:3 in TG of control animals were between 3.20% and 4.42%, and between 0.6% and 0.75%, respectively. The low contents of linoleic and linolenic acids, reflect the low incorporation of these fatty acids in the TG fraction in the adipose tissue. Fish oil did not change the proportions of C18:2 in the perirenal and intermuscular fat, whereas linoleic acid content tended to decrease in the TG fraction of the subcutaneous fat over *m. Longissimus dorsi* and at the base of the tail (Table 4). Slightly higher values of PUFA in the sweetbread, caul and at the breast plate were mostly a result of the higher proportions of C18:2 and to some extent to variations of C18:3. Probably the increased flow of long-chain polyunsaturated fatty acids from fish oil (Table 2) blocked the complete dehydrogenation of C18:2 in the rumen (Noble et al., 1974) and may have caused differential accumulation of more C18:2 in the TG of the adipose tissue.

Fish oil increased the content of C16:1, more substantially in internal fat depots – caul ($P<0.01$), perirenal fat depot ($P<0.001$) and over *m. Longissimus dorsi* ($P<0.05$). Oleic acid proportion was significantly ($P<0.05$) enhanced in perirenal fat depot, and the same tendency show the results for the other two internal fat depots, subcutaneous fat over *m. Longissimus dorsi* and at the base of the tail. As a result of the observed changes of the relative parts of palmitoleic, oleic and stearic acids (Tables 5, 6), the sum of the saturated fatty acids in TG in most of the fat depots was reduced, accompanied with a higher value of the sum of monounsaturated fatty acids. The increased formation of monounsaturated fatty acids in most of the fat depots, suggests that fish oil had an effect on the lipid metabolism of the experimental animals. The changed proportions of C16:1, C18:1 and C18:0 could be accepted as an indication, that fish oil affects the activity of stearoyl-CoA desaturase. It is shown that stearoyl-CoA desaturase is strongly influenced by the composition of the diet (Jeffcoat and James, 1977), and the location of fat depots (Barber et al.,2000). The values of the desaturase index [(C16:1+C18:1)/(C16:1+C16:0+C18:1+C18:0)], developed by Malau-Aduli et al. (1998), as an indicator for the activity of stearoyl-CoA desaturase, show a tendency for a slightly higher value in the fish oil fed animals (Tables 5 and 6).

In the current experiment, it could be expected that the higher daily fat intake from the added fish oil (41.86 vs. 32.08 g/animal), rich in polyunsaturated fatty acids, rather than the increased energy (with only 0.39 MJ/animal) affected the distribution of the body fat. In all cases, however, the added fat enhanced the flow of fish PUFA, which can change fatty acid composition of the cell membrane lipids and modify the course of several processes in the adipocytes (Fickova et al., 1997). It is quite attractive to suggest that a highest blood flow (and hence nutrient supply) to the internal and intermuscular fat depots, compared with

Table 5
Fatty acid composition (%of total fatty acids) of triacylglycerols in different fat depots in lambs

Groups [a]	Control	Experi-mental	S [b]	Control	Experi-mental	S	Control	Experi-mental	S
Internal fat depots									
Fatty acids	Sweetbread			Caul			Perirenal		
C14:0	5.55±1.11	5.84±0.62	NS	5.10±1.19	4.57±0.48	NS	5.13±1.29	5.08±0.93	NS
C15:0	0.84±0.08	0.85±0.06	NS	0.78±0.10	0.82±0.13	NS	0.82±0.06	0.83±0.09	NS
C16:0	25.04±0.42	26.18±0.31	**	25.59±1.20	25.60±0.58	NS	22.76±0.92	22.93±1.24	NS
C16:1	2.28±0.40	2.59±0.41	NS	2.63±0.14	3.04±0.13	**	2.21±0.4	3.06±0.21	***
C17:0	1.66±0.09	1.60±0.23	NS	1.67±0.07	1.72±0.15	NS	1.74±0.19	1.73±0.19	NS
C18:0	21.30±2.35	17.83±0.58	*	19.79±1.91	17.69±0.44	NS	24.26±3.47	20.15±1.13	*
C18:1	38.9±1.88	40.35±0.52	NS	39.96±2.41	41.57±0.63	NS	37.92±3.32	41.02±1.49	*
C18:2	3.75±0.41	3.83±0.51	NS	3.83±0.47	4.17±0.38	NS	4.42±0.54	4.40±0.66	NS
C18:3	0.69±0.22	0.91±0.24	NS	0.67±0.34	0.82±0.07	NS	0.75±0.03	0.80±0.12	NS
C18:2/C18:3	6.03±2.71	4.51±1.61	NS	8.21±6.75	5.15±0.79	NS	5.89±0.90	5.59±1.02	NS
SFA [c]	54.39±2.27	52.31±0.47	NS	52.91±2.36	50.39±0.53	NS	54.71±4.00	50.72±1.96	NS
MUFA [d]	41.18±2.18	42.95±0.79	NS	42.59±2.34	44.62±0.62	NS	40.13±3.66	44.08±1.61	*
PUFA [e]	4.43±0.40	4.74±0.49	NS	4.5±0.72	4.99±0.34	NS	5.17±0.52	5.2±0.69	NS
PUFA/SFA	0.08±0.01	0.09±0.01	NS	0.09±0.02	0.10±0.01	NS	0.10±0.01	0.1±0.02	NS
DSI [f]	0.47±0.02	0.49±0.01	NS	0.48±0.02	0.51±0.01	NS	0.46±0.04	0.51±0.02	*
Subcutaneous fat depots									
Fatty acids	At the breast plate			Over *m.Longissimus dorsi*			At the base of the tail		
C14:0	5.64±0.95	5.43±0.86	NS	4.14±0.92	3.94±0.63	NS	4.08±0.72	4.38±1.20	NS
C15:0	1.03±0.20	0.93±0.15	NS	0.88±0.16	0.81±0.15	NS	0.85±0.21	0.76±0.12	NS
C16:0	25.68±0.56	25.91±1.42	NS	25.75±2.44	26.88±1.63	NS	25.54±0.72	26.29±0.91	NS
C16:1	4.87±1.11	4.58±0.95	NS	3.24±0.4	3.91±0.041	*	2.53±0.71	2.95±0.39	NS
C17:0	1.57±0.28	1.57±0.18	NS	2.31±0.82	2.2±0.40	NS	2.20±0.55	2.02±0.48	NS
C18:0	10.91±3.03	10.94±2.34	NS	15.62±3.24	12.74±1.03	NS	17.13±3.18	14.79±0.66	NS
C18:1	46.42±2.95	46.48±2.37	NS	43.65±3.87	45.6±1.86	NS	42.92±2.58	44.61±2.23	NS
C18:2	3.20±1.11	3.57±0.74	NS	3.82±0.41	3.39±0.39	NS	4.06±0.54	3.47±0.58	NS
C18:3	0.69±0.07	0.59±0.16	NS	0.6±0.10	0.54±0.06	NS	0.68±0.07	0.73±0.14	NS
C18:2/C18:3	4.71±1.67	6.47±2.26	NS	6.57±1.25	6.27±0.94	NS	6.01±0.74	4.91±1.12	NS
SFA	44.82±3.27	44.78±2.16	NS	48.69±4.30	46.57±1.68	NS	49.80±3.23	48.24±1.85	NS
MUFA	51.29±3.36	51.06±2.63	NS	46.89±4.15	49.5±1.91	NS	45.46±3.11	47.57±1.97	NS
PUFA	3.89±1.11	4.16±0.83	NS	4.42±0.43	3.93±0.4	*	4.74±0.58	4.2±0.58	NS
PUFA/SFA	0.087±0.03	0.093±0.02	NS	0.09±0.01	0.08±0.1	NS	0.10±0.01	0.09±0.01	NS
DSI	0.58±0.04	0.58±0.03	NS	0.53±0.05	0.56±0.02	NS	0.52±0.04	0.54±0.02	NS

[a] Control: no added fish oil; experimental: fish oil supplemented diet (2.5% of concentrate wet weight)
[b] Significance; c SFA- Saturated fatty acids; d MUFA-Monounsaturated fatty acids
[e] PUFA-Polyunsaturated fatty acids
[f] DSI-Desaturase index [(C16:1+C18:1)/(C16:1+C16:0+C18:1+C18:0)]
NS -P>0.05; * P<0.05; ** P<0.01; *** P<0.001.

Table 6

Fatty acid composition (% of total fatty acids) of triacylglycerols in intermuscular fat depot (under m. Semimembranosus) of lambs

Fatty acids	Control[a]	Experimental	S[b]
C14:0	6.53±1.42	6.45±0.88	NS
C15:0	0.97±0.10	0.91±0.08	NS
C16:0	25.31±1.43	25.13±1.59	NS
C16:1	2.74±0.34	3.09±0.30	NS
C17:0	1.48±0.10	1.48±0.08	NS
C18:0	17.43±2.48	16.18±1.65	NS
C18:1	41.04±1.79	42.25±1.23	NS
C18:2	3.77±0.61	3.81±0.55	NS
C18:3	0.74±0.07	0.7±0.08	NS
C18:2/C18:3	5.15±0.78	5.44±0.59	NS
SFA[c]	51.71±2.16	50.15±1.04	NS
MUFA[d]	43.78±1.92	45.34±1.11	NS
PUFA[e]	4.51±0.64	4.51±0.60	NS
PUFA/SFA	0.09±0.01	0.09±0.01	NS
DSI[f]	0.51±0.02	0.52±0.01	NS

[a] Control:no added fish oil;experimental: fish oil supplemented diet (2.5% of concentrate wet weight)

[b] Significance

[c] SFA- Saturated fatty acids

[d] MUFA-Monounsaturated fatty acids

[e] PUFA-Polyunsaturated fatty acids

[f] DSI-Desaturase index [(C16:1+C18:1)/(C16:1+C16:0+C18:1+C18:0)].

NS- P>0.05.

the subcutaneous (Gregory et al., 1986) intensifies the effect of fish fatty acids and favors the elevated deposition of intermuscular (and to some extent in the sweetbread) fat in fish oil fed animals (Tables 3,4). The effect of the fish oil, however, may be limited or favored by the different stage of formation of adipocyte cells, as it was shown by Vassileva and Marinova (2007), or specific peculiarities of the lipid metabolism in the different anatomical locations (Eguinoa et al., 2003).

Therefore the reasons for the differences of the lipid deposition and fatty acid composition after fish oil supplementation still are not clear, nor the biochemical mechanism connected with the changed lipid metabolism. Undoubtedly, the observed changes in our experiment with lambs, as well as the results of experiments with other ruminants depend on the experimental design, where the breed, age, stage of development of the animals are of importance, and more studies in this aspect are needed.

Conclusion

The changes in deposition and distribution of carcass fat, and in the fatty acid composition of fat depots after fish oil supplementation could have a favorable effect on the carcass fatness and quality.

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Received August, 3, 2007; accepted for printing December, 14, 2007.