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Effect of Dietary Linseed Oil Soap on Lamb Meat

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***Abstract*—**Theexperiment was carried out with 2x5 male Merino lambs raised under intensive conditions to investigate the effect of dietary calcium soap of linseed oil on the color and fatty acid composition of *longissimusdorsi muscle*. Control lambs fed a basal diet and the experimental lambs consumed a diet supplemented with 3% calcium soap of linseed oil. The color values (L*, a*, b* a*/b* and chroma) were not influenced by dietary treatment. The MUFA proportion reduced, SFA and PUFA content did not alter. As expected, the linolenic (C18:3 n3) and thusthe n-3 content significantly improved by linseed supplement (0.47 and 0.81; 0.78 and 1.16 in control and in experimental samples, respectively). Other n-3 and n-6 fatty acids had similar valuestocontrol samples. The n-6/n-3 ratio was significantly narrower in the experimental group (6.31 vs. 9.38) but the P/S ratio did not differ betweenthe two groups.In conclusion calcium soap of linseed oil seems to be a suitable supplement form of n-3 fatty acids to improve the nutritive value of lamb meat.

***Keywords*—**calcium soap, fatty acid, lamb meat, linseed

I. Introduction

It is well known that n-3 (omega-3) fatty acids (FA) have several beneficial effects on human health, one most important is the lower risk of cardiovascular diseases [1].A great number of scientific experiments have been conducted in order to increase the PUFA, especially the n-3 content in animal products [2]-[8]. These researches demonstrated that the fatty acid profile of products (meat, egg) can be altered by dietary manipulations in monogastric animals. However, it is a more difficult challenge to modify the fatty acid composition of ruminant's products (meat, milk) by nutrition due to the rumen fermentation. Several strategies were sought to improve the n-3 PUFA content and hereby the nutritional value of intensively-reared lamb meat [9]-[12]. One of them is to involve natural unsaturated fatty acids into the diet, for example linseed rich in n-3 linolenic acid. Although PUFA undergoes high ruminalbiohydrogenation, high n-3 content (1.5-3% of total FA) was determined in lamb meat [9]-[11].

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The other possibility is to feed ruminally protected (bypass) fat.In the published studies protected form of palm oil, olive fatty acids and rapeseed oil was investigatedin lambs [13]-[15].

However these vegetable fats have lower n-3 fatty acid content than linseed, so the n-3 PUFA content could not improve appreciably.

Therefore, the aim of the present study was to investigate the influence of dietarycalcium soap of linseedoil on the color and fatty acid composition of lamb meat.

II. Materials and Methods

A. Experimental design, animals and diets

Ten male Merino lambs were used to evaluate two concentrate (five lambs per diet) containing 0 (C) or 3% calcium soap of linseed (S) (Table 1). Sunflower oil was used in the control diet to ensure iso-energetic composition. The concentrate was fed *ad libitum* and beside that one kg alfalfa hay was offered to the lambs daily. The average live weight at the beginning of the trial was 18.2±0.63 kg. The experiment lasted 60 days preceded by a 10-day adaptation period, during which lambs were gradually adapted to the experimental diets.

TABLE I
Chemical composition of concentrates

	Diets	
	C	S
Ingredients, % of DM		
Calcium soap of linseed oil	0.0	3.0
Corn	45.0	42.2
Wheat	25.0	26.1
Soybean meal	20.0	20.0
Sunflower meal	6.5	6.5
Sunflower oil	1.0	0.0
Limestone	1.5	1.2
Sodium chloride	0.5	0.5
Mineral and vitamin mixture	0.5	0.5
Total	100.0	100.0
Chemical composition, % of DM		
ME (MJ/kg DM)	12.9	13.0
Crude protein (% of DM)	20.4	20.3
Fatty acid composition, g/kg DM		
C16:0	0.33	0.32
C18:0	0.09	0.14
C18:1*cis 9*	0.84	0.92
C18:2*n-6*	1.82	1.24
C18:3*n-3*	0.05	1.39

The calcium soap of linseed oil was produced in our laboratory according to [16], which contained 51% α-linolenic acid of total fatty acids, and consequently S diet had 21 times higher n-3 content compared to control group (C). However, other properties (crude protein and ME content) of diets were similar.

At the end of the experiment all lambs were slaughtered, the average live weight was 37.6 and 37.2 kg in the C and S group, respectively (P>0.05).

B. Meat sampling and color measurement

Longissimusdorsi muscle samples (between ribs 12 and 13)were collected from the right side of each carcass in both groups to determine the color and the fatty acid profile of meat.

Color was measured with MiniScan XE Plus colorimeter (HunterLab, Inc., USA) using light source D65 and 45°/0° geometry. Measurements were carried out in three replicates in each sample. The equipment was calibrated to white tile before each session of measurements. The CIELAB L* (lightness) a* and b* (green-red and blue-yellow color indices, respectively) color space (CIE, 1976) was used to determine the color[17]; C* (chroma) was calculated according to the following equation: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$.

C. Fatty acid analysis

Lipids were extracted from the tissue and feed samples using chloroform/methanol (2:1, vol/vol). After evaporation of solvents, samples were saponified with 1 n NaOH at 100 °C. Boron-trifuorede-methanol was used for esterification of fatty acids and then the samples were solved in hexane, centrifuged, and dissolved prior to injection. The separation of fatty acids was carried out by using an Agilent Technologies 6890 N Network gaschromatograph (Agilent Technologies, Inc. Headquarters,Santa Clara, USA) equipped with Supelco SP 2560 Fused Silica Capillary Column (length: 100 m, i.d: 0.25 mm, film thickness: 0.2 μm) and a flame ionization detector. The operating conditions of the gaschromatograph were as follows: the temperature of the thermostat was from 170 to 215ºC, of the injector was 240ºC, and of the detector was 250ºC. Helium was used as carrier gas at 176.8 kPa. The flow was 35 mL/min for H, 30 mL/min for N, and 300 mL/min for air. Fatty acids were identified using Supelco 37 Component FAME Mix fatty acid standard (Catalog No. 47885-U Sigma Aldrich Chemie GmbH).

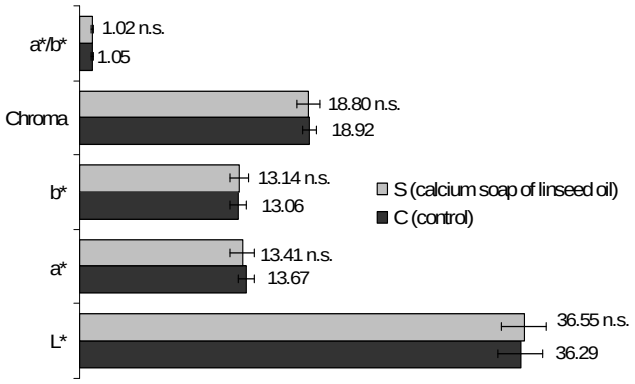
D.Statistical analysis

The effect of diet on colorand fatty acid composition was analyzed using SPSS 15.0 for Windows program package (SPSS Inc., Chicago, USA). T-test was used to test the differences between treatment means. Statistical significance was considered at $P<0.05$.

III. RESULTS AND DISCUSSION

A. Color of meat

As Fig. 1. shows L* (lightness) was found higher and a* (redness) lower in experimental (S) group compared to mean value of control (C) samples. However, neither of them was significant, and similar value was obtained in the case b* (yellowness). These results meet our expectation. The effect of other dietary supplement (fishmeal, canola meal and soy meal) at the level of 7 to 8% in lamb's dieton several properties of lamb muscles was investigatedearlier [18]. These researchersexamined not only thecolorbut also thecolor stability during storage. Their conclusion was in agreement with our result that the significant increases in muscle n-3 fatty acid content do not affect significantly the color of meat.



n.s.: not significant (P>0.05)

Fig. 1 Effect of dietary treatment on the color of muscle

Contrary to that, the dietary linseed supplementation in maternal and/or lamb diet had no influence on b* value of lamb's back fat [19]. Nevertheless a* value was lower and L* value was higher in the samples of the linseed supplemented groups compared to control group. This means that if n-3 proportion increasing in the diet, the back fat may become lighter. The authors considered this change as an improvement of fat color score.When lambs are fattened on pasture, the effect of diet on subcutaneous fat is similar than that of linseed intake [20], [21].

These observations may confirm that it is worth studying the color scores both fat and meat, if the n-3 proportion of concentrate is increased and hereby the n-3 PUFA content of carcass improves.

B. Fatty acid composition

Calcium soap is a rumen protected form of linseed oil, which hadapproximately 52% α-linolenic acid content (determined by our laboratory), so the rate of biohydrogenation of unsaturated fatty acids is lower and thus major part of them can incorporate into the muscle.

Results of our fatty acid analysis are presented in Table 2, which includes only the major fatty acids and fatty acid groups. The concentrate of the S group contained approximately 27 times higher α-linolenic acid (C18:3 n-3) than control diet. This difference caused typical alteration in fatty acid profile of muscle.

Among main fatty acid groups (SFA, MUFA, PUFA) only monounsaturated (MUFA) values differ between the experimental groups significantly ($P<0.05$). This in agreement with [10], because they also observed decreasing MUFA content when a supplement containing extruded linseed was incorporated into the lambs diet. However, if only the ewes were fed linseed diet 8 weeks long from the 11 weeks of the weaning but the lamb's diet have not included it, the MUFA content of *longissimusdorsi* was not lower than control samples (45.3 and 45.5%, respectively). However, contrary to our results the lower oleic acid (C18:1c9) content caused the MUFA proportion to decrease in all treatments. Another interesting result is published by [12], who reported higher

oleic acid and thereby higher MUFA value in linseed fed group compared to control lambs. Its reason is maybe in close relationship with the 25% alfalfa pellets ratio in the basal diet.

TABLE II
FATTY ACID COMPOSITION (MEAN±SEM) OF LONGISSIMUSDORSI MUSCLE FED A DIET WITHOUT (C) OR SUPPLEMENTED WITH CALCIUM SOAP OF LINSEED (S) (% OF TOTAL FATTY ACID CONTENT)

	C	S	Significance
C14:0	4.36±0.19	4.50±0.18	n.s.
C16:0	24.23±0.54	23.85±0.26	n.s.
C16:1	1.94±0.14	1.73±0.08	n.s.
C18:0	12.84±0.30	12.62±0.31	n.s.
C18:1 n9	29.65±0.46	29.80±0.82	n.s.
C18:1 n7	11.09±0.78	8.03±0.73	*
C18:2 n6	5.49±0.14	5.57±0.32	n.s.
C18:2 c9t11	0.49±0.07	0.66±0.07	n.s.
C18:3 n3	0.47±0.01	0.81±0.06	**
C20:4 n6	0.82±0.09	0.62±0.09	n.s.
C20:5 n3	0.07±0.01	0.08±0.02	n.s.
C22:5 n3	0.19±0.01	0.21±0.02	n.s.
C22:6 n3	0.06±0.01	0.06±0.01	n.s.
SFA[a]	44.08±0.77	43.43±0.32	n.s.
MUFA[b]	44.07±0.90	40.96±0.51	*
PUFA[c]	8.10±0.24	8.46±0.34	n.s.
n-6	7.31±0.21	7.30±0.30	n.s.
n-3	0.78±0.03	1.16±0.05	***
P/S[d]	0.18±0.01	0.20±0.01	n.s.
n6/n3	9.38±0.24	6.31±0.26	***

n.s.: not significant* P < 0.05** P < 0.01*** P < 0.001
a SFA: saturated fatty acids.
b MUFA: monounsaturated fatty acids.
c PUFA: polyunsaturated fatty acids.
d P/S: polyunsaturated/saturated fatty acids.

The saturated fatty acid (SFA)group and its major fatty acids (C16:0, C18:0) represented similar proportion in C and S samples. Similar observation was reported in other experiments in meat samples [10], [19]. Although, other authors [10], [12] found that applying linseed in the diet significantly reduced the SFA content of perirenal adipose tissues but not influenced that of the subcutaneous fat.

As presented in Table 2. dietary treatment did not increase significantly the proportion polyunsaturated fatty acids group (PUFA) but modified its composition. As it is expected, lambs fed calcium soap of linseed oil had a greater proportion (P<0.01) of linolenic acid (C18:3 n3) and hereby higher n-3 fatty acid content (P<0.001). The 3% calcium soap in the concentrate improved total fatty acids content from 0.47 to 0.81%.This means 72% increase. Compared to other studies it is considered a favorable result, because [12] achieved similar value (0.86%) with adding 97 g/day DM linseed. On the other hand, 6 or 9% of natural form of linseed supplement resulted 1.5 and 2.2% linolenic acid content, respectively [10]. Furthermore, the lambs muscle from the animals fed protected n-3 supplement diet (40 g/kg DM) contained 1.5 times much C18:3 n3 as the linseed based diet [22]. When the lambs remained at pasture during the trial, 2.2-3.7% linolenic acid of the total fatty acids were achieved independently from the dietary manipulation [21].

According to this, calcium soap of linseed was effective but its dose in the diet should be increased to achieve higher C18:3 n-3 proportion. However, this recommendation may be altered based on the result of a sensory analysis. The other components of the n-3 group (EPA, C20:5; DPA, C22:5 and DHA, C22:6) are very valuable for human nutrition. The increased total linolenic acid contentof muscle did not alter the EPA, DPA and DHA content of muscle. As EPA and DHA are the metabolites of linolenic acid, furthermore DPA is the metabolite of EPA, this suggests that the conversion efficiency in these cases may be low in ruminants. It was consistent with the results reported by [10], because they did not observed significant differences in the level of these long chain n-3 fatty acids of muscle from lamb fed 3, 6 or 9% linseed compared to control samples. Contrary to that, [19] published significant improving in the level of EPA and DPA in lambs fed linseed, but DHA content were not affected by the diet. Other study [13] presented that protected form of n-3 supplement did not, but fish oil increased EPA, DPA and DHA proportion in *longissimusdorsi muscle* of lamb compared to diet of whole linseed. The reason is that fish oil is one of the richest sources of long chain n-3 fatty acids. These results may confirm that applying of this oil in lamb's diet may be a possible method to increase the level of these fatty acids.

Control diet contained more linoleic acid (C18:2 n6) than S diet due to the low level of C18:2 n6 in the calcium soap used in our trial, and the fact that sunflower oil was added to the control concentrate to achieve isoenergetic diets. This difference did not reflected neither in the C18:2 n6 (5.49 and 5.57 in the C and S samples, respectively) nor in its metabolite (C20:4 n6) level of muscle. This result isin agreement with other authors, who observed that incorporation of C18:2 n6 into the muscle could not be influenced by higher linseed (6 or 9%) [10].Furthermore, other fat sources (unprotected rapeseed, fish meal or oil, calcium soap of olive fatty acids or palm oil, safflower or sunflower) except the protected form of canola seed could not affect it [12]-[15]. It suggests that biohydrogenation of linoleic acid may be very effective in the rumen.

The n-6/n-3 ratio is an important component of the meat quality assessment beside the amount of each fatty acid. In our trial the n-3 percentage increased due to the higher linolenic acid content and additionally, n-6 group was not influenced by calcium soap of linseed in S group. So control samples had similar amount of n-6 and lower n-3 fatty acids compared to S muscles. These changes resulted that the n-6/n-3 ratio was significantly (P<0.001) narrower in S samples than that of the control lambs (6.31 vs. 9.38, respectively). It can be considered a beneficial property for human nutrition. However, this value is higher than the recommendation of nutritionists [1], [23].

The other important aspect of nutritional value of food is the P/S (PUFA/SFA) ratio, which should be at least 0.40 according to nutritionists [23]. This value was 0.20 in S group, which should be improved to meet with human demand. However, other researchers ([10], [12], [18]) did not manage to achieve higher value using different dose of linseed or other plant origin fats (protected or unprotected form of rapeseed, sufflower, sunflower, fish meal). The P/S ratioswere between 0.05 and 0.15 in these studies, although the n-6/n-3 ratio was

narrower (1.5-4.02) than in our present study.It is an interesting fact that P/S ratio was more beneficial (0.38-0.48) in intramuscular fat of lambs if palm oil (rich in SFA and MUFA) or calcium soap of palm oil was incorporated into their diets [14]. Similar to this supplying 5% calcium soap of olive fatty acids displayed 0.43 P/S ratio in the intramuscular fatty acid profile of lambs [15]. However, these last mentioned fat sources could not result narrow n-6/n-3 ratio (18-24:1), which decrease the nutritive value of these meat.

IV. CONCLUSION

In conclusion, calcium soap of linseed seems to be a suitable supplement form of n-3 fatty acids to improve the nutritional value of lamb meat.Further work is needed to clarify the optimum level of supplementation to achieve the most beneficial fatty acid profile in terms of human nutrition without impairing the sensory properties.

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