Animal welfare, etológia és tartástechnológia

Animal welfare, ethology and housing systems

Volume 16 Issue 1

Gödöllő
2020

EFFECT OF LINSEED AND SUNFLOWER OIL SUPPLEMENTATION IN THE DIET ON HEALTH BENEFICIAL FATTY ACIDS IN INTRAMUSCULAR FAT OF LAMBS

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Received – Érkezett: 15. 11. 2019.
Accepted – Elfogadva: 04. 04. 2020.

Abstract

The effect of linseed and sunflower oil on health beneficial fatty acids composition of *Musculus longissimus lumborum et thoracis (MLLT)* of lambs was investigated. Thirty slovak dairy sheep breed ram lambs (20.11±3.06 kg) randomly allocated into three groups were fed a control diet (C) composed by 70 % concentrate and 30 % forage and two expeimental diets (5% linseed oil and 5% sunflower oil, LO and SO respectively). The experimental period was 46 days and lambs were slaughtered at 28.83±3.88 kg. Differences between dietary groups were calculated using General Linear Model of SAS 9.3. Addition of linseed oil, rich in α-linolenic acid (C18:3n-3) significantly increased rumenic acid (*c*9,*t*11-C18:2; P<0.01), C18:3n-3 (P<0.001), eicosapentaenoic acid (C20:5n-3; P<0.001), docosahexaenoic acid (C22:6n-3; P<0.05), conjugated linoleic acid (CLA; P<0.01) and decreased n-6/n-3 ratio (P<0.001). The addition of SO to the diet did not influence significantly essential and health beneficial fatty acids in lamb meat. The results of the present study suggest that linseed oil supplementation can be safely incorporated in the diets of lambs to enrich lamb meat with essential and healthy fatty acids.
Key words: lamb meat, fatty acid, linseed, sunflower, oil

Introduction

The composition of fatty acids (FA) in consumated meats play important role in the human diet in Western countries. Meat from ruminants may be an important source of healthy and essential fatty acids in human diet, as the n-3 polyunsaturated fatty acids (n-3 PUFA) with anti-inflamatory and anti-arrhytmic properties (Barceló-Coblijn and Murphy, 2009, Calder 2018) and the conjugated linoleic acid (CLA) that has received considerable attention because of its anti-carcinogenic and anti-mutagenic properties (Whigham et al. 2000; Pariza et al. 2001; Lehnen et al. 2015). CLA is the generic term of a group of positional and geometric isomers of the n-6 essential fatty acid C18:2n-6 (linoleic acid). The major CLA isomer in ruminant tissues is *c*9,*t*11-C18:2 (rumenic acid), which is synthesized mainly by endogenous desaturation of *t*11-C18:1 (vaccenic

10.17205/SZIE.AWETH.2020.1.015

acid) catalysed by steoryl-CoA desaturase. Vaccenic acid is the main intermediate of microbial biohydrogenation of dietary PUFA, namelly linoleic acid (LA) and C18:3n-3 (α-linolenic acid).

Weissová (2013) and *Horečná* (2015) reported that proportion of healthy and essential FA in meat gained from intensive finished lambs from indoor systems in Slovakia was lower than in lambs finished on pasture. The linseed and sunflower oil have a high proportion of essential FA, especially α-linolenic acid (ALA) in linseed and LA in sunflower oil. Feed content linseed oil may increase n-3 PUFA, mostly ALA and also long chain (LC) C20:5n-3 (EPA), C22:5n-3 (DPA) and C22:6n-3 (DHA), (Scollan et al. 2001; Doreau – Ferlay, 2015). According to *Kitessa et al.* (2009), the strongest increase of ALA in lamb meat is in first weeks after supplementation of linseed oil to lambs´ feed, while contents of EPA, DPA and DHA are increased only after minimum six weeks of linseed oil supplementation.

Therefore, this study was conducted to investigate the effects sunflower and linseed oil supplementation in lamb diets on fatty acid profile, particularlly essential and health beneficial fatty acid in intramuscular fat of growing lambs.

Material and methods

Experimental design, animal management and diets

Thirty ram lambs of Slovak dairy sheep breed (average live weight of 20.11 kg) were randomly allocated to the three complete ground diets (10 lambs in each group), that were composed by 70% concentrate and 30% alfalfa, suplemented with 5% linseed oil (LO) or sunflower oil (SO). Lambs from the control group (C) never received additive oil. The ingredients, chemical and fatty acid composition of diets are presented in *Table 1*. Feed was offered *ad libitum* and intake of feed was controlled daily by weighing the offered and refused feed. The trial lasted 46 days and at the end, lambs were weighted without fasting and transported to the experimental slaughterhouse, located at Slovak University of Agriculture in Nitra. The lambs were stunned and slaughtered according to the official European legislation regarding protection of animals during slaughter. Twenty-four hours after slaughter and chilling, 100 g of *Musculus longisimus lumborum et thoracis* were taken from each carcass, minced, vacuum packed, and stored at -25°C until lipid analyses were carried out.

Fatty acid analysis

The proportion of the individual FAs was analysed using capillary gas chromatography (GC). The lipids from 0.5 g minced meat samples were extracted using 2 mL chloroform-methanol mixture (2:1 vol.vol-1) during 1 hour on rotary shaker. The 1 mL of saline water was added for better separation of chloroform layer and after then, samples were centrifuged at 2000 g for 5 minutes. The lower chloroform layers with extracted lipids (1 mL) were filtered through anhydrous sodium sulphate, and then dried and stored under nitrogen at -20°C. The base-catalysed methylation procedure with a solution of sodium methoxide in methanol was used for the preparation of fatty acid methyl esters (FAME). Gas chromatographic analyses was realized by gas chromatograph Agilent Technologies 6890N with flame ionization detector (Agilent, Waldbronn, Germany) and 5973 Network mass-selective detector. FAME were separated in a capillary column 100 m x 0.25 mm i.d. x 0.2 μm film thickness of HP-88 stationary phase (J&W Scientific, Agilent Technologies, California, USA). The initial column temperature of the programmed run was set to 45°C and it was held for 2 minutes, then followed by a step up ramp of 15°C.min^{-1} to 145°C, and then of 5°C.min^{-1} to 240°C and held for 5 minutes. Helium was used as the carrier gas with a linear

velocity set at 20 cm.s^{-1}. Two µL samples, which represented approximately 10 mg.mL^{-1} FAME, were injected using a split 50:1 at injection temperature 300°C. Separated fatty acids were identified by reference materials (Supelco 37 Component FAME mix, PUFA No. 3 from menhaden oil, Sigma, Aldrich, Germany), published retention data and mass spectrometric measurements.

***Table 1*: Ingredients, chemical and fatty acid composition of the experimental diets**

	Diets		
	C	LO	SO
Feed ingredient[1]			
Dehydrated chopped alfalfa	30.0	30.0	30.0
Corn meal	25.0	6.0	6.0
Barley grain	16.0	22.9	22.9
Soybean meal	11.0	9.0	9.0
Rapeseed meal	8.0	8.0	8.0
Malted barley	3.9	10.0	10.0
Sugar beet pulp	2.0	5.0	5.0
Premix	3.0	3.0	3.0
Sodium chloride	0.6	0.6	0.6
Calcium carbonate	0.5	0.5	0.5
Linseed oil	-	5.0	-
Sunflower oil	-	-	5.0
Chemical composition[2]			
Dry matter[3]	922.5	929.3	930.0
Crude protein	168.9	184.3	184.4
Ether extract	24.7	73.3	76.1
Neutral detergent fiber (NDF)	243.6	281.7	280.2
Acid detergent fiber (ADF)	161.4	173.7	174.1
Crude ash	88.3	95.2	95.8
Fatty acid composition[4]			
C16:0	14.1	11.0	9.0
C18:0	2.3	4.3	3.4
*c*9-C18:1	24.6	18.9	61.3
C18:2n-6	43.9	23.4	15.8
C18:3n-3	8.8	38.9	5.8

C – control; LO – linseed oil; SO – sunflower oil; [1] g/100 g; [2] g/kg dry matter; [3] g/kg; [4] g/100g FAME

The chromatograms were published response factors of flame ionization detector for FAME (Ackman, 2000). The fatty acid composition of IMF was detected in grams of each individual FAME per 100 g of sum detected FAME. The average relative standard deviation of the FAME analysed with a proportion above 0.5 g 100 g^{-1} was 1.1 % for the whole analytical procedure and the five replicate samples.

Statistical analysis

The experimental data were evaluated using an analysis of variance and a general linear model procedure as implemented in SAS 9.3. Least-squares means were compared using a Scheffe test.

Results and discussion

Results concerning composition of selected fatty acids in intramuscular fat of lambs are presented in *Table 2*. The supplementation with linseed oil increased the proportion of *c*9*t*11-C18:2, which is important isomer of CLA, essential C18:3n-3 and LC n-3 PUFA, namely EPA and DHA. Linseed oil can be considered as significant source of n-3 PUFA in the diet, when it increased ALA more than fourfold and EPA more than twofold. Proportions of DPA and DHA were more similar to results in C group. It can be caused by low activity of the elongase enzyme in elongation C18 ≥ C20 and following desaturation of ALA on more effective LC n-3 PUFA. Similar results after linseed oil supplementation are reported *Le et al.* (2019), who mentioned almost twofold higher proportion of ALA and increased proportion of EPA, whereas proportion of DPA and DHA were similar to control group. Aproximatelly twofold higher proportion ALA and slightly higher proportion EPA and DPA in *M. longissimus thoracis* of lambs after linseed supplementation reported *Andrés et al.* (2014). Likewise *Urrutia et al.* (2015) reported almost twofold higher proportion of ALA after 5% supplementation of linseed oil, however they did not find out differences in EPA content of intramuscular fat of lambs.

***Table 2*: Effect of dietary supplementation with linseed and sunflower oil on the on health beneficial fatty acids in intramuscular fat of lambs**

FA composition (g/100g FAME)	C	LO	SO	S. E.	*P*-values
*t*11-C18:1	0.393	0.359	0.404	0.0394	0.697
C18:2n-6	6.68	6.73	7.37	0.461	0.502
*c*9, *t*11-C18:2	0.167^{b}	0.279^{a}	0.202^{b}	0.0230	0.006
C18:3n-3	0.396^{b}	1.77^{a}	0.389^{b}	0.0758	<0.001
C20:5n-3	0.174^{b}	0.413^{a}	0.212^{b}	0.0395	<0.001
C22:5n-3	0.422	0.632	0.576	0.0765	0.152
C22:6n-3	0.055^{b}	0.090^{a}	$0.070^{a.b}$	0.0109	0.090
Total CLA	0.188^{b}	0.297^{a}	0.217^{b}	0.0229	0.007
n-3 PUFA	0.657^{b}	2.32^{a}	0.703^{b}	0.1056	<0.001
LC n-3 PUFA	0.261^{b}	0.552^{a}	0.314^{b}	0.0525	0.001
n-6 PUFA	9.63	9.42	11.58	0.810	0.134
LC n-6 PUFA	2.95^{b}	2.69^{b}	4.21^{a}	0.423	0.039
n-6 PUFA / n-3 PUFA	15.02^{a}	4.14^{b}	16.97^{a}	0.798	<0.001
LC n-6 PUFA / LC n-3 PUFA	11.87^{a}	4.97^{b}	13.96^{a}	0.819	<0.001

C – control; LO – linseed oil; SO – sunflower oil; S. E. – standard error; $^{a, b, c}$ means in the same row with different superscripts differ significantly (P<0.05)

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Linseed oil supplementation increased important FA groups, namely ∑n-3 PUFA, ∑LC n-3 PUFA and ∑CLA. Higher proportion of ∑LC n-3 PUFA can be caused by the higher level of by-pass lipids and their protection from excessive lipolysis and extensive biohydrogenation in rumen (*Chikunya et al*. 2004). Differences in rumen biohydrogenation proces between groups can caused also higher CLA proportion in *MLLT* of lambs in LO group. Increasing of CLA after linseed product supplementation reported also *Majewska et al.* (2004) and *Kamel et al.* (2018), whereas *Urrutia et al.* (2015), *Andrés et al.* (2014) and *Giannico et al.* (2009) found no differences between groups. It can be due to diferent biohydrogenation proces of fatty acids in rumen dependent on different experimental diets. Only linseed oil supplementation positively affected n-6 PUFA/n-3 PUFA a LC n-6 PUFA/ LC n-3 PUFA ratios, when these ratios were at the level recomen by the health organizations in terms of suitability for human health. The ratio n-6 PUFA/n-3 PUFA was more than threefold lower after linseed supplementation, what influenced LC n-6 PUFA/ LC n3 PUFA ration too. The similar ratios dicreasing after linseed supplementation reported *Abuelfatah et al.* (2016) in goat kid and *de la Fuente et al.* (2014) in lambs. *Urrutia et al.* (2015) noted twofold dicreasing of n-6 PUFA/n-3 PUFA ratio after 5% linseed oil supplementation. *Le et al*. (2019) and Majewska et al. (2016) found no differences in n-6 PUFA/n-3 PUFA ratio after linseed supplementation. The high proportion of α-linoleic acid in intramuscular fat of LO lambs significantly caused level of LA/ALA ratio, where the level was fourfold lower in comparision to C lambs.

The sunflower oil supplementation had no significant effect on proportion of specific polyunsaturated FAs, only sum of LC n-6 PUFA was significant higher in SO compared to C. In accordance with *de la Fuente et al.* (2014), the proportion of LA was not influenced by addition of vegetable oils, because the LA proportion is more influenced by animal genotyp and slaughter weight of lambs (*Bessera et al.* 2004).

Conclusion

This study demonstrated that feeding lambs a diet supplemented with linseed oil significantly increased rumenic acid, CLA and omega 3 fatty acids as essential α-linoleic, eicosapentenoic, doxosahexaenoic acids and decreased n-6/n-3 and LA/ALA ratio in lamb meat. This study demonstrated also that, unlike sunflower oil supplementation of lamb diets had no effect on essential and health beneficial fatty acids in meat of lambs.

Founding

This project was supported by the KEGA grant No. 015SPU – 4/2019 and the VEGA grant No. 1/0364/15.

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