Influence of selenate and linseed oil on fatty-acid and amino-acid profiles in the liver, muscles, fat tissues and blood plasma of sheep

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ABSTRACT

Twenty individually-housed male Polish Merino lambs with an initial body weight of 25±2 kg were used in the experiment. Each group comprised 5 lambs. Sheep were fed the control or experimental diets enriched in 5% linseed oil (LO), 2 ppm selenium as selenate (SE) or combined addition of 5% LO and 2 ppm selenium (LOSE). The effects of the experimental diets on organs and body weight gain (BWG) of the sheep were small, but the LOSE treatment increased BWG and the weight of M. biceps femoris, M. longissimus dorsi, and the liver. The diets enriched in LO with or without SE decreased the concentration of atherogenic and thrombogenic saturated fatty acids in muscles, liver and perirenal fat tissues. The LO treatment increased the concentration of conjugated linoleic acid (CLA) isomers in the subcutaneous and perirenal fat tissues, liver, M. longissimus dorsi and blood plasma in comparison with the control. Dietary LO with or without SE resulted in negligible changes in the concentration of CLA isomers in *M. biceps femoris*. The diet enriched in SE decreased the concentration of CLA isomers in both muscles compared with control muscles, whereas it increased in the plasma, liver and perirenal fat tissue. There was a LO and SE interaction for the CLA isomer concentration in the perirenal fat tissue and plasma; this treatment resulted in an increase of the CLA isomer concentration in these tissues compared with the control. The diets enriched in LO or with LO and SE increased the concentration of polyunsaturated fatty acids (PUFA) in the subcutaneous fat tissue, plasma, and perirenal fat tissue. Feeding the diet with LO increased the concentrations of *cis9cis12*C18:2 and *cis9cis12*C18:3 in the subcutaneous fat tissue, muscles and plasma in comparison with the control. LO supplementation to sheep increased the concentration of essential (Σ E-AA), non-essential (Σ NE-AA) and sulphur (Σ S-AA) amino acids in the muscles; the diet enriched in LO with or without SE decreased Σ E-AA, $\Sigma NE-AA$, $\Sigma S-AA$ and ΣAA in the liver. These diets increased the nutritional value of meats as the ratio of SFA/MUFA and SFA/PUFA decreased, while the concentration of Σ E-AA increased. The diet containing SE with or without LO resulted in an increase in the Se concentration in meat, plasma, and liver compared with the control.

KEY WORDS: linseed oil, selenate, fatty acid, amino acid, muscle, liver, fat tissue, blood plasma, sheep

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INTRODUCTION

The purpose of modifying the fatty acid composition of meat or milk is to produce high-quality products meeting dietary recommendations for increased intake of polyunsaturated fatty acids (PUFA), especially n-3PUFA, and reduced intake of atherogenic and thrombogenic saturated fatty acids (A-SFA and T-SFA). Indeed, many epidemiological and experimental studies (Niedźwiedzka et al., 2006; Murphy et al., 2007) documented that A-SFA (C12:0, C14:0 and C16:0) and T-SFA (C14:0, C16:0 and C18:0) possessed atherogenic and thrombogenic properties, while α -linolenic acid and its products of elongation and desaturation (i.e. long-chain n-3PUFA) improved anti-inflammatory status, immune response, as well as benefited the cardiovascular system by decreasing platelet aggregation, serum triglycerides and cholesterol (Raes et al., 2004; Flachowsky et al., 2006). On the other hand, a higher dietary content of n-6PUFA results in a pro-inflammatory status, therefore, it is recommended to maintain the n-6PUFA/n-3PUFA ratio below 4. Recent investigations documented that elevating the PUFA content in a diet stimulated oxidative deterioration in meat of ruminants and monogastric animals. Considering these arguments, an adequate concentration of n-3PUFA in diets together with antioxidants, like selenium, is essential for the good health of domestic animals and humans.

Diverse inorganic Se compounds, like selenate or selenite, are used as nutritional sources of Se. These compounds are metabolized to intermediates and then utilized for the formation of SeCys SeCystRMA to pair with the codon of Se-Cys for synthesis of proteins containing Se-Cys (e.g., the glutathione peroxidase family, selenoprotein P, or thioredoxin reductase). The principal physiological roles of half of Se-proteins are to maintain the appropriate metabolism of arachidonic acid and low concentrations of pre-oxides or free radicals within cells, thus decreasing oxidative stress in living organisms (Tapiero et al., 2003; Shweizer et al., 2005). Glutathione peroxidases (GPx) in particular provide a second line of defense against hydroperoxides, which can damage lipids in membranes as well as other cell structures. GSH-Px acts synergistically with tocopherol in the regulation of lipid peroxidation. Recent studies documented that phospholipid hydroperoxide GPx, in particular, interacted more directly than cytosolic and mitochondrial GPx in protecting PUFA from peroxidation damage (Crespo et al., 1995; Tapiero et al., 2003). A positive correlation was observed between concentrations of unsaturated FA and the dietary content of Se (Crespo et al., 1995; Tanguy et al., 2003; Yu et al., 2008).

Considering the above, we hypothesized that dietary inorganic Se and a vegetable oil rich in n-3PUFA would improve the nutritive properties of food derived from domestic ruminants. Therefore, the aim of our pilot investigations was to study the influence of a diet enriched in linseed oil (as a precursor of FA

containing conjugated double bonds) and selenate (Se) on the profile of fatty acids (FA) and amino acids (AA) in muscles, liver, fat tissues and blood plasma of sheep.

MATERIAL AND METHODS

Animals and diets

Twenty male Polish Merino lambs with an average initial body weight (BW) of 25 ± 2 kg were allotted to 4 groups of 5 animals and housed individually. During a 1-week preliminary period the animals were given *ad libitum* access to the standard concentrate-hay diet (Czauderna et al., 2004a,b). This diet contains, g: crude protein 120, crude fibre 120 and 11 MJ metabolizable energy in 1 kg DM. The concentration of Se in the standard concentrate-hay diet was 0.1 ppm. After a 1-week preliminary period, the lambs were fed for 35 days the standard concentrate-hay diet (Control), the experimental diets enriched in either 5% linseed oil (LO) or 2 ppm selenium as sodium selenate (SE), or the experimental diet with the combined addition of 5% LO and 2 ppm selenium (LOSE). The fatty acid profile of LO is shown in Table 1. The rations were fed *ad libitum*.

Fatty acids	Concentration, µg/g
C8:0	26.0
C10:0	35.3
C12:0	9.62
C14:0	18.0
C16:0	1405
C18:0	872
C18:3n-3	5892
C18:3n-6	894
C18:2n-6	4962
<i>c9</i> C18:1	4169
<i>c6</i> C18:1	539
CLA isomers ¹	109
SFA	2413
MUFA	4718
PUFA	11749
FA ²	18880

Table 1. The fatty acid composition in linseed oil

the concentration sum of: 1CLA isomers, 2all assayed fatty acids (FA)

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Chemicals

All of the chemicals used for saponification of samples, solvents used for eluting, and Waters HPLC equipment (USA) were as described previously (Czauderna and Kowalczyk, 2002). A CLA isomer mixture (95-97%), and isomers *cis9,trans11* (*c9t11*CLA) (95-97%) and *trans10,cis12* (*t10,c12*CLA) (95-97%) were supplied by Larodan Fine Chemicals AB (Sweden). Other fatty acid standards and sodium selenate (Na₂SeO₄) were provided by Sigma (USA).

Underivatized conjugated linoleic acid (CLA) isomers and other fatty acids containing conjugated double bonds (CFA) in hydrolysates were determined directly according to Czauderna et al. (2003a). Other FA as well as CLA isomers and CFA were derivatized and then quantified according to Czauderna et al. (2002b). Amino acid concentrations in all assayed samples were determined according to Czauderna et al. (2002a).

Amino acid concentrations in muscles and livers were analysed in pooled samples prepared by combination of all livers, *M. biceps femoris* and *M. longissimus dorsi* from sheep fed the same diet.

Statistical analysis

Statistical analyses of the effects of dietary Se or LO on fatty acid concentrations in sheep tissues and amino acids in blood plasma were conducted using the nonparametric Mann-Whitney U test for comparing independent experimental groups, while statistical analyses of interactions between LO and Se were performed using two-factorial ANOVA analysis (LO x Se). Statistica ver. 6 and Microsoft Office Excel software were used (Statistica, 2002). Differences were considered significant at P<0.05.

RESULTS

Dietary administration of LO and/or SE resulted in no lesions or symptoms of harmful effects in sheep fed the experimental diets. Experimental data on feed intake, body weight gain (BWG), feed conversion efficiency (FCE), weight of the liver, *M. biceps femoris* and *M. longissimus dorsi* after 35 days of feeding the experimental diets are summarized in Table 2. Results showed that feeding sheep the diet supplemented with LO slightly increased (P>0.05) the weight of *M. biceps femoris* and *M. longissimus dorsi* as Well as BWG and FCE and consequently decreased feed intake. There was an OL x SE interaction (P=0.039) because sheep fed the diet enriched in OL and SE had increased BWG (P=0.039) and liver (P=0.049) and *M. biceps femoris* (P=0.043) weights. On the other hand, only minute effects of the extra selenate in the diet (SE) on feed intake, FCE, BWG and weight of both muscles were observed, whereas this diet resulted in a more distinctive but not statistically significant decrease in liver weight.

		Gro	oup			ANOVA					
Item			LO ³	LO+SE ⁴	S	statistical effects					
	control	SE^2			S	SE)	OL×SE		
					SEM	Р	SEM	Р	Р		
Feed intake, kg	39.0	38.9	37.1	37.6	0.41	NS	0.46	0.03	NS		
BWG, kg	6.6ª	6.9ª	9.5 ^b	9.2 ^{ab}	1.8	NS	1.1	NS	0.039		
FCE	0.166	0.178	0.255	0.244	0.021	NS	0.020	NS	NS		
Liver, g	618 ^{ab}	563ª	625 ^{ab}	659 ^{ab}	52	NS	31	NS	0.049		
M. biceps femoris, kg	4.1	4.3	4.7	4.6	205	NS	166	NS	0.043		
M. longissimus dorsi, g	371	373	417	385	47	NS	36	NS	NS		

Table 2. Effects of diets on the feed intake, body weight gain (BWG), the conversion efficiency¹ (FCE) and masses of the liver, *M. biceps femoris* and *M. longissimus dorsi* of sheep

¹kg body weight gain/kg feed intake; sheep group fed diet enriched in: ²selenate (2 ppm Se), ³ 5% linseed oil (w/w), ⁴selenate (2 ppm Se) and 5% linseed oil (w/w); NS - P>0.05

The influence of dietary LO and SE on the SFA concentrations in tissues of sheep. The current study also investigated the relationship between the experimental diets and the saturated fatty acids (SFA) in assayed tissues of sheep (Tables 3, 4 and 5). In both muscles, the perirenal fat tissue and the liver, A-SFA

Fatty acids		Gr	oup			Inter- action			
			-		SE		LO		OL×SE
	control	SE	LO	LO+SE	SEM	Р	SEM	Р	Р
M. biceps femor	is								
A-SFA	1.93ª	1.24°	1.35 ^{bc}	1.71 ^{ab}	0.12	NS	0.104	NS	0.002
T-SFA	3.04 ^a	2.01°	2.22 ^{bc}	2.81 ^{ab}	0.18	NS	0.158	NS	0.003
LA^2	1.34 ^{cb}	1.11 ^b	1.60 ^{ab}	1.89ª	0.13	NS	0.114	0.008	NS
γ-LNA ²	0.001ª	0.001ª	0.002ª	0.001ª	0.0002	NS	0.0003	NS	NS
α -LNA ²	0.20 ^b	0.14 ^b	0.23 ^{ba}	0.35ª	0.03	NS	0.025	0.001	0.016
CLA ³	0.35ª	0.18 ^b	0.31ª	0.31ª	0.03	NS	0.03	NS	NS
CFA ³	0.116 ^{ab}	0.091ª	0.073ª	0.156 ^b	0.016	NS	0.015	NS	NS
$\Sigma t C18:1^{4}$	0.119 ^{ab}	0.063°	0.072^{ac}	0.138 ^b	0.013	NS	0.012	NS	0.003
<i>c9</i> C18:1	3.02 ^a	1.92 ^b	1.76 ^b	2.42 ^{ab}	0.19	NS	0.19	NS	0.002
SFA/MUFA ⁵	0.52 ^b	0.63ª	0.38°	0.42°	0.03	0.02	0.02	0.001	NS
SFA/PUFA5	1.99ª	1.62 ^b	1.22°	1.25°	0.10	NS	0.11	0.001	0.046

Table 3. Effects of the diets enriched in linseed oil (LO) and/or selenate (SE) on the concentration (mg/g) of fatty acids (FA) and amino acids (AA) in *M. biceps femoris* of sheep ¹

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		Gro	oup			Inter- action				
Fatty acids							LO		OL×SE	
•	control	SE	LO	LO+SE	SEM	Р	SEM	Р	Р	
ΣFA^5	11.1ª	6.82 ^b	10.5 ^{ab}	12.8ª	0.98	NS	0.77	NS	0.020	
$\sum E-AA^{6}$	298	320	312	293	-	-	-	-	-	
\sum NE-AA ⁶	308	358	321	327	-	-	-	-	-	
cysteine	14.2	15.4	16.3	15.9	-	-	-	-	-	
taurine	56	61	62	55	-	-	-	-	-	
methionine	7.23	11.57	7.27	8.98	-	-	-	-	-	
lysine	51	57	56	50	-	-	-	-	-	
\sum S-AA ⁷	77	88	86	80						
$\sum_{i=1}^{n} AA^{6}$	606	678	633	621	-	-	-	-	-	
M. longissimus	dorsi									
A-SFA	2.43ª	1.67 ^{ab}	1.70 ^b	1.53 ^b	0.19	NS	0.17	NS	NS	
T-SFA	3.80 ^a	2.74 ^b	2.75 ^b	2.55 ^b	0.26	NS	0.25	NS	NS	
LA	1.06 ^a	0.93ª	1.12 ^a	0.79ª	0.07	0.045	0.07	NS	NS	
γ - LNA	0.001	_8	0.001	_8	0.0002	NS	0.0002	NS	NS	
α-LNA	0.13 ^a	0.11 ^a	0.15 ^a	0.11ª	0.01	NS	0.01	NS	NS	
CLA	0.35 ^{ab}	0.20 ^c	0.40 ^a	0.24 ^{bc}	0.03	0.007	0.033	NS	NS	
CFA	0.107 ^a	0.067 ^{ab}	0.066 ^b	0.079 ^{ab}	0.011	NS	0.012	NS	NS	
ΣtC18:1	0.109 ^a	0.090 ^{ab}	0.096 ^{ab}	0.091 ^b	0.008	NS	0.007	NS	NS	
<i>c9</i> C18:1	3.05 ^a	2.28 ^{ab}	2.11 ^b	1.87 ^{ab}	0.25	NS	0.24	NS	NS	
SFA/MUFA	0.67ª	0.70^{ab}	0.37 ^b	0.46 ^b	0.04	NS	0.038	0.001	NS	
SFA/PUFA	3.16 ^a	2.69 ^{ab}	2.16 ^b	2.91 ^{ab}	0.15	NS	0.159	NS	0.019	
ΣFA	11.47ª	8.16 ^a	12.14 ^a	9.52ª	0.81	0.03	0.78	NS	NS	
∑E-AA	267	307	284	282	-	-	-	-	-	
\sum NE-AA	307	347	323	315	-	-	-	-	-	
cysteine	2.46	8.45	9.34	8.96	-	-	-	-	-	
taurine	54	60	57	55	-	-	-	-	-	
methionine	12.57	15.46	14.22	12.60	-	-	-	-	-	
lysine	53	58	52	53	-	-	-	-	-	
∑S-AA	69	84	81	77	-	-	-	-	-	
$\overline{\Sigma}AA$	574	654	608	597	-	-	-	-	-	

Table 3 continued

¹ means in rows with different letters are significantly different at the ^{a,b} P<0.05; ²LA - linoleic acid; α-LNA - α-linolenic acid; γ-LNA - γ-linolenic acid; ³CLA - the sum of CLA isomers; CFA - non-CLA conjugated fatty acids; ⁴Σ*t*C18:1 - the sum of *t6*C18:1, *t7*C18:1, *t9*C18:1 and *t11*C18:1; ⁵ SFAMUFA, SFA/PUFA and ΣFA - the ratio of SFA to MUFA, the ratio of SFA to PUFA and the sum of all FA, respectively; ⁶ the sum of essential (ΣE-AA), non-essential (ΣNE-AA) and all amino acids (ΣAA); ⁷ the sum of methionine, cysteine and taurine; ⁸ below the quantification limit (L₀); ⁹NS - P>0.05 and T-SFA concentrations decreased (usually statistically significantly; P<0.05) with the addition of LO to the diet. Concomitantly with this, acid concentrations decreased, values of the concentration ratios of SFA/MUFA and SFA/PUFA also decreased (P<0.05) in these sheep tissues. Similarly, the diet with extra selenate resulted in a decrease of the A-STA and T-SFA in *M. biceps femoris* (P<0.05), but usually statistically non-significantly in *M. longissimus dorsi* and the liver. Feeding sheep the diet enriched in LO resulted in an increase (P<0.05) in the concentrations of SFA, A-SFA and T-SFA in the subcutaneous fat tissue and blood plasma, whereas changes of values of the concentration ratios of SFA/MUFA and SFA/PUFA in these tissues were inconsistent with changes of the concentrations of SFA, A-SFA and T-SFA; the ratios were usually lower in comparison with those of these fatty acids in the control (Tables 4 and 5). The diet with extra selenate resulted in an increase (P<0.05) in the concentration of A-SFA in blood plasma and subcutaneous fat tissues.

There were LO×SE interaction effects (P<0.01) on the concentrations of A-SFA and T-SFA in *M. biceps femoris* and blood plasma. The simultaneous addition of LO and SE to the diet decreased the A-SFA and T-SFA concentrations in the liver (P<0.05), *M. longissimus dorsi* (P<0.05), while only slightly decreased the concentration of these acids in *M. biceps femoris* and the perirenal fat tissue in comparison with the concentrations in the concentration in the subcutaneous fat tissue (P<0.05), but statistically non-significant in blood plasma compared with the control group.

The influence of dietary LO and SE on the concentrations of CLA isomers and CFA in tissues of sheep. The LO treatment increased (P<0.05) the concentration of CLA isomers in both the fat tissues and liver, whereas it insignificantly elevated it in *M. longissimus dorsi* and blood plasma in comparison with the control tissues. On the other hand, dietary LO, regardless of the presence of extra selenate in the diet, resulted in negligible changes in the concentration of CLA isomers in *M. biceps femoris*. The diet enriched in only SE decreased (P<0.05) the concentration of CLA isomers in both muscles compared with the control, whereas increased (P<0.05) it in the blood plasma, liver and, insignificantly, in the perirenal fat tissue (P=0.02). There was a LO and SE interaction for the CLA isomer concentration in the perirenal fat tissue (P=0.03) and blood plasma (P=0.04); this treatment resulted in an increase of the CLA isomer concentration in these tissues compared with the control.

The effect of dietary LO and/or SE on the CFA concentration was analysed in the assayed tissues of sheep. The LA supplement decreased (P<0.05) the CFA concentration in *M. longissimus dorsi* and tended to decrease it in *M. biceps femoris* (P=0.07), whereas it increased (P<0.05) it in the subcutaneous fat tissue compared

		Gr	oup			Inter- action			
Fatty acids ²		01	oup			SE	Ι	.0	OL×SE
	control	SE	LO	LO+SE	SEM	Р	SEM	Р	Р
Liver									
A-SFA	5.80ª	5.56ª	3.20 ^b	2.78 ^b	0.43	NS	0.42	0.001	NS
T-SFA	12.4ª	11.0 ^{ab}	8.2 ^{ab}	6.60 ^b	1.1	NS	1.0	0.007	NS
LA	2.91ª	3.08 ^a	3.98 ^b	3.15 ^a	0.18	NS	0.32	NS	NS
γ-LNA	0.11ª	0.13ª	0.68 ^b	0.63 ^b	0.08	NS	0.09	0.001	NS
α-LNA	0.32 ^{at}	0.39ª	0.25 ^b	0.25 ^b	0.03	NS	0.03	NS	NS
CLA	1.04 ^a	1.73 ^b	1.57 ^b	0.92ª	0.19	NS	0.18	NS	NS
CFA	2.40 ^a	0.37 ^b	2.36ª	0.84 ^b	0.28	0.003	0.37	NS	NS
Σ <i>t</i> C18:1	0.39ª	0.54 ^{ab}	0.45 ^b	0.39ª	0.03	NS	0.02	NS	0.04
C9C18:1	6.12 ^a	5.70 ^{ab}			0.39	NS	0.37	0.006	NS
SFA/MUFA	0.58ª	0.62ª	0.17 ^b		0.07	NS	0.06	0.001	NS
SFA/PUFA	3.54ª	2.61 ^b	1.78 ^b		0.31	NS	0.34	0.001	NS
ΣFA	41.9ª	37.1ª	66.9 ^b	49.2ª	2.9	0.034	3.7	0.001	NS
∑E-AA	270	282	242	249	-	-	-	-	-
∑NE-AA	281	298	248	262	-	-	-	-	-
cysteine	6.45	3.45	3.55	3.30	-	-	-	-	-
taurine	41	45	38	42	-	-	-	-	-
methionine	9.15	4.73	5.09	4.72	-	-	-	-	-
lysine	42	45	38	40	-	-	-	-	-
∑S-AA	57	53	47	50	-	-	-	-	-
∑AA	550	580	490	511	-	-	-	-	-
Subcutaneous	fat tissue								
SFA	9.89 ^b	26.8ª	29.8ª	32.7ª	3.2	0.03	3.6	0.01	NS
A-SFA	7.02 ^b	18.1ª	17.1ª	19.5ª	1.8	0.01	1.9	0.02	NS
T-SFA	9.59 ^b	26.4ª	29.4ª		3.1	0.03	3.6	0.01	NS
LA	1.34 ^b	6.77ª	7.39		1.0	0.04	1.12	0.02	NS
γ-LNA	0.14 ^a	0.12ª	0.11		0.01	NS	0.01	NS	NS
α-LNA	5.12ª	2.91ª	8.57		0.95	NS	1.03	0.003	NS
CLA	4.79ª	4.26 ^a	6.11		0.64	NS	0.70	NS	NS
CFA	0.10^{b}	0.05 ^b	0.27		0.01	0.01	0.03	0.0004	NS
Σ <i>t</i> C18:1	2.64°	3.40 ^b	4.26		0.48	NS	0.67	0.008	0.048
<i>c9</i> C18:1	26 ^b	37ª	33ª	44 ^a	3	0.04	5	NS	NS
MUFA	77 ^a	84 ^a	169 ^{cb}	157 ^b	3	0.02	9	0.004	NS
PUFA	6.77 ^b	9.94 ^{ab}			1.7	NS	2.1	0.006	NS
SFA/MUFA	0.62ª	0.26 ^b	0.22		0.05	0.001	0.05	0.001	0.001
SFA/PUFA	1.52 ^b	2.87ª	1.97		0.20	0.01	0.09	NS	0.006
$\Delta 9$ -index ³	0.75 ^{ab}	0.83ª	0.72	2 ^b 0.76 ^{ab}	0.02	0.046	0.02	NS	NS

Table 4. Effects of the diets enriched in linseed oil (LO) and/or selenate (SE) on the concentration (mg/g) of fatty acids (FA) and amino acids (AA) in the liver and subcutaneous fat tissues of sheep¹

¹ means in rows with different letters are significantly different at ^{a,b}P<0.05

² abbreviations for fatty acids and other items see Table 1

³ the $\Delta 9$ - desaturase index - the concentration ratio: *cis*-9C18:1/(C18:0+*cis*-9C18:1)

⁴NS - P>0.05

		C				Inter- action			
Fatty acids ²		G	roup			SE	LC)	OL×SE
	control	SE	LO	LO+SE	SEM	P	SEM	Р	P
Perirenal fat									
SFA	23.3ª	22.9ª	17.3ª	17.6 ^a	1.4	NS	1.3	0.02	NS
A-SFA	12.9ª	14.0 ^a	10.2 ^{ab}	8.89 ^b	0.91	NS	0.71	0.005	NS
T-SFA	20.9ª	20.0ª	15.8 ^b	16.9ª	1.2	NS	1.2	NS	NS
LA	2.90 ^b	4.70 ^a	3.00 ^b	4.10 ^{ab}	0.35	0.01	0.254	NS	NS
γ-LNA	0.061ª	0.032 ^b	0.030 ^b	0.031 ^b	0.006	NS	0.006	NS	NS
α-LNA	0.34ª	0.20 ^b	0.16 ^b	0.19 ^b	0.03	NS	0.032	0.04	NS
CLA	1.62 ^b	2.70 ^{ab}	3.05ª	2.41 ^{ab}	0.22	NS	0.23	NS	0.03
CFA	_3	0.037ª	_3	0.306 ^b	0.04	0.001	0.04	0.001	0.01
Σ <i>t</i> C18:1	1.59 ^b	2.33ª	1.64 ^b	1.82 ^{ab}	0.14	0.049	0.11	NS	NS
<i>c9</i> C18:1	17.3 ^b	31.4ª	13.5 ^b	19.5 ^b	2.3	0.001	1.341	0.01	NS
MUFA	79ª	81 ^a	106 ^b	84^{ab}	7	NS	7	NS	NS
PUFA	3.61ª	5.38ª	4.30 ^a	5.58ª	0.43	0.03	0.37	NS	NS
SFA/MUFA	0.31ª	0.29 ^{ab}	0.16 ^c	0.22 ^{bc}	0.02	NS	0.02	0.001	NS
SFA/PUFA	6.71ª	4.40 ^b	4.09 ^b	3.37 ^b	0.51	0.02	0.51	0.006	NS
$\Delta 9$ -index	0.63 ^b	0.78ª	0.65 ^b	0.69 ^b	0.02	0.001	0.014	NS	0.03
Blood plasma	!								
SFA	10.1°	30.6ª	21.7 ^b	16.0 ^{cb}	2.6	0.01	1.8	NS	0.01
A-SFA	5.17 ^b	15.1ª	8.20 ^b	6.48 ^b	1.31	0.002	0.63	0.03	0.01
T-SFA	8.87°	29.3ª	20.6 ^b	15.2°	2.6	0.003	1.8	NS	0.01
LA	2.23 ^b	7.14 ^a	6.08 ^a	3.73 ^b	0.68	NS	0.58	NS	0.01
γ - LNA	0.07^{a}	0.11^{ab}	0.28 ^{bd}	0.13 ^b	0.01	0.01	0.05	0.001	0.01
α-LNA	0.50ª	0.52^{ab}	0.57 ^b	0.72 ^b	0.03	NS	0.04	0.001	0.01
CLA	1.17 ^b	4.74 ^a	2.32 ^{ab}	2.06 ^{ab}	0.66	NS	0.24	NS	0.04
CFA	_3	_3	_3	-3	-	-	-	-	-
Σ <i>t</i> C18:1	2.18 ^b	2.04 ^b	9.60ª	6.57ª	0.78	NS	1.15	0.001	NS
<i>c9</i> C18:1	2.99 ^b	10.2ª	4.88 ^b	3.95 ^b	1.10	0.02	0.41	NS	0.01
MUFA	6.18 ^a	5.37ª	16.9 ^b	12.7 ^b	7.1	0.01	1.7	NS	0.01
PUFA	2.83°	9.77 ^{ab}	12.3ª	7.42 ^b	0.98	NS	1.25	0.01	0.01
SFA/MUFA	1.67 ^a	0.73 ^b	1.37 ^a	1.31ª	0.13	0.01	0.10	NS	0.02
SFA/PUFA	3.71ª	3.18 ^a	1.77 ^b	2.31 ^b	0.19	NS	0.24	0.001	0.02
$\Delta 9$ -index	0.41ª	0.40^{a}	0.27 ^b	0.32 ^{ab}	0.02	NS	0.02	0.01	NS
ΣFA	26.1ª	92.9 ^b	53.2°	82.6°	10.1	0.003	4.3	NS	0.001
∑E-AA	0.21ª	0.18 ^a	0.23ª	0.20ª	0.011	NS	0.014	NS	NS
∑NE-AA	0.33ª	0.35 ^{ab}	0.42 ^b	0.39 ^{ab}	0.015	NS	0.02	NS	NS

Table 5. Effects of the diets enriched in linseed oil (LO) and/or selenate (SE) on the concentration (mg/g) of fatty acids (FA) and amino acids (AA) in the perirenal fat tissue and plasma blood of sheep¹

continued on the next page

Fatty acids ²		Gro	oup		S	Inter- action			
			1		S	E	LO		OL×SE
	control	SE	LO	LO+SE	SEM	Р	SEM	Р	Р
taurine	0.023 ^{ab}	0.026^{a}	0.031^{b}	0.025ª	0.001	NS	0.001	NS	NS
homo-cysteine	0.0001°	0.016 ^a	0.012 ^b	0.010^{b}	0.001	0.001	0.001	NS	NS
methionine	0.008ª	0.007^{a}	0.008 ^a	0.007 ^a	0.001	NS	0.001	NS	NS
Se-cysteine	0.0010°	0.015ª	0.0012 ^b	0.015 ^a	0.0002	0.001	0.002	0.001	0.01
lysine	0.031ª	0.030ª	0.042 ^b	0.037 ^b	0.002	NS	0.003	NS	NS
\sum S-AA ⁴	0.031ª	0.049 ^b	0.051 ^b	0.042ª	0.002	0.001	0.004	0.003	0.01
∑AA	0.54ª	0.53 ^{ab}	0.65 ^b	0.59 ^{ab}	0.026	NS	0.034	NS	NS

Table 5. continued

¹ means in rows with different letters are significantly different at ^{a,b} P<0.05

 2 abbreviations for fatty acids and other items see Tables 1 and 2; ³ the concentration of an assayed compound was below the quantification limit (L_Q); ⁴ the concentration sum of methionine, taurine and *homo*-cysteine; ⁵ NS - P>0.05

with the control. The perirenal fat tissue and blood plasma CFA was undetectable in these tissues of sheep fed the control diet and the diet enriched in LO. The diet containing LO and SE resulted in a decrease (P<0.05) in the CFA concentration in the liver and, but not significantly, in *M. longissimus dorsi* (P=0.17) compared with the control tissues. There was an LO and SE treatment interaction (P<0.01) for the CFA concentration in the perirenal fat tissue. The SE supplement decreased (P<0.05) CFA concentrations in the liver and subcutaneous fat tissue (P=0.01), whereas they decreased, but not significantly, in *M. longissimus dorsi* (P=0.17) and *M. biceps femoris* (P=0.41).

The influence of dietary LO and SE on the concentrations of monounsaturated fatty acids in tissues of sheep. In the present study, supplemental LO decreased (P < 0.05) the concentration of c9C18:1 in both muscles, liver and, but not significantly, in the perirenal fat tissue compared with the control. On the other hand, this diet increased (P < 0.05) the c9C18:1 concentration in the subcutaneous fat tissue and, insignificantly (P=0.11), in plasma compared with the control. There was an LO and SE treatment interaction for the c9C18:1 concentration in M. biceps *femoris* (P=0.002) and blood plasma (P=0.01). The diet enriched in SE decreased the concentration of this acid in *M. biceps femoris* (P<0.05), but decreased it, insignificantly however, in the liver and *M. longissimus dorsi*. On the other hand, this diet resulted in an increase (P<0.05) in the c9C18:1 concentration in both fat tissues and blood plasma. Supplemental LO increased the sum of *trans* octadecenoic acid concentrations ($\Sigma tC18:1$) in the liver, subcutaneous fat tissue, plasma and, but not significantly, in the perirenal fat tissue compared with the control. The diet enriched in LO resulted in an increase (P<0.05) in the concentration of MUFA in the fat tissues, plasma, as well as decreased (P<0.05) the concentration ratio of SFA/MUFA in both muscles, the liver, both fat tissues and, but not significantly, in plasma compared with the control. There was an LO and SE treatment interaction for the ratio of SFA/MUFA in the subcutaneous fat tissue (P=0.001) and plasma (P=0.02); the diet enriched in LO and SE resulted in a decrease of this ratio in all assayed tissues compared with the control group.

The influence of dietary LO and SE on the concentrations of polyunsaturated fatty acids in tissues of sheep. The diets enriched in LO or simultaneously with LO and SE increased (P<0.05) the concentration of PUFA in the subcutaneous fat tissue, blood plasma and, not significantly, in the perirenal fat (P=0.13 and 0.09, respectively). Feeding the diet containing LO increased the concentrations (P<0.05) of *cis9,cis12*C18:2 (LA; *c9,c12*C18:2) and *c9,c12,c15*C18:3 (α-LNA) in the subcutaneous fat tissue, blood plasma and, not significantly, in both muscles in comparison with the control tissues. On the other hand, the diet enriched in LO, regardless of the presence of SE, decreased (P<0.05) the concentration of α -LNA in the perirenal fat tissue and, not significantly, in the liver. The experimental diets also changed the concentration of c6, c9, c12C18:3 (y-LNA), however, a significant increase (P<.05) of the γ -LNA concentration was found in the liver and blood plasma of lambs fed the diet enriched in LO, regardless of the presence of SE. Dietary LO resulted in a decrease (P<0.05) in the concentration ratio of SFA/PUFA in both muscles and fat tissues, as well as in the liver and blood plasma compared with the control. Similarly, adding SE to the diet enriched in LO decreased the SFA/ PUFA ratio in all assayed tissues compared with the control, however, these changes were in some cases not significant (P>0.05). The two-factorial interaction between LO and SE was usually significant (P<0.05) for this ratio of fatty acids in assayed tissues, with the exceptions of the perirenal fat tissue and liver. The diet enriched in SE resulted in an increase (P<0.05) in the concentration of PUFA in the blood plasma and tended to increase in the perirenal fat tissue (P=0.09) and subcutaneous fat tissue (P=0.08) in comparison with the control. This diet decreased the ratio of SFA/PUFA in the liver (P < 0.05), both fat tissues (P < 0.05), M. biceps femoris (P<0.05), M. longissimus dorsi (P=0.07), and, but non-significantly, in the blood plasma (P=0.21).

As can be seen from the data presented in Tables 4 and 5, the diets enriched in LO, regardless of the presence of SE, did not significantly change the yield of Δ 9-desaturation in both fat tissues. On the other hand, SE supplementation increased the yield of Δ 9-desaturation in the perirenal fat tissue (P<0.05) and subcutaneous fat tissue (a tendency: P=0.09). As expected, the higher yield of Δ 9-desaturation in both fat tissues resulted in an increase (P<0.05) in the concentrations of *c*9C18:1 and LA in them.

The diet enriched in LO increased (P<0.05) the concentration sum of all assayed fatty acids (Σ FA) in the liver and blood plasma compared with the control. Similarly,

SE supplementation resulted in a decrease in the Σ FA concentration in *M. biceps femoris* (P<0.05), *M. longissimus dorsi* (P=0.11), the liver, both muscles, whereas it increased the concentration of Σ FA in blood plasma (P<0.05).

The influence of dietary LO and SE on the concentrations of amino acids in tissues of sheep. As can be seen from the data summarized in Tables 3, 4 and 5, the experimental diets generated substantial changes in amino acid (AA) concentrations in both muscles, the liver and blood plasma. AA concentrations in the muscles (Table 3) and liver (Table 4) were analysed in pooled samples prepared by combination of all livers, M. biceps femoris and M. longissimus dorsi from sheep fed the same diet. SE supplementation resulted in an increase in the sum of essential amino acids (Σ E-AA), non-essential amino acids (Σ NE-AA) and sulphur amino acids (Σ S-AA) in both muscles and the liver. On the other hand, this diet showed a negligible influence (P>0.05) on the concentrations of Σ E-AA, Σ NE-AA and the sum of all amino acids (Σ AA) in blood plasma (Table 5). LO administered to sheep also increased the concentrations of Σ E-AA, Σ NE-AA and Σ S-AA in both muscles (Table 3), as well as significantly increasing Σ NE-AA, Σ S-AA and ΣAA in the blood plasma, whereas the diet enriched in LO, regardless of the presence of SE, decreased Σ E-AA, Σ NE-AA, Σ S-AA and Σ AA in the liver. From the data shown in Tables 3, 4 and 5, it results that the concentration of cvsteine increased in the muscles of sheep fed the diet enriched in SE and/or LO and decreased in the liver. The concentration of this amino acid was below the quantification limit in blood plasma of sheep fed the control and experimental diets. The diet containing SE increased the concentration of methionine in both muscles, whereas all experimental diets decreased the concentration of this amino acid in the liver. The results presented in Tables 3, 4 and 5 demonstrate that the concentration of lysine in the muscles and liver was slightly increased by SE supplementation, and significantly increased (P < 0.05) in the blood plasma of sheep fed the diets enriched in LO, regardless of the presence of SE.

DISCUSSION

In the current study and our previous investigations (Czauderna et al., 2004a,b), no macroscopic lesions or toxic symptoms of dietary 5% LO and 2 ppm selenium (as selenate) were observed in sheep fed diets enriched in LO and/or SE. Diets containing up to 2 mg selenium per kg would not be toxic for animals, especially for ruminants (Tinggi, 2003; McDowell et al., 2005). Furthermore, in contrast to selenide and selenite, selenate is less reactive and assimilative; consequently, selenate is also less toxic for animals and humans. Therefore, selenium, as selenate, is a significantly better tolerated chemical form of selenium for livestock compared with dietary selenite or selenide and seleno-cysteine, in particular. Only chronic dietary inorganic seleno-compounds, especially selenate and selenide, at rates of more than 5 mg Se per kg can be hepatotoxic and teratogenic in humans and animals (Tapiero et al., 2003; Tinggi, 2003). The LD₅₀ is about 5 mg selenium per kg body weight for animals, thus, this corresponds to about 50 mg Se/kg.

The present trial demonstrates the ability of supplemented LO to increase BWG of examined sheep as well as the weight of *M. biceps femoris* and *M. longissimus dorsi*. Moreover, no negative effect on BWG, FCE and weight of the liver and both muscles was found in sheep fed the diet enriched in LO and SE. Values of FCE and BWG summarized in Table 2 document that the diet enriched in LO most efficiently stimulated the efficiency of animal production. Similarly, the diet containing LO and SE effectively increased the body weight of sheep. Thus, our current study is consistent with our previous works (Czauderna et al., 2003b, 2004c; Korniluk et al., 2007) showing that diets enriched in selenium (as organic or inorganic seleno-compound) and a precursor of FA containing conjugated double bonds had a similar effect on FCE and BWG of such monogastric animals as laboratory rats (Czauderna et al., 2003b; Korniluk et al., 2007).

The results show that the experimental diets improved the nutritional quality of M. biceps femoris, M. longissimus dorsi and the liver, as the concentrations of A-SFA, T-SFA in these tissues decreased compared with the controls. A similar effect was found in the perirenal fat tissue when sheep were fed a diet enriched in LO, regardless of the presence of extra selenate. Considering the above results, it can be concluded that these dietary additives possess antiatherogenic properties. Moreover, the diet enriched in LO, regardless of the presence of extra selenate, improved the ratio of SFA/MUFA and SFA/PUFA in both muscles, and in the liver and perirenal fat tissue. As can be seen from the results summarized in Tables 3, 4 and 5, improvement in the nutritional value of these tissues correlated with increasing concentrations of MUFA and PUFA in the liver and muscles (Czauderna et al., 2004a,b), both fat tissues, and blood plasma. Selenate supplementation to the LO-enriched diet slightly deteriorated the ratio of SFA/MUFA and SFA/PUFA in both muscles. According to our previous studies (Czauderna et al., 2008), an increase in the ratio of SFA/MUFA and SFA/PUFA in muscles may be related to the higher yield of biohydrogenation in the rumen of sheep fed diets containing selenate. Our recent *in vitro* studies documented that addition of selenate to ruminal fluids decreased the concentrations of CLA isomers and t/lC18:1 in fluids in comparison with the control fluid (Wasowska et al., 2006). In accordance with our recent studies, a decrease in the concentration of CLA isomers and their metabolites (CFA, i.e.: c6,c9,c11C18:3, c6,t10,c12C18:3, c8,c11,t13C20:3, c8,t12,c14C20:3, c5, c8, c11, t13 C20:4 or c5, c8, t12, c14 C20:4) was found in both muscles and the subcutaneous fat tissue of sheep fed the diet enriched in selenate. Our results

regarding the abundance of conjugated fatty acids in the liver are also consistent with these findings. Indeed, we found a decrease in the concentration of CLA isomer metabolites in the liver of sheep fed the diet containing selenate. Moreover, the concentration of CLA isomers and their metabolites in this organ of sheep fed the diet enriched in LA and SE was compared with the control group and the group of sheep fed the diet with LO. The increase in CLA isomer concentrations in the liver, *M. longissimus dorsi*, both fat tissues, and blood plasma led us to suggest that the yield of the biohydrogenation of formed CLA isomers in the rumen was reduced by LO supplemented to the diet compared with the control sheep. Addition of Se to the diet enriched in LO elevated the yield of biohydrogenation of CLA isomers in the rumen compared with sheep fed the diet containing LO.

As can be seen from data summarized in Tables 3, 4 and 5 and our recent studies, LO supplementation resulted in a decrease in Δ 9-desaturation in both muscles, the liver, blood plasma and subcutaneous fat tissue (Czauderna et al., 2004a,b). Considering the above, it can be concluded that unsaturated fatty acids contained in dietary LO lowered the yield of desaturation of FA in tissues of sheep compared with the control animals. SE added to the diet with LO slightly elevated the yield of Δ 9-desaturation (a positive interaction) in the tissues of sheep compared with sheep fed the diet enriched in LO. In accordance with these results, we found that selenate supplementation increased the yield of Δ 9-desaturation in all assayed tissues (Czauderna et al., 2004a,b) compared with sheep fed the diet containing only LO.

The results from this study indicate that the Σ S-AA concentration in *M*. *biceps femoris*, *M. longissimus dorsi* and blood plasma increased with LO and/ or SE supplementation. The finding that these diets fed to sheep increased the concentrations of Σ E-AA, methionine and taurine in both muscles (Table 3) is valuable for nutritionists in the context of research on improving the nutritional quality of meat of ruminants for human health.

CONCLUSIONS

Feeds enriched in linseed oil, regardless of the presence of selenate, can be permanently used to increase the concentration of fatty acids containing conjugated double bonds (e.g., CLA isomers) in tissues of ruminants without adversely influencing performance. The experimental diets enriched in LA with or without selenate increased the nutritional value of *M. biceps femoris* and *M. longissimus dorsi* as the ratio of SFA/MUFA and SFA/PUFA decreased, as well as increased the concentration of essential amino acids (i.e.: histidine, valine, leucine, *iso*-leucine, lysine, methionine, threonine and phenylalanine). Moreover, the diet containing selenate, regardless of the presence of LO, resulted in a \sim 2-fold increase in the Se concentration in *M. biceps femoris*, *M. longissimus dorsi*, blood plasma and \sim 10-fold in the liver of sheep compared with the control animals. Therefore, the results of our short-term study constitute important information for nutritionists carrying out further investigations to improve the nutritional quality of feed for ruminants and humans. On the other hand, further research is necessary to determine if dietary selenate and other vegetable oils induce changes in profiles of fatty acids and amino acids in ruminant meat that are more beneficial to human health.

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