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# Dietary linseed oil and selenate affect the concentration of fatty acids and selenium in the spleen, pancreas, and kidneys of lambs

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ABSTRACT

The purpose of the investigation was to determine the short-term influence of the addition of linseed oil (LO) and Se as selenate (SeVI) on the concentration of fatty acids (FA), especially conjugated linoleic acid (CLA) isomers, in the spleen, pancreas, and kidneys of lambs. The experiment was carried out on 20 lambs (25±2 kg) allotted to 4 groups of 5 animals each. For 35 days the lambs were fed a basal diet composed of concentrate and hay (control group; C) or supplemented with: 5% linseed oil (group LO), 2 ppm Se as SeVI (group SE); combined addition of LO and SeVI (group LOSE). The results showed that short-term feeding of lambs with the diet enriched with LO, irrespective of the presence of SeVI, increased body weight gain and feed conversion efficiency compared with the control and SE groups. Feeding LO and SeVI most effectively stimulated the accumulation of Se in the spleen and pancreas. The diet with LO most efficiently increased the level of *cis9trans11*CLA (*c9t11*CLA), the sum of *ct/tc*CLA, *tt*CLA, all CLA isomers, and FA containing conjugated double bonds in the spleen and pancreas. The *c9t11*CLA/*t10c12*CLA ratio was highest in all assayed organs of lambs fed the diet with SeVI. The diet with LO, irrespective of the presence of SeVI, increased the concentration sum of monounsaturated FA and all FA in all organs compared with the C and SE groups. The LO and LOSE treatments decreased the values of the atherogenic and thrombogenic indexes in the spleen and pancreas compared with the control and SE groups and increased the concentration of polyunsaturated FA in all organs as compared with the same groups. Feeding lambs the diet enriched in LO or SeVI increased the value of the Δ9-desaturase index in the pancreas compared with the control and LOSE groups, while decreased values of this index were found in the spleen and kidneys of lambs fed the LO, SE, or LOSE diet. Our results of feeding diets with LO and SeVI constitute useful information for nutritionists carrying out further investigations to improve performance and the nutritional quality of feed for ruminants.

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## INTRODUCTION

The different geometric and positional isomers of conjugated linoleic acid (CLA) differ in their influence on the health of mammals (Belury, 2002; Park and Pariza, 2007; Park, 2009; Husvéth et al., 2011). The *cis9trans11*CLA (*c9t11*CLA) isomer possesses anti-carcinogenic properties (Ip et al., 1999; Whitlock et al., 2006), while the *trans10cis12*CLA (*t10c12*CLA) isomer decreases the body fat content of animals in a dose-dependent fashion (Petersen et al., 2002; Park and Pariza, 2007). The significant physiological effects of CLA isomers on mammals have stimulated efforts to establish methods of increasing the concentrations of CLA isomers in the body of mammals. The CLA isomer concentration in ruminants is higher compared with non-ruminants, while among ruminants, ovine tissues are characterized as having the highest CLA isomer content, especially *c9t11*CLA (Szumacher-Strabel et al., 2004). Previous studies demonstrated that the CLA isomer content in the body of sheep is altered by oil supplemented to the diet (Czauderna et al., 2004a,b; Raes et al., 2004; Szumacher-Strabel et al., 2004; Niedźwiedzka et al., 2008). Moreover, in numerous studies it has been found that diets enriched with vegetable oils, fish oils, or fish meal rich in polyunsaturated fatty acids (PUFA) decreased the content of saturated fatty acids (SFA) in the body of ruminants and their milk and increased the content of valuable monounsaturated fatty acids (MUFA) (*cis*-MUFA and *trans9*C18:1 or *trans7*C18:1), CLA isomers, other PUFA, and non-CLA fatty acids containing conjugated double bonds (CFA) (Raes et al., 2004; AbuGhazaleh, 2008; Cho and Kim, 2011; Kupczyński et al., 2011). Linseed oil (LO) should be incorporated into animal diets because of its unique fatty acid composition, including especially a high level of essential linolenic acid (*c9c12c15*C18:3; αLNA). Our previous studies documented that diets enriched with LO resulted in an increase in the level of unsaturated fatty acids (UFA), especially PUFAn-3 and sum of CFA (ΣCFA) in the liver, adipose tissues, and muscles of sheep (Czauderna et al., 2004a,b; Niedźwiedzka et al., 2008). Importantly, dietary αLNA is the essential substrate (a precursor) for biosynthesis of long-chain PUFA n-3 (LPUFAn-3) in animal and human tissues (Niedźwiedzka et al., 2008). Fortunately, increasing the concentration of LPUFA, especially LPUFAn-3, and decreasing the content of atherogenic and thrombogenic SFA (A-SFA and T-SFA) in the body of ruminants and food derived from them, without lowering their organoleptic quality, is an effective way of helping humans to meet nutritional guidelines recommending increasing consumption of unsaturated fatty acids (Ulbricht and Southgate, 1991;

Murphy et al., 2007). Unfortunately, numerous studies have indicated that an increase in the concentration of UFA, especially PUFA, in tissues stimulates oxidative stress in animals and humans (Devasagayam et al., 2003; González and Tejeda, et al., 2007). Considering these facts, an adequate content of LO in diets together with antioxidants, like selenium (Se) compounds, is crucial for the good health of farm animals and humans (Tapiero et al., 2003; Burk and Hill, 2005; Navarro-Alarcon and Cabrera-Vique, 2008). Indeed, Se compounds have the ability of removing reactive oxygen species and radicals (Rayman, 2004; Burk and Hill, 2005). Half of the known seleno-proteins have been implicated in antioxidant functions, and seleno-cysteine is in the active centers of Se-enzymes that carry out redox reactions (Tanguy et al., 2003; Suzuki, 2005). Consequently, in numerous studies on animals it was found that the level of PUFA in their tissues was positively correlated with the content of Se in the diet (Crespo et al., 1995; Tanguy et al., 2003; Yu et al., 2008). Thus, we hypothesized that the addition of selenate, an unreactive inorganic Se compound, to a diet enriched with LO would stimulate the accumulation of UFA in the internal organs of sheep. Considering the above, the aim of current study was to investigate the short-term effect of a diet enriched with selenate (SeVI), as an antioxidant, and LO on the concentrations of fatty acids, Se, Zn, Fe, Ca and Mg in the spleen, pancreas, brain and kidneys of sheep. These organs were selected for investigation as they are physiologically important for mammals.

## MATERIAL AND METHODS

### *Animals, housing, diets and experimental design*

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. The lambs were housed and handled in accordance with protocols approved by the Local Animal Care and Use Committee. During a one-week preliminary period the lambs were given *ad libitum* access to a standard concentrate-hay diet with a vitamin and mineral premix (the basal diet; Table 1). This basal diet contained: 120 g crude protein, 120 g crude fibre, and 11 MJ metabolizable energy per kg dry mater (DM). The total concentration of Se in the basal diet was 0.1 ppm. After the preliminary period, for 35 days the lambs were fed the basal diet (control group), the basal diet enriched in either 5% LO (group LO) or 2 ppm Se as sodium selenate (SeVI) (group SE), or the experimental diet with the combined addition of 5% LO and 2 ppm Se as SeVI (group LOSE). The fatty acid (FA) profile of LO and the other components of the diet are shown

in Table 1. The diets were adjusted weekly to ensure *ad libitum* access to feed; the lambs were weighed weekly. The animals were slaughtered at the end of the 35-day experiment. The spleen, pancreas, and kidneys were removed, weighed and frozen. Data on the relative body weight gain (BWG, %) of lambs, feed

Table 1. The chemical composition of a concentrate, hay, vitamins and a mineral mixture in the basal diet (C) and linseed oil (LO) fed to lambs

| Measured analyte | Meadow hay[6] | Concentrate | | | Linseed oil |
|---|---|---|---|---|---|
| | | barley meal | soyabean meal | wheat starch | |
| The basal diet[1,2] composition, g/kg D M | 360 | 360 | 165 | 90 | - |
| *The chemical composition of the basal diet, g/kg* | | | | | |
| dry matter (DM) | 914.8 | 875.1 | 911.8 | 873.2 | - |
| crude protein | 132.0 | 95.6 | 450.8 | 8.8 | - |
| crude fibre | 245.9 | 42.7 | 60.9 | - | - |
| crude fat | 32.9 | 15.1 | 23.1 | 0.87 | - |
| ash | 62.6 | 18.0 | 63.3 | 1.2 | - |
| *Fatty acid, mg/kg DM* | | | | | |
| C18:0 | 25.3 | 43.7 | 67.9 | 39.2 | 872 |
| C20:0 | 5.31 | 1.07 | 2.98 | 0.54 | 26.8 |
| C22:0 | 5.85 | 1.03 | 3.56 | 0.28 | 20.1 |
| SFA | 278 | 222 | 388 | 110 | 2413 |
| A-FA | 208 | 160 | 305 | 64.6 | 1432 |
| T-FA | 230 | 197 | 372 | 93.8 | 2295 |
| αLNA | 1.60 | 1.72 | 0.58 | 4.36 | 5892 |
| γLNA[3] | 113 | 105 | 315 | 15.9 | 894 |
| *C9c12*C18:2[4] | 161 | 294 | 807 | 44.0 | 4962 |
| *C*6C18:1 | 4.50 | 130 | 64.2 | 108 | 539 |
| *C*9C18:1 | 41.0 | 88.1 | 209 | 46.7 | 4169 |
| MUFA | 50.3 | 221 | 274 | 157 | 4718 |
| PUFA | 276 | 401 | 1123 | 64.3 | 11749 |
| ΣFA[5] | 681 | 856 | 1880 | 339 | 19070 |

[1] 25 g/kg DM of the basal diet; 1 kg of vitamin and mineral mixture containing; IU/kg: vitamins: A 500.000, $D_3$ 125.000, E as α-tocopherol 25.000; mg: Co as carbonate 42, I as iodate 10, Se as sodium selenite 6; g: Ca 285, P 16, Na 56, Fe as sulphate 1, Cu as cupric sulphate 0.5, Mn as sulphate 5.8, Zn as sulphate 7.5; [2] the basal diet contains; g: crude protein 120, crude fibre 120, 11 MJ metabolizable energy in 1 kg dry mater (DM); [3] *C6c9c12*C18:3 (γ-linolenic acid; αLNA)
[4] linoleic acid (LA); [5] the sum of all assayed fatty acids; [6] metabolizable energy in 1 kg DM of hay: 10.59 MJ/kg

conversion efficiency (FCE; kg body weight gain/kg feed intake), mean body weight gain per day (BWG/day), and mass of removed spleens, pancreases, and kidneys after 35 days of feeding the experimental diets are summarized in Table 2. For quantification of fatty acids and elements (Se, Zn, Mg, Ca and Fe), samples of spleen, pancreas and kidneys were lyophilized and the obtained residues were

stored in sealed tubes at -20°C until analysed. The concentrations of all fatty acids and elements were calculated from lyophilized spleen, pancreas, and kidney samples.

Table 2. Effect of dietary LO and SeVI on the feed intake (kg/group), the body mass gain (BMG, %)[1], the feed conversion efficiency (FCE)[2], the mean body mass gain/day (BMG/day), the concentration of Se, Zn, Mg, Ca and Fe of whole blood (µg/g), average mass[3] of the spleen, pancreas and kidneys of lambs

| Parameters | Groups[4] | | | |
|---|---|---|---|---|
| | C | LO | SE | LOSE |
| Feed intake, kg/group | $39.0^{a}$ | $37.1^{a}$ | $38.9^{\alpha}$ | $37.6^{\alpha}$ |
| BMG, % | $27.5^{a}$ | $38.9^{a}$ | $27.8^{\alpha}$ | $35.7^{\alpha x}$ |
| FCE | $0.166^{a}$ | $0.255^{a}$ | $0.178^{b}$ | $0.244^{b}$ |
| BMG/day[5] | $0.189^{a}$ | $0.271^{a}$ | $0.197^{\alpha}$ | $0.263^{b\alpha}$ |
| Spleen, g/kg | 2.52 | 2.60 | $2.46^{\alpha}$ | $2.54^{\alpha}$ |
| Pancreas, g/kg | $1.24^{a}$ | $0.97^{a}$ | $1.23^{\alpha}$ | $1.06^{\alpha}$ |
| Kidneys, g/kg | $2.91^{\alpha}$ | $2.86^{\alpha}$ | $3.06^{\beta}$ | $2.86^{\beta}$ |
| Blood[6] | | | | |
| Se | $0.65^{a}$ | $0.55^{\alpha}$ | $0.93^{a}$ | $0.84^{\alpha x}$ |
| Zn | 8.71 | 9.63 | 8.76 | 8.78 |
| Mg | 115 | 103 | 105 | 99 |
| Ca | $456^{\alpha}$ | 358 | $325^{\alpha}$ | 350 |
| Fe | 1622 | 1712 | 1656 | 1650 |

[1] BMG, % = $(m_{35days} - m_{initial})*100))/m_{initial}$, where: $m_{35days}$ - the average body mass of sheep fed the experimental diets for 35 days of study; $m_{initial}$ - the average initial body mass (kg); [2] kg body mass gain/kg diet intake; [3] masses derived from fresh internal organs per 1 kg of final mass of lambs; [4] C - the basal diet composed of concentrate and hay; LO - the diet with 5% linseed oil; SE - the diet with 2 ppm Se as SeVI; LOSE - the diet with combined addition of LO; and SeVI; [5] kg mean body mass gain per day (BMG/day = $(m_{35days} - m_{initial})/35$); [6] freeze-dried samples of whole blood

*Chemicals*

HPLC-grade acetonitrile and n-hexane were purchased from Lab-Scan (Dublin, Ireland), other reagents were of analytical grade (POCh, Poland). A CLA isomer mixture, *c9t11*CLA, *t10c12*CLA and other fatty acid standards and sodium selenate ($Na_2SeO_4$) were provided by Sigma (USA). Acetone, dichloromethane, glacial acetic acid, methanol were purchased from POCh (Gliwice, Poland). Linseed oil was provided by APA Polska, Kobylnica near Poznań (Poland). All other chemicals were of analytical grade and organic solvents were of HPLC grade. Water used for the preparation of mobile phases and chemical reagents was prepared using an Elix™ water purification system (Millipore, Canada). The mobile phases were filtered through a 0.45 µm membrane filter (Millipore) and then de-gassed for 2-3 min *in vacuum* with utrasonication prior to use.

*Chromatographic equipment and analytical methods*

A Waters (USA) HPLC 625LC system for quantification of underivatized CLA isomers and other CFA was used. The system comprised a 515 pump, a 712 WISP autosampler, a Waters 996 photodiode array detector and two ion-exchange columns loaded with silver ions (250 × 4.6 mm Chrompac ChromSpher 5 μm Lipids columns; the Netherlands) in conjunction with a 10 × 3 mm guard column. CLA isomers and other CFA in hydrolysates were determined directly according to Czauderna et al. (2003).

Base- and acid-catalyzed methylation was introduced for preparation of methyl esters of other fatty acids (FA) in processed biological samples (Czauderna et al., 2007). FA methyl esters (FAME) were then quantified using gas chromatography according to Czauderna et al. (2009). The analyses of all FAME were performed on a SHIMADZU GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica capillary column (120 m × 0.25 mm i.d. × 0.25 μm film thickness; SHIM-POL, quadrupole mass selective (MS) detector (Model 5973N) and injection port. FAME identification was validated based on electron impact ionization spectra of FAME and compared with authentic FAME standards and the NIST 2007 reference mass spectra library.

Selenium (*via* $Se^{82}$) in freeze-dried tissue samples of spleen, pancreas, kidneys, and whole blood of sheep was determined by the CPI-MS method after tissue sample digestion using a mixture of 65% $HNO_3$ and 30% $H_2O_2$ (2:1, v/v) (Wysocka et al., 2003). Concentrations of Zn, Mg and Ca in freeze-dried samples of all assayed tissues of lambs were determined by flame (air-acetylene) atomic absorption spectrometry (PU9100X Atomic Absorption Spectrometer, UNICAM, Philips) (Czauderna et al., 2005).

*Statistical analyses*

Results are presented as means of 5 individually analysed lyophilized spleens, pancreases, and kidneys of lambs. Mean values in columns having the same superscripts are significantly different at $^{a,b}$ $P<0.05$ and $^{A,B}$ $P<0.01$, while differences at $^{\alpha,\beta}$ $P\leq0.1$ are denoted as tendencies. These one-factorial statistical analyses of the effects of LO or SeVI in the diets were conducted using the non-parametric Mann-Whitney U test for comparing independent experimental groups. Statistical analyses of interactions between LO and SeVI (i.e. LO × SeVI) were performed using two-factorial ANOVA analysis; the interactions were significant at the $^{x,y}$ $P<0.05$ and $^{X,Y}$ $P<0.01$ levels, respectively. Statistical analyses were performed using the Statistica v. 6 software package (2002; www.statsoft.pl).

## RESULTS AND DISCUSSION

### *Effects of the experimental diets on masses of spleen, pancreas and kidneys, and concentrations of Se, Zn, Mg, Ca and Fe in these organs*

The diets enriched in 5% LO and/or 2 ppm SeVI resulted in no macroscopic lesions, physiological disorders or pathological changes in the spleen, pancreas, kidneys, liver (Czauderna et al., 2004b), muscles or in any other tissues of sheep (Czauderna et al., 2004a; Niedźwiedzka et al., 2008). Indeed, only prolonged consumption of inorganic Se compounds, selenite in particular, at rates of more than 5 ppm Se can be hepatotoxic and teratogenic in humans and animals; furthermore, in contrast to selenide and selenite, selenate is less reactive and toxic in living organisms (Tapiero et al., 2003; Tinggi, 2003; McDowell et al., 2005). Despite these facts, in accordance with a regulation of the European Commission (EC, no. 1831/2003), rations administered continuously to farm animals should contain no more than 0.5 ppm Se. Moreover, in contrast to selenite, selenate (SeVI) added to diets does not react with the components of the ration, or with components of the digesta in the digestive tract of farm animals. Therefore, we argue that the more stable selenate (SeVI) is a better source of selenium in the ration compared with selenite. An extruded mixture of linseed and wheat bran is a good source of linolenic acid; however, the more expensive linseed oil (OL) is a better source of linoleic acid for farm animals.

Considering the above, we carried out a short-term (35-day) study on lambs fed a diet enriched with 2 ppm Se as SeVI and/or 5% LO. The experimental data on body mass gain (BWG), feed conversion efficiency (FCE), mean body mass gain per day (BWG/day), mass of the spleen, pancreas, and kidneys after 35 days of feeding the experimental diets are summarized in Table 2. The results showed that short-term feeding of the diet supplemented with LO to lambs significantly increased ($P<0.05$) BWG, FCE, and BMG/day, so consequently decreased feed intake (Niedźwiedzka et al., 2008) compared with the control group and lambs fed the diet with SeVI. Similarly, the diet enriched in LO and SeVI elevated BWG (a statistically significant interaction; $P<0.05$) and FCE in comparison with the control or SE diets. Interestingly, the extra SeVI in the diet (SE) resulted in a minute increase in BWG and FCE. These results thus document that short-term feeding the diet containing 2 ppm Se as SeVI is not harmful to lambs and also does not reduce the mass of spleen, pancreas or kidneys (Table 2). Our previous studies also indicated that the diet enriched with 2 ppm Se as SeVI does not reduce the mass of *M. biceps femoris* and *M. longissimus dorsi* (Niedźwiedzka et al., 2008). On the other hand, the diet supplemented with LO, regardless of the presence of SeVI, decreased the mass of the pancreas compared with the control and SE groups.

The current study also investigated the relationship between the experimental diets and concentrations of Se, Zn, Fe, Ca and Mg in the spleen, pancreas, kidneys, and whole blood of sheep. As can be seen from the results presented in Tables 3, 4 and 5, the diet enriched in LO and SeVI most efficiently stimulated the accumulation of Se in the spleen and pancreas, whereas the accumulation of Se was practically the same in the kidneys of lambs fed the diet containing both LO and SeVI and the diet enriched with only SeVI. These results suggest that Se is preferentially accumulated in the kidneys, as the addition of LO to the diet with Se did not change the amount of Se in this internal organ compared with the diet containing SeVI only. Indeed, the kidneys are a special site of plasma glutathione peroxidase activity because many free radicals and other harmful products stimulating oxidative stress are formed there (Artur et al., 1996). On the other hand, the diet enriched with only LO slightly decreased the concentration of Se in the spleen, pancreas, kidneys, and whole blood (Table 2). Moreover, the addition of LO to the diet containing SeVI also reduced the concentration of Se in blood in comparison with the SE group. These results are consistent with our previous studies in which the concentration of Se was lowest in the liver, heart, and muscles (*M. longissimus dorsi* and *M. biceps femoris*) of sheep fed a diet enriched in LO (Czauderna et al., 2004a,b). Considering the above, we suggest that dietary LO exerted oxidative stress and therefore the concentration of Se, an essential element of anti-oxidative Se-enzymes, was lowest in the internal organs and muscles of sheep fed the diet containing LO. On the other hand, all of the experimental diets showed a negligible effect on accumulation of Zn and Mg in the spleen, pancreas, and kidneys. The results the presented study document that all of the experimental diets decreased the concentration of Mg and Ca in whole blood of lambs compared with the control group. The addition of LO to the diet, irrespective of the presence of SeVI, decreased the concentration of Ca in the spleen and pancreas, whereas the diet with only SeVI stimulated the accumulation of Ca (+22%) in the pancreas compared with the control group. The results of our study document that changes in blood Ca concentrations (Table 2) correlate well with changes of Ca concentrations in the spleen (Table 3). A similar good correlation was found between the concentration of Ca in the pancreas and in the blood of sheep fed the diet with LO, regardless of the presence of SeVI.

Feeding the diets enriched in LO and/or SeVI did not consistently or significantly change the concentration of Fe in the spleen and blood, whereas it slightly decreased the level of Fe in the pancreas and kidneys in comparison with the control group.

Considering the current results and our previous studies (Czauderna et al., 2004a,b; Niedźwiedzka et al., 2008), we suggest that none of the experimental diets stimulated abnormal deposition of Ca (calcification), Mg, Zn, or Fe in sheep organs.

Table 3. Effect of dietary LO and SeVI on concentrations (μg/g) of Se, Zn, Mg, Ca, Fe and selected fatty acids in the spleen of lambs, μg/g

| Measured analyte | Groups[1] | | | |
|---|---|---|---|---|
| | C | LO | SE | LOSE |
| Se | 1.145 | $1.070^{\alpha}$ | 1.108 | $1.550^{\alpha}$ |
| Zn | 42 | 42 | $45^{\alpha}$ | $41^{\alpha}$ |
| Mg | 669 | 682 | 659 | 681 |
| Ca | 190 | 168 | 135 | 133 |
| Fe | 3869 | 4012 | 3344 | 4285 |
| ΣCLA | 251 | $411^{a}$ | $229^{a}$ | 295 |
| *t10c12*CLA | $1.83^{A}$ | $11.2^{ABa}$ | $1.24^{Ca}$ | $3.67^{bax}$ |
| *c9t11*CLA | 170 | $188^{\alpha}$ | 164 | $110^{\alpha}$ |
| *ct/tc*CLA | 206 | $289^{a}$ | 180 | $184^{a}$ |
| *cc*CLA[2] | - | - | - | - |
| *tt*CLA | 45 | 122 | $49^{\alpha}$ | $111^{\alpha}$ |
| $R_{c9t11CLA/t10c12CLA}$[3] | $93^{Aa}$ | $17^{aC}$ | $132^{ABC}$ | $30^{B}$ |
| ΣCFA | $411^{abc}$ | $1177^{a}$ | $1257^{b}$ | $1162^{cX}$ |
| ΣCLA+ΣCFA | $662^{ab}$ | $1588^{a}$ | $1486^{b}$ | 1457 |
| C8:0 | 13 | 14 | 16 | 15 |
| C10:0 | $19^{AB}$ | $3^{A}$ | $3^{B}$ | $3^{X}$ |
| C12:0 | $4^{AB}$ | $18^{A}$ | $14^{AB}$ | $14^{x}$ |
| C14:0 | $103^{ab}$ | $167^{a}$ | $171^{b}$ | 141 |
| C16:0 | $2064^{\alpha}$ | $2373^{\alpha}$ | 2668 | 2238 |
| C18:0 | $1734^{\alpha\beta}$ | $2138^{\alpha}$ | $2183^{\beta}$ | 2030 |
| C20:0 | 24 | 25 | 29 | 25 |
| C22:0 | $5^{a}$ | $8^{a}$ | 7 | 9 |
| A-SFA[4] | $2172^{\alpha}$ | 2558 | $2854^{\alpha}$ | 2394 |
| $A_{SFAindex}$[5] | $0.165^{a}$ | $0.090^{a}$ | $0.200^{A}$ | $0.096^{A}$ |
| T-SFA[6] | $3902^{\alpha}$ | 4679 | $5023^{\alpha}$ | $4410^{x}$ |
| $T_{SFAindex}$[7] | 0.892 | 0.538 | 1.196 | 0.587 |
| SFA | $3971^{\alpha}$ | 4750 | $5093^{\alpha}$ | 4478 |
| αLNA | $29^{a}$ | $53^{a}$ | $24^{b}$ | $53^{b}$ |
| γLNA | $151^{A}$ | $430^{AB}$ | $144^{BC}$ | $484^{C}$ |
| LA | $1056^{\alpha}$ | 1142 | $871^{\alpha a}$ | $1155^{a}$ |
| *c6*C18:1 | 2494 | 2644 | 2945 | 2383 |
| *c9*C18:1 | $4085^{A}$ | $12600^{AB}$ | $4023^{BC}$ | $10429^{C}$ |
| Σ*t*C18:1[8] | 151 | 198 | 186 | 185 |
| MUFA | $6731^{A}$ | $15442^{Aa}$ | $7156^{ab}$ | $13001^{b}$ |
| PUFA | $1315^{a}$ | $1688^{ab}$ | $1093^{bc}$ | $1765^{c}$ |
| SFA/PUFA[9] | $3.07^{a}$ | $2.81^{b}$ | $4.72^{abc}$ | $2.62^{cX}$ |
| Δ9-index[10] | $0.59^{\alpha}$ | $0.55^{\alpha}$ | 0.57 | 0.54 |
| ΣFA | $12681^{A}$ | $23460^{AB}$ | $14829^{Ba}$ | $20704^{a}$ |

[1] see Table 2; [2] below quantification limit ($L_Q$ - defined as 10 times the average noise level) (Czauderna et al., 2003); [3] the concentration ratio of *c9t11*CLA and *t10c12*CLA; [4] atherogenic saturated fatty acids: the concentration sum of C12:0, C14:0 and C16:0; [5] the atherogenic index = (C12:0+4*C14:0+ C16:0)/(MUFA+PUFAn-6+PUFAn-3) (Ulbricht and Southgate, 1991); [6] thrombogenic saturated fatty acids: the concentration sum of C14:0, C16:0 and C18:0; [7] the thrombogenic index = (C14:0 +C16:0+ C18:0)/(0.5*MUFA + 0.5*PUFAn-6 + 3*PUFAn-3 + PUFAn-3/PUFAn-6) (Ulbricht and Southgate, 1991); [8] a sum of *trans*C18:1 (*t6*C18:1, *t7*C18:1, *t9*C18:1 and *t11*C18:1); [9] the ratio of SFA and PUFA; [10] Δ9-desaturase index = *c9*C18:1/(*c9*C18:1 + C18:0)

*Effects of dietary LO and/or SeVI on the concentration of CLA isomers in the spleen, pancreas, and kidneys*

It was found that dietary LO most efficiently increased the concentration of *c9t11*CLA, the sum of *ct/tc*CLA, *tt*CLA, and all CLA isomers (ΣCLA) as well as the sum of ΣCLA and ΣCFA (Σ(CLA+CFA)) in the spleen and pancreas compared with the control sheep and other experimental groups (Tables 3 and 4). Moreover, detailed analysis of these results showed that dietary LO most efficiently increased the concentration of *t10c12*CLA in the spleen, pancreas, and kidneys (i.e, 512%, 249%, and 26% higher than in the control organs, respectively). On the other hand, the accumulation of ΣCLA and Σ(CLA+CFA) in the kidneys was stimulated by the simultaneous addition of LO and SeVI to the diet as compared with the control, LO and SE groups. As can be seen from the data presented in Tables 3, 4 and 5, the diet enriched in LO most efficiently increased the concentration of *t10c12*CLA in the spleen, pancreas and kidneys (i.e.: 512%, 249%, and 26%, respectively). Considering the above, we argue that dietary LO stimulated the isomerization of dietary *c9c12*C18:2 (LA) into *t10c12*C18:2 in the rumen of sheep. The addition of LO to the diet with SeVI also increased the capacity of isomerization compared with the SeVI diet, therefore the concentration of *t10c12*CLA in all examined organs in lambs of the LOSE group was higher than in the SE group. In contrast, the addition of SeVI to the diet most efficiently decreased the yield of this isomerization, so the concentration of *t10c12*CLA in all assayed organs was smaller than in the control, LOSE, and LO groups. As expected, therefore, the concentration ratio ($R_{c9t11\text{CLA}/t10c12\text{CLA}}$) of *c9t11*CLA to *t10c12*CLA was higher in all assayed organs of lambs fed the diet with SeVI than in the control, LO, and LOSE groups. Consequently, the addition SeVI to the diet with LO increased the values of the $R_{c9t11\text{CLA}/t10c12\text{CLA}}$ ratio in all organs in comparison with the LO group, although these values were lower than in group SeVI. On the other hand, dietary LO most efficiently decreased values of $R_{c9t11\text{CLA}/t10c12\text{CLA}}$ in all examined organs compared with the control, SE, and LOSE groups.

Feeding LO with or without SeVI increased the concentration of *cc*CLA in kidneys and that of CFA in the spleen compared with the control group. Therefore, we suggest that CFA are preferentially accumulated in the spleen when diets enriched in LO or SeVI are fed.

Table 4. Effect of dietary LO and SeVI on concentrations (μg/g) of Se, Zn, Mg, Ca, Fe and selected fatty acids (μg/g) in the pancreas of lambs

| Measured analyte | Groups[1] | | | |
|---|---|---|---|---|
| | C | LO | SE | LOSE |
| Se | 0.609$^{a}$ | 0.604$^{\alpha\beta}$ | 1.003$^{a\beta}$ | 1.124$^{\alpha}$ |
| Zn | 40 | 38 | 39 | 41 |
| Mg | 881$^{\alpha}$ | 815$^{\alpha\beta}$ | 856 | 882$^{\beta}$ |
| Ca | 416 | 343 | 508$^{\alpha}$ | 326$^{\alpha}$ |
| Fe | 75$^{\alpha}$ | 64 | 50$^{\alpha}$ | 60 |
| ΣCLA | 1480 | 2 174$^{Aa}$ | 1020$^{A}$ | 1392$^{a}$ |
| *t10c12*CLA | 12.0$^{a}$ | 41.9$^{Aab}$ | 2.23$^{Ac}$ | 14.3$^{bc}$ |
| *c9t11*CLA | 1399 | 1461$^{ab}$ | 841$^{b}$ | 821$^{a}$ |
| *ct/tc*CLA | 1497 | 1694$^{Aa}$ | 872$^{A}$ | 930$^{a}$ |
| *cc*CLA | - | - | - | - |
| *tt*CLA | 167$^{ab}$ | 481$^{Aa}$ | 148$^{AB}$ | 462$^{Bb}$ |
| $R_{c9t11CLA/t10c12CLA}$ | 117$^{AB}$ | 35$^{AC}$ | 377$^{BDC}$ | 57$^{D}$ |
| ΣCFA | 53$^{a}$ | 25$^{a}$ | 34$^{b}$ | 27$^{cX}$ |
| ΣCLA+ΣCFA | 1533 | 2199$^{A}$ | 1054$^{A\alpha}$ | 1419$^{\alpha}$ |
| C8:0 | 16 | 17 | 15 | 17 |
| C10:0 | 21 | 43 | 36 | 35 |
| C12:0 | 20$^{a\alpha}$ | 63$^{a}$ | 44$^{\alpha}$ | 42$^{x}$ |
| C14:0 | 301$^{\alpha}$ | 443$^{\alpha}$ | 366 | 394 |
| C16:0 | 6407$^{AB}$ | 3207$^{A\alpha}$ | 2659$^{B}$ | 2237$^{\alpha x}$ |
| C18:0 | 3603$^{A\alpha}$ | 2710$^{\alpha\beta}$ | 2124$^{A}$ | 1965$^{\beta}$ |
| C20:0 | 51$^{AB}$ | 19$^{A}$ | 21$^{B}$ | 9 |
| C22:0 | 11$^{AB}$ | 5$^{A}$ | 5$^{B}$ | 3 |
| A-SFA | 6729$^{Aa}$ | 3714$^{a}$ | 3071$^{A}$ | 2674 |
| $A_{SFAindex}$ | 0.170$^{Aa}$ | 0.067$^{A}$ | 0.106$^{a}$ | 0.074$^{A}$ |
| T-SFA | 10313$^{Aa}$ | 6361$^{a}$ | 5151$^{A}$ | 4598$^{x}$ |
| $T_{SFAindex}$ | 0.839 | 0.301 | 0.466 | 0.302 |
| SFA | 10431$^{Aa}$ | 6511$^{a}$ | 5274$^{A}$ | 4706 |
| αLNA | 91$^{A}$ | 635$^{AC}$ | 131$^{BC}$ | 566$^{B}$ |
| γLNA | 1983$^{Aa}$ | 460$^{A}$ | 1040$^{a\alpha}$ | 647$^{\alpha X}$ |
| LA | 3378$^{a}$ | 4313$^{ab\alpha}$ | 3265$^{ab}$ | 3618$^{a\alpha}$ |
| *c6*C18:1 | 6185 | 8065 | 8057 | 6782 |
| *c9*C18:1 | 7936$^{A}$ | 24923$^{ABa}$ | 8148$^{Bb}$ | 15451$^{ab}$ |
| Σ*t*C18:1 | 518$^{a}$ | 542 | 755$^{a}$ | 584 |
| MUFA | 14661$^{A}$ | 33533$^{ABa}$ | 16959$^{B\alpha}$ | 22817$^{a\alpha}$ |
| PUFA | 5648$^{a}$ | 5563 | 4565$^{a}$ | 4986 |
| SFA/PUFA | 1.847$^{ab}$ | 1.170$^{a}$ | 1.155$^{b}$ | 0.944 |
| Δ9-index | 0.63$^{ab}$ | 0.74$^{a\alpha}$ | 0.79$^{b}$ | 0.54$^{b\alpha}$ |
| ΣFA | 32471$^{ab}$ | 47803$^{ac}$ | 27848$^{b}$ | 33932$^{c}$ |

[1] see Table 2

Table 5. Effect of dietary LO and SeVI on concentrations (μg/g) of Se, Zn, Mg, Ca, Fe and selected fatty acids in the kidneys of lambs, μg/g

| Measured analyte | Groups[1] | | | |
|---|---|---|---|---|
| | C | LO | SE | LOSE |
| Se | $2.230^{\alpha}$ | $2.135^{\beta\delta}$ | $3.268^{\alpha\beta}$ | $3.265^{\delta}$ |
| Zn | 50 | $53^{\alpha}$ | 53 | $47^{\alpha}$ |
| Mg | $671^{\alpha}$ | $674^{\alpha\beta}$ | 667 | $661^{\beta}$ |
| Ca | 510 | $557^{\alpha}$ | 538 | $493^{\alpha}$ |
| Fe | 126 | 124 | 109 | 109 |
| ΣCLA | 678 | 666 | 691 | 805 |
| *t10c12*CLA | 7.67 | $9.68^{A}$ | $2.45^{A}$ | 4.17 |
| *c9t11*CLA | 539 | $390^{\alpha}$ | $533^{\alpha}$ | 492 |
| *ct/tc*CLA | 577 | 503 | 569 | 589 |
| *cc*CLA | $2.82^{A}$ | $12.4^{AB}$ | 9.0 | $2.67^{Bx}$ |
| *tt*CLA | $98^{A}$ | $151^{Aa\alpha}$ | $114^{ab}$ | $214^{b\alpha}$ |
| $R_{c9t11\text{CLA}/t10c12\text{CLA}}$ | $70^{A}$ | $40^{ABa}$ | $218^{AB}$ | $118^{Da}$ |
| ΣCFA | $303^{ab}$ | $142^{ac}$ | $257^{b}$ | $294^{Xcx}$ |
| ΣCLA+ΣCFA | 981 | $808^{\alpha}$ | 948 | $1099^{\alpha}$ |
| C8:0 | 7 | 7 | 7 | 7 |
| C10:0 | 29 | $27^{a}$ | 35 | $46^{a}$ |
| C12:0 | $18^{\alpha}$ | $25^{a}$ | $35^{\alpha\beta}$ | $55^{a\beta}$ |
| C14:0 | $3174^{AB}$ | $980^{A}$ | $1576^{B}$ | $1192^{X}$ |
| C16:0 | $1439^{A}$ | $1441^{a}$ | $2942^{A}$ | $2237^{a}$ |
| C18:0 | $1156^{\alpha}$ | 1265 | $1824^{\alpha}$ | 1530 |
| C20:0 | $11^{A}$ | $9^{B\alpha}$ | $19^{AB}$ | $16^{\alpha}$ |
| C22:0 | $1^{A}$ | $2^{\alpha}$ | $4^{A}$ | $4^{\alpha}$ |
| A-SFA | $1632^{A}$ | $2444^{\alpha}$ | $4554^{A}$ | $3487^{\alpha x}$ |
| $A_{SFAindex}$ | $0.089^{Aa}$ | $0.150^{a}$ | $0.324^{Ab}$ | $0.151^{Ab}$ |
| T-SFA | $2770^{A}$ | $3684^{\alpha}$ | $6343^{A\alpha}$ | $4962^{x}$ |
| $T_{SFAindex}$ | $0.409^{A}$ | 0.336 | $1.556^{AB}$ | $0.415^{B}$ |
| SFA | $2838^{A}$ | $3756^{\alpha}$ | $6445^{A}$ | $5093^{\alpha x}$ |
| αLNA | $102^{A}$ | $247^{AB}$ | $116^{BC}$ | $285^{C}$ |
| γLNA | $501^{A}$ | $1731^{A\alpha}$ | $495^{Ba\alpha}$ | $2135^{B\alpha}$ |
| LA | 2379 | 2705 | 2684 | 2892 |
| *c6*C18:1 | $4303^{A}$ | $3734^{B}$ | $5665^{A}$ | $5421^{B}$ |
| *c9*C18:1 | $5455^{A}$ | $11826^{Aa}$ | $6136^{Ba}$ | $14308^{B}$ |
| Σ*t*C18:1 | 242 | 250 | 219 | 323 |
| MUFA | $10002^{A}$ | $15813^{AC\alpha}$ | $12092^{BD\alpha}$ | $20051^{CDa}$ |
| PUFA | $3164^{A}$ | $5869^{A}$ | $3515^{a}$ | $5571^{a}$ |
| SFA/PUFA | $0.90^{Aa}$ | $0.80^{Ba}$ | $1.87^{ABC}$ | $0.89^{CX}$ |
| Δ9-index | 0.79 | $0.74^{\alpha}$ | 0.76 | $0.78^{\alpha}$ |
| ΣFA | $16984^{Ab}$ | $25438^{A\alpha}$ | $23945^{b}$ | $32418^{\alpha}$ |

[1] see Table 2

*Effects of dietary LO and/or SeVI on the concentration of other fatty acids in the spleen, pancreas, and kidneys*

The results of the study documented that the addition of LO to the diet, irrespective of the presence of SeVI, increased the concentration sum of MUFA and all assayed fatty acids (ΣFA) in all organs compared with the control and SE groups. On the other hand, all experimental diets increased the concentration of A-SFA, T-SFA and SFA in the spleen and kidneys compared with the control group. A similar effect of these experimental diets was found in subcutaneous fat tissue and blood plasma (Niedźwiedzka et al., 2008). In contrast, the experimental diets decreased the concentration of A-SFA and T-SFA in the pancreas, liver, muscles (*M. biceps femoris* and *m. Longissimus dorsi*), and perirenal fat tissue (Niedźwiedzka et al., 2008) compared with the control group. Considering our present and previous results, we argue that the effect of additives in the diet on the concentration of A-SFA and T-SFA is dependent on the type of internal organ and tissue of sheep.

As can be seen from the results summarized in Tables 3 and 4, the LO and LOSE treatments decreased the values of the atherogenic and thrombogenic indices ($A_{SFA}$ index, $T_{SFA}$ index) in the spleen and pancreas compared with the control and SE groups. Indeed, the significant increase in the concentration of MUFA in the spleen and pancreas resulted in a decrease in the values of the $A_{SFA}$ and $T_{SFA}$ indices in the spleen and pancreas of lambs fed the LO or LOSE diet compared with the control or SE group. On the other hand, all of the experimental diets usually increased the concentrations of C16:0, C18:0 and, especially, C14:0 ($P<0.01$) in the kidneys compared with the control animals (Table 5). As a consequence, the values of the $A_{SFA}$ and $T_{SFA}$ indices increased in the kidneys of sheep fed the LO and LOSE diets with the exception of the $T_{SFA}$ index in the kidneys of sheep fed the diet containing LO. Interestingly, the addition of SeVI to the diet most efficiently increased the concentrations of C14:0, C16:0 and C18:0, whereas least efficiently increased the level of MUFA and PUFA in the kidneys compared with the LO or LOSE groups. Therefore, SeVI added to the diet most effectively increased the values of the $A_{SFA}$ and $T_{SFA}$ indices in the kidneys in comparison with other organs of lambs fed the control, LO, and LOSE diets.

The present studies show that the experimental diets resulted in an increase in the concentration of MUFA in the pancreas and, especially, in the spleen and kidneys compared with the control group. A similar effect of these experimental diets was found in subcutaneous and perirenal fat tissues (Niedźwiedzka et al., 2008). Moreover, the addition of LO to the diet, irrespective of the presence of SeVI, usually more efficiently elevated the concentration of MUFA and especially *c9*C18:1 in all internal organs and tissues of sheep compared with the control

and SE groups. Interestingly, the diet enriched in LO with or without SeVI increased the concentration of the sum of *trans*C18:1 (Σ*t*C18:1) in all assayed organs compared with the control group. Similarly, the LO or LOSE treatment increased the concentration of PUFA, especially linolenic acid (αLNA) in all assayed organs compared with the control and SE groups. A similar effect of these treatments was also found in the liver, muscles, blood plasma, perirenal and subcutaneous fat tissues of sheep (Czauderna et al., 2004a,b; Niedźwiedzka et al., 2008). Concomitantly with this, the LO or LOSE treatment decreased the value of the concentration ratio of SFA to PUFA (SFA/PUFA) in the spleen, kidneys and, especially, in the pancreas compared with the control and SE group. On the other hand, SeVI added to the diet decreased the concentration of PUFA, especially linoleic (LA) or γ-linolenic acid (γ-LNA) in the spleen and pancreas compared with the control group. The current results are consistent with our recent studies, in which SeVI treatment also decreased the concentration of PUFA in the liver, *M. biceps femoris* and *M. longissimus dorsi* compared with the control, LO and LOSE groups (Czauderna et al., 2004a,b; Niedźwiedzka et al., 2008). Considering the above, we suggest that this short-term SeVI treatment exerts a pro-oxidative effect, so the concentration of PUFA decreases in the spleen, pancreas, kidneys, liver and both muscles, and the values of the SFA/PUFA ratio increase in the spleen, kidneys and subcutaneous fat tissue (Niedźwiedzka et al., 2008) compared with the control, LO, and LOSE groups.

As can be seen from the results summarized in Tables 3, 4 and 5, feeding lambs the diet enriched with LO or SeVI statistically increased ($P<0.05$) the value of the Δ9-desaturase index in the pancreas compared with the control and LOSE groups, whereas small decreases in the values of this index were found in the spleen and kidneys of lambs fed the LO, SE, or LOSE diet. Interestingly, the increase in the value of the Δ9-desaturase index in the pancreas negatively correlated with concentration of C18:0 and C16:0 (the substrates of Δ9-desaturase) in this organ of lambs fed the diet enriched in LO or SeVI (Table 3). On the other hand, the small decrease in the value of Δ9-desaturase index in the spleen and kidneys resulted in an increase in the concentration of C18:0 and C16:0 in these organs of lambs fed the diet with LO, SeVI or combined addition of LO and SeVI.

## CONCLUSIONS

Our current studies show that short-term addition of 2 ppm Se as selenate (SeVI) can be used to increase the concentration of Se in tissues of farm ruminants without adversely influencing performance or causing physiological disorders in the spleen and pancreas, in particular. Feeding linseed oil (LO) and SeVI

most effectively stimulated the accumulation of Se (an essential element) in the spleen and pancreas. This is especially important in relation to the spleen, which has important roles in regard to erythrocytes and the immune system, while the pancreas plays important roles in the digestive and endocrine systems of mammals. The current study and our previous investigations documented that the diets enriched in LO and SeVI most effectively decreased the SFA/PUFA ratio in the spleen, pancreas, as well as in the muscles of lambs. This study therefore provides useful information for nutritionists carrying out further investigations aimed at improving farm animal health, performance, and the nutritional quality of feed for ruminants. Further investigation is necessary to determine if dietary selenate and other vegetable oils induce changes in the profiles of fatty acids and other essential elements in ruminant meat that are beneficial to human health.

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