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Organic and Inorganic Selenium Compounds Affected Lipidomic Profile of Spleen of Lambs Fed with Diets Enriched in Carnosic Acid and Fish Oil

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Simple Summary: The spleen, traditionally associated with their role in the immune surveillance and blood cells turnover, nowadays has been known to be engaged in metabolic control processes, e.g. in metabolism of lipids. While input of energy sources is essential during animal ratio' formulation exploring the lipid composition of the spleen and its potential modulation by antioxidant supplements becomes particularly relevant. Our results may be practically applied in food industry as they may provide animal food ensuring the nutritional requirements of the underprivileged consumers. Moreover, our findings could bridge the existing knowledge gap about the interplay of diet and lipids composition in the spleen. As this organ is considered to have the essential role in the development of atherosclerosis, obesity, nonalcoholic liver disease, nonalcoholic steatohepatitis and fatty liver, understanding the function of this internal organ may be a starting point to develop efficient prevention strategies in order to counteract these disorders.

Abstract: The purpose of these studies was to investigate effect of selenate (Se^{6}), selenized yeast (Se^{Ye}) and carnosic acid (CA) supplementation to the diet containing fish oil (F-O) and rapeseed oil (R-O) on contents of fatty acids (FA), malondialdehyde (MDA), tocopherols (Ts) and total cholesterol (TCh) in lambs' spleens. 24 male lambs (4 groups per 6 animals) have been fed: the control diet - the basal diet (BD) enriched in F-O and R-O; the CA diet - BD enriched in F-O, R-O and CA; the Se^{Ye}CA diet - BD enriched in F-O, R-O, CA and Se^{Ye}; the Se^{6}CA diet - BD enriched in F-O, R-O, CA and Se^{6}. Dietary modifications affected profiles of FA in spleens. The Se^{Ye}CA and Se^{6}CA diets increased the docosapentaenoic acid preference in Δ4-desaturase, hence the higher content of docosahexaenoic acid was found in spleens of Se^{Ye}- or Se^{6}-treated lambs than in spleens of animals receiving the CA and control diets. Experimental diets reduced the level of atherogenic FA in the spleen in comparison with the control diet. The experimental diets supplemented with Se^{Ye} or Se^{6} increased levels of TCh and Ts in spleens in comparison with the CA and control CA diets. The present studies documented that Se^{6}, Se^{Ye} and CA influenced the metabolism of FA, Ts and cholesterol in spleens.

Keywords: selenium; carnosic acid; ovine spleen; fatty acids; tocopherols

1. Introduction

The spleen, derived from mesenchymal tissue, is the largest lymphatic organ found in all vertebrates [1] . Spleen (the multipurpose internal organ) have very important physiological roles in regard to red blood cells, storage of blood, the center of the blood defense system, the immune system as well as formation of lymphocytes and eliminating senescent erythrocyte cells [2–4]. Moreover, spleen controls physiologically essential processes, e.g. metabolism of metals, albuminoids and, what recently is gaining increasing attention, metabolism of lipids. The latter encompasses digestion and absorption, transportation through the blood, as well as biosynthesis [5].

There are several mechanisms presumably implicated in spleen regulation of lipid metabolism. The most well-known is theory of splenic lipid reservoir, which covers both the volume of spleen and the activity of macrophage. Hence, the presence of a mononuclear system of phagocytes active against fractions of lipids; biosynthesis of anti-oxLDL antibodies together with the removal of antigen-antibody species, interference with the lipid peroxidation in the liver (liver-spleen axis), activity of lipoprotein lipase, shifts in expression of microRNAs involved in regulation of genes correlated with high density lipoprotein (HDL) metabolism, platelet pathway and immune-mediated mechanism are also involved in lipid metabolism regulation [5]. Additionally, splenic connection with the propagation of certain diseases connected with the lipid disorders (like Niemann-Pick's disease, Gaucher's disease, Fabry disease or gangliosidoses) were also reported [6]. Total splenectomy may unfavorably affect levels of plasma lipids (triglycerides, cholesterols, fatty acids (FA)) and lipoproteins [7,8], and thus lead to the development of atherosclerosis and other cardiovascular disorders. The influence of spleen in lipids metabolism was confirmed in rats, rabbits and dogs models [9–11]. However, to the Authors best knowledge, no research were undertaken in ruminants' model. That is why our current experiment, aiming on evaluation of lipidomic profile of ovine spleen, seems to be up to date and valid.

Determinations of FA profiles of lambs' spleen were previously performed e.g. to confirm the possible routes of supply of FA to lymph. The most abundant lipid classes were phospholipids (phosphatidylethanolamine, phosphatidylcholine), free cholesterol and triacylglycerols. In each lipid class, the amounts of the essential fatty acids (EFA) were lower than in the corresponding lipids of plasma or lymph [12].

Lymph nodes and the spleen jointly make up the majority of peripheral immune tissues, and earlier investigations have documented that spleen tissue is very sensitive to changes of concentrations of selenium (Se) in diets [4]. In fact, this element is the part of glutathione peroxidases (GPx), which selenoenzymes functioning as stimulators of the immune system, responsible for scavenging if free radicals in tissues, thus reducing oxidative damage [13]. In fact, dietary deficiency of Se stimulated the inflammation and oxidative stress in spleen tissues, and so, disturbances immune activity of the spleen [4,14]. Low levels of Se in diet reduced the expression of seleno-proteins and Se contents, obstructed the thioredoxin and glutathione antioxidant systems, as well as caused disturbance of the redox balance in the spleen. Too low Se contents in tissues stimulated the HIF-1α and NF-κB transcription factors, increasing pro-inflammatory cytokines (like IL-1β, IL-6, IL-8, IL-17 or TNF-α), reducing anti-inflammatory cytokines (e.g.: IL-10, IL-13 or TGF-β) and stimulating expression of the downstream genes iNOS and COX-2, thus causing inflammation [4,14,15]. Additionally, insufficient doses of Se stimulated apoptosis (*via* the mitochondrial apoptotic pathway), up-regulated apoptotic genes, and down-regulated anti-apoptotic genes (*Bcl-2*) (at the mRNA level). On the other hand, diets containing high concentrations of Se stimulated oxidative stress (i.e. oxidative damage) as well as reduced immune responses in splenocytes [16–18]. Therefore, the optimal concentration of Se-compounds in diets is very important for proper functioning spleen.

Recent studies have shown that dietary supplementation with n-3 polyunsaturated FA (n-3PUFA), especially n-3 long-chain PUFA (n-3LPUFA), suppressed pro-inflammatory cytokine production, increased the number of lymphocyte cells and stimulated formation of immunocompetent cytokines in the spleen, which have a crucial role in anti-tumor and anti-infection activities [19–21]. However, higher contents of unsaturated fatty acids (UFA) in internal organs and tissues stimulated the oxidative stress in animals' body [22,23]. Oxidative stress caused by reactive nitrogen (RNS) and oxygen (ROS) species damage lipids, proteins as well as cellular RNA and DNA. Carbonyl compounds, especially malondialdehyde (MDA), the naturally occurring by-products of PUFA peroxidation and prostaglandin synthesis, are known to be detrimental for health [22,24]. Taking into account that the principal physiological functions of more than half of Se-enzymes (e.g., GPx, selenoprotein P or thioredoxin reductase) are to maintain the proper oxidative-antioxidant balance, low contents of ROS and RNS and free radicals within cells [25], an adequate amount of Se should be delivered in ration.

However, not only quantity but also chemical form of Se is of utmost importance. Organoselenium compounds (particularly seleno-methionine derived from Se^{Ye}) are more efficiently incorporated in the mammalian organisms than inorganic seleno-compounds (like Se^{4} or Se^{6}) [24]. Seleno-methionine (Se-Met) derived from Se^{Ye} is metabolized to inorganic seleno-compounds or accumulated into ruminal microbiota and animal tissue proteins as Se-Met (as a replacement of methionine (Met)) or seleno-cysteine [26].

Considering the different metabolism of seleno-compounds and thus, their physiological role, which cannot be limited only to antioxidant properties, we claim that an additional antioxidant should be added to diets rich in n-3LPUFA, e.g. containing fish oil (F-O). The herbal nutraceutical, carnosic acid (CA), which is one of the diterpenes present in rosemary, [27] was chosen in this study. As it has been found previously CA introduced in the ruminants ration improved production parameters (like diet intake, feed conversion efficiency or live weight gain) and the growth and/or activity of rumen microorganisms [28–30]. Furthermore, our previous studies showed that simultaneous supplementation of Se (as Se^{Ye} or Se^{6}) with CA affected the biosynthesis yield of Se-proteins as well as the profile of lipid compounds in rumen microbiota [26,31,32], blood [28], muscles [33], adipose tissues [34,35] and internal organs like the brain [36], kidneys [37], heart [38] and pancreas [39] of lambs. That is why we also hypothesized that the bioaccumulation of FA, total cholesterol (TCh), tocopherols (Ts) and the MDA concentration in the ovine spleen depend upon seleno-compound added to the diet containing F-O and CA. Therefore, the principal objective of these studies was to evaluate the effect of Se^{Ye} and Se^{6} on the lipidomic profile in the ovine spleen. It is of importance not only from the point of view of ruminant physiology and welfare but also human nutrition, especially in undeveloped countries, as the spleen, classified as giblets, may be considered as inexpensive source of bioactive lipids for humans at risk of malnutrition.

2. Materials and Methods

2.1. Lambs, housing, experimental scheme, diets and sampling

Our research was accepted by the Third Local Commission of Animal Experiment Ethics - approval number: 41/2013 (the Warsaw University of Life Sciences; 8 Ciszewskiego street; Warsaw 02-786; Poland). Our manuscript does not contain human studies. All experiments were carried out on 24 Corriedale male lambs (the initial average body weight (BW) of 23.3 ± 2.1 kg) selected from 110 animals, according to their BW and age (82–90 days). Preliminary feeding and all nutritional experiments on lambs and then spleen collections were conducted in special animal laboratory rooms at the Kielanowski Institute of Animal Physiology and Nutrition, Jabłonna (Polish Academy of Sciences; Poland). Welfare guidelines of animals were strictly adhered to throughout the 3 weeks of preliminary and whole experimental period. Selected sheep were divided into four equinumerous groups of six lambs each, housed in a climate controlled and ventilated facility (20 – 22°C) and given freely access to tap water throughout whole experiment. All animals were housed singly in adjacent pens (height, length, and width of pens were respectively 150, 170 and 130 cm) and had visual contact with other lambs. During the 3 weeks of preliminary period, animals had free and *ad libitum* access to a basal diet (BD) supplemented with the vitamin and mineral-premix (20 g in 1 kg of the BD), R-O) (20 g R-O in 1 kg of the BD) and odorless F-O (10 g F-O in 1 kg of the BD) (Table 1). The BD contains: meadow hay (~36.0%), barley meal (~16.5%), soybean meal (~36.0%), wheat starch (~9%) and a mixture of vitamins and minerals (the premix – ID number: a PL 1 405 002 p). The control and all experimental diets were iso-proteinous and iso-energetic. Details of chemical composition of all ingredients in the BD were as previously described [37].

After 3 weeks of preliminary period, 5 weeks of experimental period were carried out during which animals received the BD containing 1% F-O and 2% R-O (the control diet) or 3 experimental diets enriched in 1% F-O, 2% R-O and antioxidant(s) (i.e. 0.1% CA without/with 0.35 ppm selenium as Se^{Ye} or Se^{6}). The control and all experimental diets (Table 1) were suppled to lambs at 7:30 am and 4:00 pm (in equal amounts). The amount of the diets was adjusted each week according to nutritional requirements of lambs and their BW to avoid refusal of offered diets. The average feed intake was

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1.08 kg diet/lamb/day during the whole experimental period. Thus, each animal ate 37.8 kg of the experimental diets or the control diet. After 5 weeks of experimental period, ewes were rendered unconscious by intramuscular injections of 2-4 mg xylazine/10 kg of BW and then lambs were immediately slaughtered (in accordance with the guidelines No: 1099/2009 of the European Union Council Regulations (EC)). Then the spleen was removed from each animal. All collected spleens were individually homogenized and finally stored at 32°C (in tightly sealed vials).

Table 1. The experimental scheme and the extra ingredients in the experimental and control diets, LW (the live weight, kg) of sheep, BWG (body weight gain, kg), spleen weight (g), spleen index (g/kg) and FCE (feed conversion efficiency, kg/kg) of sheep.

The experimental scheme		The live weight (LW)			Spleen weight		FCE [5]
Group/diet	Ingredients added to 1 kg of the basal diet (BD)	Initial LW kg[1]	Final LW kg[2]	BWG kg	g[3]	Spleen index[4] g/kg final LW	kg/kg
Control[6]	20 g R-O and 10 g F-O	30.6 ± 2.4	37.7 ± 2.1^{ab}	7.1 ± 0.4^{ab}	75.8 ± 3.3^{a}	2.01^{a}	0.189^{ab}
CA[7]	20 g R-O, 10 g F-O and 1 g CA	30.6 ± 2.6	37.2 ± 2.3^{b}	6.6 ± 0.4^{b}	75.5 ± 3.1^{a}	2.03^{a}	0.174^{b}
Se^{Ye}CA[7]	20 g R-O, 10 g F-O, 1 g CA and 0.35 mg Se as Se^{Ye}	30.3 ± 2.7	36.8 ± 2.7^{b}	6.5 ± 0.4^{b}	88.6 ± 3.5^{b}	2.41^{b}	0.174^{b}
Se^{6}CA[7]	20 g R-O, 10 g F-O, 1 g CA and 0.35 mg Se as Se^{6}	30.3 ± 3.0	38.5 ± 3.1^{a}	8.2 ± 0.4^{a}	72.0 ± 3.1^{a}	1.87^{a}	0.215^{a}

BWG = final LW – Initial LW. Means with different superscripts (a,b) within columns are significantly different at $p \leq 0.05$. [1] The initial live weight of sheep (mean ± SD) after the preliminary period; for the whole preliminary period animals receiving the control diet. [2] The average live weight of sheep (mean ± SD) receiving the diets for 5 weeks of experimentation. [3] The average weight of the ovine spleens. [4] The relative weight of the ovine spleen (g/kg) = spleen weight (g)/the final LW of sheep (kg) [4]. [5] FCE for 5 weeks of experimentation; FCE = [BWG, kg]/[diet intake, kg]. [6] The Se level in 1 kg of the control diet: 0.16 mg Se/kg. [7] Se levels in the CA, Se^{Ye}CA and Se^{6}CA diets (mg Se/kg diet): 0.16, 0.51 and 0.51, respectively. The Se levels in 1 kg of meadow hay, and soybean and barley meals were: 0.003 mg, 0.020 mg and 0.016 mg, respectively; the Se level in wheat starch was below the limit of detection.

2.2. Reagents, chemicals and dietary supplements

GC-grade n-hexane (≥99%), GC-grade methanol (≥99.9%) and acetonitrile (HPLC-grade; ≥99.9%) were purchased from Lab-Scan (Dublin; Ireland). Isomers of conjugated linoleic acid (C18:2 isomers; CLA isomers), sorbic acid (as the internal standard; C6:2), nonadecanoic acid (as the internal standard; C19:0), and a mixture of 37 methylated fatty acid standards, α-tocopheryl acetate, α-, δ- and γ- forms of tocopherol, 2,6-ditert-butyl-pcresol, cholesterol, 25% aqueous 1,5-pentanedialdehyde solution, 1,1,3,3-tetramethoxy-propane (99%), 2,4-dinitrophenylhydrazine (including ~30% water), the methanolic solution of 25% BF_3 and trichloroacetic acid were obtained from Sigma Aldrich (St Louis, MO; USA). NaOH, KOH, $CHCl_3$, CH_2Cl_2, Na_2SO_4 and NaCl were obtained from Avantor Performance Materials (Gliwice; Poland). Other chemical reagents were of analytical grade. Water applied for the preparation of all reagents was obtained from an Elix™ water purification system (Millipore; Canada).

CA was obtained from Hunan Geneham Biomedical Technology Ltd (Changsha Road, Changsha, Hunan, China). The vitamin and mineral premix (ID No: a PL 1 405 002 p) was supplied from POLFAMIX OK (Trouw Nutrition; Poland). Se^{Ye} (highly selenized *Saccharomyces cerevisiae* yeast) was purchased from Sel-Plex (Alltech In.; Nicholasville, KY; USA); approx. 83% of the total amount of Se in Se^{Ye} is in the chemical form of Se-Met, while approx. 5% of Se is in the chemical form of Se-Cys; these Se-amino acids are in proteins of yeast. R-O and odourless F-O, purchased from Company AGSOL (Pacanów; Poland), were stored at ~4°C in a dark place in tightly closed bags.

2.3. Pre-column methods and chromatography instruments

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2.3.1. Fatty acid extraction and methylation of fatty acids

The finely homogenized spleens (45-60 mg) from each lamb were hydrolyzed according to our previous published method [40]. Nonadecanoic acid (C19:0) was added to each processed spleen. Next, mild acid- and base-catalysed estereffications were used for the synthesis of fatty acids methyl esters (FAME) in analyzed spleens. Next, methylated FA in assayed spleens were analyzed using capillary gas chromatography and mass spectrometry (GC-MS) [40]. GC-MS analyses were performed using a Shimadzu GCMS-QP2010 Plus EI, a BPX70 fused silica column (120 m x 0.25 mm i.d. x 0.25 mm film thickness) and a mass detector (Model 5973 N). FA (as FAME) identification in spleens was validated using the electron impact ionization spectra, and compared to retention times of FAME standards as well as the reference mass spectra library (NIST 2007) [41]. Determination of FAME contents in analytical samples was based on total ion current (TIC mode) chromatograms or/and selected ion monitoring (SIM mode) chromatograms [40].

2.3.2. Lipid quality indices

Atherogenic (${}_{index}A^{SFA}$) [42], modified atherogenic (${}_{index}A^{SFA}$/Toc) [39], and thrombogenic (${}_{index}T^{SFA}$) [42] indices were calculated according to the FA concentrations using the following formulae:

$$ {}_{index}A^{SFA} = (C16:0 + 4 \times C14:0 + C12:0)/(\Sigma n\text{-}3PUFA + \Sigma n\text{-}6PUFA + \Sigma MUFA) $$

$$ {}_{index}A^{SFA/Toc} = {}_{index}A^{SFA}/(0.05 \times C\delta T + 0.15 \times C\gamma T + 1.36 \times C\alpha TAc + 1.49 \times C\alpha T), $$

$$ {}_{index}T^{SFA} = (C18:0 + C16:0 + C14:0)/[(3 \times \Sigma n\text{-}3PUFA + 0.5 \times \Sigma n\text{-}6PUFA + 0.5 \times \Sigma MUFA)/\Sigma n\text{-}6PUFA) $$

where: MUFA – monounsaturated fatty acids; CδT, CγT, CαTAc and CαT – contents of δ-tocopherol, γ-tocopherol, α-tocopheryl acetate and α-tocopherol, respectively; 0.05, 0.15, 1.36 and 1.49 are biological activity coefficients of assayed tocopherols [43]. The concentration sum of atherogenic (A-SFA) and thrombogenic (T-SFA) saturated fatty acids (SFA) were calculated using the following formulae:

$$ A\text{-}SFA = C16:0 + C14:0 + C12:0 $$

$$ T\text{-}SFA = C18:0 + C16:0 + C14:0 $$

The spleen index was calculated as spleen weight (g)/lamb weight (kg)[4,44]. The ratio of hypocholesterolemic/hypercholesterolemic fatty acids (h/HCh ratio) was calculated using the following equation [45]:

$$ h/HCh\ ratio = (13C22:1 + c11C20:1 + c14C18:1 + c12C18:1 + c9C18:1 + c7C18:1 + DPA + c7c10c13c16C22:4 + EPA + c11c14C20:2 + AA + c6c9c12C18:3 + \Sigma LNA + LA)/(C16:0 + C14:0) $$

2.3.3. Chromatographic analysis of TCh, α-TAc, tocopherols and MDA in spleens

Total cholesterol (TCh), α-tocopheryl acetate (α-TAc), α-tocopherol (α-T), δ-tocopherol (δ-T) and γ-tocopherol (γ-T) were analyzed in spleen samples (60-80 mg) using a liquid chromatograph (UFLC-DAD; Shimadzu, Tokyo; Japan) including two LC-pumps (LC-20ADXP), an autosampler (SIL-20ACXR), a communications bus module (CBM-20A), a column oven (CTO-20A), a degasser (DGU-20A5), a SPD-photodiode array detector and a Kinetex C18-column (Phenomenex; the particle size: 2.6 μm; Hydro-RP, 100 Å, 150 mm × 2.1 mm i.d.; Torrance, CA; USA) [46].

Concentrations of malondialdehyde (MDA) in ovine spleens (50-70 mg) were chromatographically quantified after pre-column saponification followed by derivatization [22]. Concentrations of derivatized MDA in assayed spleens were determined applying a liquid chromatograph (UFLC-DAD; Shimadzu, Tokyo; Japan) equipped with a Synergi C18-column (Phenomenex; the particle size: 2.5 μm; Hydro-RP, 100 Å, 100 mm × 2 mm i.d.; Torrance, CA; USA).

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2.4. Statistical analyses

Statistical analyses were carried out applying the Statistica 12.5 PL software package (StatSoft Inc., Tulsa, OK, USA). The results are presented as means and standard errors of means, except for live weight (LW) of animals, feed conversion efficiency and spleen weight (mean ± standard deviation). The Shapiro-Wilk test was applied to analyze the normality of the data distribution. The impact of the experimental diets on the examined parameters in ovine spleens for variables with normal distribution was tested applying one-way ANOVA and Tukey's Honestly Significant Difference test. All results for variables without normal distribution were tested applying the Kruskal-Wallis test, which is a non-parametric equivalent of one-way ANOVA, with a post-hoc multiple comparison test. The acceptable level of statistical significance was established at $p \leq 0.05$.

3. Results

Our observation showed that no damaging symptoms (e.g. diarrhea and vomiting) in the control and all experimental lambs, as well as no visual pathological changes, acute toxicity or toxic changes of Se (as Se^{Ye} or Se^{6}) in the spleen and other organs (like the liver, kidneys, pancreas and brain), muscles and adipose tissues. Se^{6} and CA added to the ovine diet significantly increased the FCE, final LW and BWG of sheep in comparison to the CA and Se^{Ye}CA diets (Table 1). On the other hand, the Se^{Ye}CA diet most efficiently elevated spleen weight and the values of spleen index in comparison with the Se^{6}CA, CA and control diets.

3.1. Contents of SFA and MUFA in ovine spleens

Results reflecting the concentration of selected SFA in the spleen of sheep are presented in Table 2. Compared to the control diet, all experimental diets substantially reduced the C17:0 and C18:0 contents in ovine spleens. Similarly, the experimental diets with CA, irrespective of the presence of , Se^{Ye}CA and Se^{6}CA diets tend to decrease the C16:0 content in the spleen in comparison with the control diet, whereas the Se^{Ye}CA and CA diets decreased ($p \leq 0.05$) in the contents of C22:0 and C24:0 in the spleen compared to the Se^{6}CA and control diets. The Se^{6}CA and CA diets decreased ($p \leq 0.05$) the content of A-SFA, and did not influence ($p > 0.05$) T-SFA, the concentration sum of all assayed FA (ΣFA) and all assayed SFA (ΣSFA) in the ovine spleens as compared with the control diet. Compared to the control diet, the Se^{6}CA diet decreased the ratios of T-SFA/ΣFA ($p \leq 0.05$), ΣSFA/ΣUFA ($p \leq 0.05$) and ΣSFA/ΣMUFA ($p \leq 0.05$) and did not exert any impact on the ratios ($p > 0.05$) of A-SFA/ΣFA, ΣSFA/ΣPUFA and ΣSFA/ΣFA in the ovine spleens. Moreover, the Se^{6}CA diet most efficiently reduced the values of indexASFA and indexTSFA in the spleen in comparison with the Se^{Ye}CA, CA and control diets. The experimental diet containing only CA also significantly elevated the content ratios ($p \leq 0.05$) of ΣSFA/ΣPUFA or did not influence the ratios ($p > 0.05$) of ΣSFA/ΣUFA and ΣSFA/ΣFA in the spleen when compared with the Se^{Ye}CA, Se^{6}CA and control diets.

Table 2. The contents (μg/g spleen) of selected individual saturated fatty acids (SFA), content sums of all analysed SFA (ΣSFA)[1], all analysed FA (ΣFA), values of the atherogenic [42] ($_{index}A^{SFA}$) and thrombogenic index [42] ($_{index}T^{SFA}$) and the content ratios of ΣSFA to the content sums of UFA (ΣSFA/ΣUFA), PUFA (∑SFA/ΣPUFA), MUFA (ΣSFA/ΣMUFA) and ΣFA (ΣSFA/ΣFA) in the ovine spleens.

Additive: / **Group/diet:**	**-**	**CA**	**CA and Se^{Ye}**	**CA and Se^{6}**	**SEM**	**p value**
Item	**Control**	**CA**	**Se^{Ye}CA**	**Se^{6}CA**		
C10:0	0.9	0.5	1.0	0.9	0.3	0.41
C12:0	1.0	0.7	0.6	1.1	0.4	0.29
C14:0	56.1	48.2	61.6	57.3	1.9	0.37
C15:0	0.4	0.2	0.3	0.3	0.2	0.19

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C16:0	4452	3796	3968	3566	97	0.09
C17:0	112^{c}	49^{a}	84^{b}	85^{b}	5	0.04
C18:0	6119^{b}	5183^{a}	5284^{a}	5086^{a}	49	0.04
C20:0	1.0	0.8	0.7	1.1	0.1	0.29
C22:0	16.4^{c}	8.6^{a}	14.1^{b}	19.5^{c}	0.3	0.03
C24:0	56.4^{b}	39.2^{a}	45.7^{a}	60.7^{b}	0.5	0.04
A-SFA	4 509^{b}	3 845^{a}	4 031^{ab}	3 625^{a}	98	0.04
A-SFA/ΣFA	0.2106	0.2171	0.2191	0.2000	0.0022	0.13
T-SFA	10 627	9 028	9 314	8 709	198	0.13
T-SFA/ΣFA	0.5009^{b}	0.5099^{c}	0.5062^{bc}	0.4806^{a}	0.0017	0.04
$_{index}A^{SFA}$	0.4585^{b}	0.4700^{c}	0.4762^{c}	0.4179^{a}	0.0007	0.04
$_{index}T^{SFA}$	1.0399^{c}	0.9925^{bc}	0.8737^{b}	0.7852^{a}	0.0010	0.03
ΣSFA	10 816	9 127	9 460	8 877	223	0.09
ΣFA	20 959	17 655	18 350	18 150	667	0.11
ΣSFA/ΣUFA	1.0660^{bc}	1.0697^{c}	1.0636^{b}	0.9567^{a}	0.0014	0.04
ΣSFA/ΣPUFA	2.3420^{a}	2.6507^{c}	2.5652^{b}	2.2345^{a}	0.0041	0.03
ΣSFA/ΣMUFA	1.8919^{c}	1.7787^{b}	1.8039^{b}	1.6728^{a}	0.0059	0.04
ΣSFA/ΣFA	0.5102	0.5156	0.5143	0.4898	0.0082	0.37

SEM = standard error of the mean; sig - Statistical significances. Means in rows sharing the different superscript letter (a, b or c) are significantly different at $p \leq 0.05$. [1]The content sum of saturated fatty acids (ΣSFA) = C8:0+C10:0+C11:0+C12:0+C13:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C22:0+ C24:0.

Results concerning the contents of MUFA in the ovine spleen are presented in Tables 3 and 4. The CA diet significantly reduced ($p \leq 0.05$) the amounts of c9C14:1, c9C16:1, c12C18:1 and t11C18:1 in the spleen as compared with the Se^{Ye}CA, Se^{6}CA and control diets. The Se^{6}CA diet substantially increased ($p \leq 0.05$) indices of the Δ9-desaturation of C16:0, t11C18:1, the total Δ9-desaturation (ΣΔ9index) of C18:0, C16:0, C14:0 and t11C18:1 and the total Δ9-, Δ6-, Δ5- and Δ4-desaturation ($\Sigma^{\Delta 9,6,5,4}$FAindex) of FA in the spleen when compared with the control diet (Tables 4 and 5). Similarly, the Se^{Ye}CA, Se^{6}CA and CA diets increased the values of $\Sigma^{\Delta 9,6,5,4}$FAindex and the content ratio of ΣMUFA/ΣFA in the spleen in comparison with the control diet (Table 3).

Table 3. The contents (μg/g spleen) of selected individual monounsaturated FA (MUFA) and indices of Δ9-desaturation of FA and total FA desaturation in the ovine spleens.

Item / Additive: Group/diet:	-	CA	CA and Se^{Ye}	CA and Se^{6}	SEM	p value
	Control	CA	Se^{Ye}CA	Se^{6}CA		
c9C14:1	62.9^{c}	33.9^{a}	48.2^{b}	67.3^{c}	3	0.03
c9C16:1	115^{a}	99^{a}	143^{b}	153^{b}	7	0.04
c10C16:1	7.08	7.58	7.08	8.24	0.83	0.53
t11C18:1	263^{c}	155^{a}	210^{b}	237^{bc}	11	0.03
c9C18:1	4 698	4 317	4 151	4 137	159	0.32
c12C18:1	537^{a}	471^{a}	622^{b}	639^{b}	20	0.04
c11C20:1	33^{a}	36^{a}	62^{b}	65^{b}	5	0.03
ΣMUFA [1]	5 716	5 130	5 243	5 305	51	0.37
ΣMUFA/ΣFA	0.274^{a}	0.291^{b}	0.287^{b}	0.292^{b}	0.003	0.03

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$^{C18:0}\Delta9_{index}$ [2]	0.436	0.453	0.441	0.449	0.004	0.13
$^{C16:0}\Delta9_{index}$ [3]	0.0274^{a}	0.0265^{a}	0.0346^{b}	0.0407^{b}	0.0010	0.02
$^{C14:0}\Delta9_{index}$ [4]	0.529^{b}	0.413^{a}	0.439^{a}	0.540^{b}	0.003	0.03
$^{t11C18:1}\Delta9_{index}$ [5]	0.103^{a}	0.201^{d}	0.133^{b}	0.167^{c}	0.002	0.02
$\Sigma\Delta9_{index}$ [6]	0.310^{a}	0.328^{b}	0.315^{a}	0.330^{b}	0.002	0.04
$^{\Sigma\Delta9,6,5,4}FA_{index}$[7]	0.502^{a}	0.516^{b}	0.527^{b}	0.516^{b}	0.002	0.03

abbreviations for FA and other items see Table 2. Means with different superscripts within a row are significantly different at $p \leq 0.05$. [1] The content sum of MUFA (ΣMUFA) = c7C16:1+c9C16:1+t11C18:1+c6C18:1+c7C18:1+c9C18:1+c11C18:1+c12C18:1+ c11C20:1+c11C22:1+c13C22:1. [2] Δ9-desaturaturation of C18:0 index: $^{C18:0}\Delta9_{index}$ = c9C18:1/(C18:0+c9C18:1). [3] Δ9-desaturaturation of C16:0 index: $^{C16:0}\Delta9_{index}$ = c9C16:1/(C16:0+c9C16:1). [4] Δ9-desaturaturation of C14:0 index: $^{C14:0}\Delta9_{index}$ = c9C14:1/(C14:0+c9C14:1). [5] Δ9-desaturaturation of *trans*11C18:1 (t11C18:1) index: $^{t11C18:1}\Delta9_{index}$ = c9t11C18:2/(t11C18:1+c9t11C18:2). [6] Index of Δ9-desaturaturation of C18:0, C16:0, C14:0 and *t11*C18:1: $^{\Sigma}\Delta9_{index}$ = (c9C18:1+c9C16:1+c9C14:1+ c9t11C18:2)/(c9C18:1+c9C16:1+c9C14:1+c9t11C18:2+t11C18:1+C14:0+C18:0+C16:0). [7] Total FA desaturation index (i.e., Δ9-, Δ6-, Δ5- and Δ4-desaturation of FA): $^{\Sigma\Delta9,6,5,4}FA_{index}$ = (ΣMUFA+ΣPUFA)/(C16:0+C18:0+ C20:0+C22:0+C24:0+ΣMUFA+ΣPUFA).

Table 4. The contents (μg/g spleen) of selected individual polyunsaturated FA (PUFA), the content ratios of analysed PUFA to ΣFA, indices of elongases and desaturases and hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H-Ch ratio) in the ovine spleens.

Item / Additive: / Group/diet:	-	CA	CA and Se^{Ye}	CA and Se^{6}	SEM	p value
	Control	CA	Se^{Ye}CA	Se^{6}CA		
c9t11CLA	30.3^{a}	38.9^{b}	32.1^{ab}	47.6^{c}	0.6	0.04
c9c12C18:2 (LA)	682^{c}	550^{a}	600^{ab}	645^{bc}	13	0.03
c9c12c15C18:3 (αLNA)	10.6^{b}	5.5^{a}	7.2^{a}	17.0^{c}	0.5	0.02
c11c14C20:2	41.0^{b}	21.6^{a}	18.4^{a}	37.9^{b}	0.8	0.02
c8c11c14C20:3	68.4^{bc}	49.9^{a}	61.6^{ab}	76.0^{c}	2.9	0.03
c5c8c11c14C20:4 (AA)	2,904^{b}	2,141^{a}	2,203^{a}	2,389^{a}	32	0.05
c5c8c11c14c17C20:5 (EPA)	91.4^{c}	54.4^{a}	72.7^{b}	75.9^{b}	4.1	0.04
c7c10c13c16c19C22:5 (DPA)	438	404	443	478	12	0.17
c4c 7c10c13c16c19C22:5 (DHA)	164^{a}	135^{a}	211^{b}	204^{b}	8	0.04
Σn-3PUFA[1]	704^{b}	599^{a}	734^{bc}	774^{c}	12	0.04
Σn-6PUFA[2]	3,653^{c}	2,741^{a}	2,865^{ab}	3,111^{b}	29	0.04
ΣPUFA[3]	4,428^{c}	3,400^{a}	3,649^{ab}	3,971^{b}	34	0.03
Σn-6PUFA/Σn-3PUFA	9.258^{c}	4.911^{b}	3.960^{a}	4.013^{a}	0.005	0.02
Σn-6LPUFA	2,972^{b}	2,191^{a}	2,265^{a}	2466^{ab}	36	0.04
Σn-3LPUFA	693	593	727	757	14	0.27
ΣLPUFA[4]	3,665^{b}	2,784^{a}	2,992^{a}	3,223^{ab}	24	0.04
Σn-6LPUFA/Σn-3LPUFA	4.286^{c}	3.695^{b}	3.117^{a}	3.256^{a}	0.009	0.02
Σn-3LPUFA/ΣFA	0.0359^{ab}	0.0340^{a}	0.0395^{c}	0.0417^{d}	0.0002	0.04
ΣLPUFA/ΣFA	0.175^{b}	0.158^{a}	0.163^{a}	0.178^{b}	0.002	0.02
ΣPUFA/ΣFA	0.215	0.194	0.200	0.218	0.002	0.06
ΣPUFA/ΣSFA	0.427^{b}	0.377^{a}	0.390^{a}	0.448^{b}	0.003	0.04

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ΣUFA/ΣSFA	0.938	0.935	0.940	1.045	0.007	0.07
n−6ElongC20/C18index[5]	0.0567^{c}	0.0378^{b}	0.0298^{a}	0.0555^{c}	0.0002	0.03
n−3ElongC22/C20index[6]	0.721^{a}	0.888^{c}	0.862^{b}	0.864^{bc}	0.003	0.05
$\Delta 4_{index}$[7]	0.272^{b}	0.250^{a}	0.323^{d}	0.297^{c}	0.001	0.02
$\Delta 5_{index}$[8]	0.977	0.977	0.973	0.969	0.002	0.43
h/H-Ch ratio) [45]	2.250^{b}	2.219^{a}	2.207^{a}	2.600^{c}	0.003	0.02

CLA = conjugated linoleic acid; other abbreviations for FA and other items see Tables 2 and 3. Means with different superscripts within a row are significantly different at $p \le 0.05$. [1] The content sum of n-3PUFA; Σn-3PUFA = αLNA+c6c9c12c15C18:4+Σn-3LPUFA (i.e., Σn-3LPUFA = c11c14c17C20:3+c8c11c14c17C20:3+EPA+DPA+DHA). [2] The content sum of n-6PUFA; Σn-6PUFA = LA+c6c9c12C18:3+Σn-6LPUFA (i.e., Σn-6LPUFA = c11c14C20:2+c8c11c14C20:3 +AA+c7c10c13c16C22:4). [3] The content sum of PUFA; ΣPUFA = ΣCLA+Σn-3PUFA+Σn-6PUFA. [4] The content sum of LPUFA; ΣLPUFA = Σn-6LPUFA+Σn-3LPUFA. [5] The elongase index of C18:0: n-6ElongC20/C18index = c11c14C20:2/(c11c14C20:2+LA). [6] The elongase index of C20:0: n-3ElongC22/C20index = DPA/(DPA+EPA). [7] Δ4-desaturation of DPA index = DHA/(DPA+DHA). [8] Δ5-desaturation of C20:3n-6 index = AA/(c8c11c14C20:3+AA).

Table 5. The contents of total cholesterol (TCh; μg/g spleen), tocopherols (μg/g spleen) and MDA (μg/g spleen)[1] and values of the modified atherogenic index ($_{index}A^{SFA/\Sigma Toc}$) and PUFA peroxidation index ($^{\Sigma PUFA}MDA_{index}$) in the spleen of ewes fed the experimental and control diets.

Item	Group/diets				SEM	p value
	Control	CA	$Se^{Ye}CA$	$Se^{6}CA$		
TCh	223^{b}	120^{a}	308^{c}	260^{bc}	23	0.04
δ-tocopherol (δ-T)	1.07	0.33	0.38	0.65	0.05	0.09
γ-tocopherol (γ-T)	0.36	0.23	0.17	0.24	0.04	0.42
α-tocopherol (α-T)	3.83^{a}	4.65^{a}	12.11^{b}	10.93^{b}	0.06	0.04
α-tocopheryl acetate (α-TAc)	0.11	0.11	0.28	0.28	0.04	0.19
Σ(α-T+α-TAc)	3.93^{a}	4.75^{a}	12.39^{b}	11.22^{b}	0.07	0.03
Σall-Ts[2]	5.36^{a}	5.31^{a}	12.93^{b}	12.10^{b}	0.07	0.03
$_{index}A^{SFA/\Sigma Toc}$[39]	0.0769^{c}	0.0659^{b}	0.0258^{a}	0.0250^{a}	0.0005	0.03
MDA	4.52	4.45	3.97	3.62	0.12	0.37
$^{\Sigma PUFA}MDA_{index}$ [3]	1.021^{b}	1.309^{d}	1.087^{c}	0.911^{a}	0.014	0.03

MDA – malondialdehyde; other abbreviations for FA and other items see Tables 2–4. Means with different superscripts within a row are significantly different at $p \le 0.05$. [1] The content of MDA (C_{MDA}) was determined immediately after the spleen homogenization. [2] The content sum of all analysed tocopherols: $C_{\Sigma all\text{-}Ts}$ = α-Tac+α-T+δ-T+γ-T. [3] $^{\Sigma PUFA}MDA_{index}$ = MDA (ng/g)/ΣPUFA (μg/g).

3.2. PUFA concentrations in the ovine spleens

The obtained results showed (Table 4) that the $Se^{6}CA$ diet increased the contents of c9t11CLA, α-linolenic acid (αLNA), docosahexaenoic acid (DHA) and the concentration sum of all assayed n-3PUFA (Σn-3PUFA) in the ovine spleens as compared to the CA and control diets ($p \le 0.05$). In contrast, the experimental diet with only CA significantly reduced ($p \le 0.05$) indices of the C18:0 elongase and Δ4-desaturation of docosapentaenoic acid (DPA) as well as the levels of LA, c11c14C20:2, αLNA, c8c11c14C20:3, arachidonic acid (AA), eicosapentaenoic acid (EPA), Σn-3PUFA, Σn-6PUFA, ΣPUFA and Σn-6LPUFA in the ovine spleens as compared with the control diet. Similarly, the $Se^{Ye}CA$ diet significantly decreased ($p \le 0.05$) the levels of LA, αLNA, c11c14C20:2, AA, EPA, Σn-6PUFA and Σn-6 LPUFA in the ovine spleen in comparison with to the control diet. Compared to the CA and control diets, lower concentration ratios ($p \le 0.05$) of Σn-6PUFA/Σn-3PUFA and Σn-6 LPUFA/Σn-3LPUFA were found in the spleen of ewes fed the $Se^{Ye}CA$ and $Se^{6}CA$ diets. The

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substantially higher ratio of Σn-3LPUFA/ΣFA and the index of Δ4-desaturation of DPA were found in the spleen of animals fed the Se^{Ye}CA and Se^{6}CA diets than fed the CA and control diets ($p \leq 0.05$). The Se^{6}CA diet increased the ratio of hypocholesterolemic/hypercholesterolemic fatty acids (h/H-Ch ratio) in the ovine spleens in comparison to other diets.

3.3. Concentrations of tocopherols, TCh and MDA in the ovine spleens

CA added to the diet caused a significant ($p \leq 0.05$) decrease in the TCh content in the ovine spleens as compared with the Se^{Ye}CA, Se^{6}CA and control diets (Table 5). In contrast, the experimental diets supplemented with Se^{6} or Se^{Ye} significantly decreased the levels of α-T, the content sums of α-TAc and α-T (Σ (α-T+α-TAc)), as well as all detected tocopherols (Σall-Ts) when compared to the CA and control diets. Compared to the CA diet, the Se^{6}CA and Se^{Ye}CA diets decreased the modified atherogenic index ($_{index}A^{SFA}$/Toc) [39]in the ovine spleens. Compared to the control diet, all experimental diets decreased the $_{index}A^{\Sigma SFA/\Sigma Toc}$ values in the spleen of sheep. The Se^{6}CA diet significantly decreased ($p \leq 0.05$) values of the PUFA peroxidation index ($^{\Sigma PUFA}MDA_{index}$) in the spleen in comparison with the Se^{Ye}CA , CA and control diets. On the other hand, the CA diet increased ($p \leq 0.05$) the $^{PUFA}MDA_{index}$ value in the ovine spleens when compared with the control, Se^{Ye}CA and Se^{6}CA and control diets.

4. Discussion

The control diet containing F-O and R-O as well as all experimental diets including CA, F-O and R-O without/with Se (as Se^{6} or Se^{Ye}) did not affect negatively the total health conditions and especially welfare of sheep. Furthermore, the results of current investigations were confirmed by previous research documented that neither spleen injuries symptoms nor toxic symptoms and macroscopic lesions of 10 g of F-O, 20 g of R-O and 0.35 mg of Se (as Se^{6} or Se^{Ye}) added to 1 kg of the BD were observed in lambs [28,35,36,39,46]. In fact, dietary supplementation with 1% F-O and 2% plant sunflower oil reduced the numbers of ruminal *Butyrivibrio* C18:0-producers, and influenced the numbers of *Streptococcus bovis*, *Selenomonas ruminantium*, methanogens and protozoa, but not the total number of bacteria in a rumen [47]. Moreover, previous research showed that dietary supplementation of 1% F-O without or with 0.1% CA, regardless of the presence of Se^{6} or Se^{Ye}, changes composition of ruminal microbiota and, so, FA metabolism, so, decreased the biohydrogenation yield of C18-UFA and stimulated bacterial isomerization of UFA [28,46]. Similarly, diets including up to 2 mg of Se in kilogram of the BD would not be toxic for sheep and cows whereas long-term administration of diets containing Se^{6} or especially Se^{4} (selenite) or selenides at doses of more than 5 mg of Se per kilogram of the BD can be teratogenic as well as hepatotoxic [24,48]. Indeed, these physiological effect of dietary Se were confirmed in the spleen, as well as previously in adipose tissues, muscles and other internal organs (like kidneys, pancreas, heart or brain) [28,32,33,36,37,39,46]. Furthermore, lack of the detrimental health impact of dietary Se can be due to the fact, Se^{6} used in our investigation, is relatively less reactive and toxic in mammals [24]. Moreover, Se-Met (the main Se-compound in Se^{Ye}) is the less physiologically-active chemical form of Se, therefore, dietary supplementation with Se^{Ye} is considered as safe storage mode for Se [24].

The mammals' spleen is the very important immune organ, possessing different immunocompetent cytokines, which effectively stimulate anti-cancer as well as anti-infective functions. The higher spleen weight as well as the higher value of the spleen index observed in the lambs receiving the Se^{Ye}CA diet may suggests that Se-Met (derived from Se^{Ye}) is predominantly accumulated in spleen proteins instead of Met [24]. These Se-Met containing proteins in the spleen have no impact on important biochemical reactions, particularly protein or enzyme biosynthesis [49]. Moreover, Se-Met containing proteins in the spleen are less effective in the detoxification of RNS, ROS or other radicals in comparison to Se-Cys-enzymes biosynthesized primarily from SeVI supplemented to the SeVICA diet [50]. As a consequence, compared to sheep receiving the Se^{6}CA diet, Se-Cys-enzymes deficiency in the spleen of sheep receiving the Se^{Ye}CA diet caused redox imbalance. Therefore, the current researches are consistent with studies Yan et al. [44] showing that

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the value of the spleen index decreased significantly ($p \leq 0.05$) with increasing dietary supplementation with superoxide dismutase (SOD).

Moreover, the present researches showed that especially the $Se^{Ye}CA$ diet significantly stimulated the TCh incorporation in the ovine spleen, which was also observed in pancreases and kidneys [37,39]. It is well established that elevated levels of cholesterol and atherogenic SFA (as well atherogenic index) in tissues are associated with increases oxidative stress, LDL-cholesterol concentration as well as they activate the inflammation process [51,52]. Similarly, a high-cholesterol diet increased ROS generation and formation in mitochondria and decreased the level of glutathione (efficient a free radical scavenger and a key the antioxidant) [52]. These was confirmed by our results obtained on lambs fed particularly with the $Se^{Ye}CA$ diet. Indeed, the level of MDA tended to decrease ($p > 0.05$) and the index values of $^{\Sigma PUFA}MDA_{index}$ and $_{index}A^{SFA}$ in the spleen of animals receiving the $Se^{Ye}CA$ diet were lower ($p \leq 0.05$) than in the spleen of ewes receiving the $Se^{6}CA$ diet. The earlier results [37,39] obtained for pancreas and kidneys also seem to support above mentioned assumptions. Thus, we argued that the higher levels of ROS, RNS or other radicals in the spleen of the $Se^{Ye}CA$ treated lambs may cause inflammation of this internal organ [20]. Therefore, we supposed that the levels of TNF-α, IL-1β, IL-6, IL-8 or IL-17 (the pro-inflammatory cytokines [4]) in the lambs' spleen of the $Se^{Ye}CA$ group were higher than that in the $Se^{6}CA$ group. So, the higher spleen index observed in the $Se^{Ye}CA$-treated lambs may suggests the increased amounts of splenocytes, and thus, the enhanced immunoreaction, or state similar to hypersplenism, when macrophages in the spleen contain a large amount of fat due to hyper-active phagocytosis [5].

Our current results indicated that diets enriched with extra Se-compounds exert the lipogenic effects. In fact, the $Se^{6}CA$ and $Se^{Ye}CA$ diets significantly stimulated or tend to stimulate the incorporation of TCh in the ovine spleens when compared to the CA and control diets, which was observed also in kidneys [37]. Indeed, cholesterol as well as seleno-proteins used isopentenyl pyrophosphate for Sec-tRNA and isoprenoid synthesis [53]. In contrast, compared to the $Se^{Ye}CA$ and $Se^{6}CA$ diets, the CA diet reduced the concentration of TCh in the spleen, kidneys, heart, subcutaneous fat and fat located between thigh muscles of sheep [34,37,38]. So, these results is consistent with previous studies [54] indicating that CA reduced the BWG and concentrations of triglyceride, TCh and glucose in experimental animals. Really, CA reduces the nuclear level of SREBPs (i.e., sterol regulatory element-binding proteins) as well as downregulates their target genes, hence reducing the yield of the de novo-biosynthesis of cholesterol and fatty acids; dietary CA stimulates the degradation of mature SREBPs-form [55].

This study revealed that the $Se^{Ye}CA$ and $Se^{6}CA$ diets statistically significant increase the yield of Δ9-desaturation of C16:0 in the ovine spleen in comparison with the CA and control diets. So, the present research indicated that the $Se^{Ye}CA$ diet and particularly the $Se^{6}CA$ diet affected a substrate preference in the Δ9-desaturase in the ovine spleens. In fact, the Δ9-desaturase prefers acyl-CoA [56] with lengths of saturated fatty acid containing 16-carbons (i.e. formation of palmitoyl-CoA desaturase) in the spleen of animals receiving the $Se^{Ye}CA$ and $Se^{6}CA$ diets as compared with the CA and control diets. Additionally, the $Se^{Ye}CA$ and Se^{6} CA diets increased the concentrations of α-T and stimulated DPA preference in the Δ4-desaturase, hence the higher level of DHA was found in the spleen of the Se^{6} or Se^{Ye}-treated lambs than in animals fed the CA or control groups. Thus, our current study is in agreement with earlier researches [57,58] in which the Δ4-desaturase capacity and DHA content correlated with the level of Se-dependent hormones and enzymes, as well as tocopherol concentrations in animal tissues [59]. Indeed, tocopherols and Se-compounds play the essential roles in Δ4-, Δ5- and Δ6-desaturations of UFA by involving in the microsomal electron transport chain and in a peroxidase group of the desaturase complex [59].

In contrast, the CA diet decreased C14:0 and C16:0 (as the substrates) preference in the Δ9-desaturation, whereas increased t11C18:1 preference in the Δ9-desaturation in the ovine spleens in comparison with the $Se^{Ye}CA$ and $Se^{6}CA$ diets. Thus, we claimed that compared to C14:0 and C16:0, t11C18:1 shows greater affinity to Δ9-desaturase in the spleen of ewes receiving the CA diet. However, Se^{Ye} and particularly Se^{6} added to experimental diets with CA stimulated C14:0 and C16:0

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preference in the Δ9-desaturation in the spleen as well as values of Δ9-desaturase index in body fat of sheep [34] as compared with the CA and control diets.

The present and our previous researches showed that the Se^{Ye}CA and Se^{6}CA diets increased the accumulation of TCh and Σall-Ts in the spleen, heart and subcutaneous fat [34,38] in comparison with the CA and control diets. So, we claimed that dietary SeY or SeVI spared of tocopherols as well as easy peroxidized long-chain highly UFA in lambs' tissues. In fact, dietary Se^{Ye} and Se^{6} are utilized for biosynthesis of Se-dependent antioxidant enzymes which prevent against peroxidation of UFA (particularly highly unsaturated long-chain FA) in mammalian organisms [49,58,60]. Thus, Se-enzymes decreased content of free radicals mediated peroxidation and synergistically with tocopherols regulated lipid peroxidation in mammalian tissues [61].

In this study it was shown, that all experimental diets decreased spleen content of fatty acids responsible for atherogenesis [51]. Indeed, particularly the Se^{6}CA diet, decreased the content of A-SFA, values of the modified atherogenic index ($_{index}A^{SFA/Toc}$) and $_{index}T^{SFA}$, whereas improved the value of the h/H-Ch ratio in the ovine spleen in comparison with the control diet.

5. Conclusions

The present studies indicated the modulatory effect of seleno-compounds and CA supplemented to diets containing F-O on lipid compound metabolism as well as oxidative stress in the ovine spleen, without adverse effects and disruption in animal physiology. The experimental diets (particularly the Se^{6}CA diet) reduced spleen content of fatty acids responsible for atherogenesis and improved the value of the hypocholesterolemic/hypercholesterolemic FA ratio; thus, all experimental diets improve nutritional status of internal organ, which may be considered as edible giblets. That is why obtained results seem promising not only when animal welfare, physiology and health are considered, but also when human nutrition is taken into account.

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Abbreviations

AA – arachidonic acid; A-SFA - atherogenic saturated fatty acids; BD - basal diet; BWG - body weight gain; CA - carnosic acid; CLA – conjugated fatty acids isomers; COX-2 – cyclooxygenase 2; DAD - photodiode array detector; DHA – docosahexaenoic acid; DPA – docosapenaenoic acid; EFA – essential fatty acids; EPA – eicosapentaenoic acid; FA - fatty acids; FAME – FA methyl esters; F-O - fish oil; GC - gas chromatography; GPx - glutathione peroxidase; HDL – high density lipoprotein; HIF-1α - hypoxia-inducible factor 1α; HSD - Honestly Significant Difference; IL-10 – interleukin 10; IL-13 – interleukin 13; IL-17 – interleukin 17; IL-1β – interleukin 1β; IL-6 – interleukin 6; IL-8 – interleukin 8; iNOS - inducible nitric oxide synthase; LDL – low density lipoprotein; LA – linoleic acid; αLNA – alpha linolenic acid; LW - live weight; MDA – malondialdehyde; Met – methionine; MS - mass spectrometry; MUFA - monounsaturated FA; n-3LPUFA - n-3 long-chain PUFA; NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells; PUFA – polyunsaturated FA; RNS – reactive nitrogen species; R-O - rapeseed oil; ROS - reactive oxygen species; RP - reversed-phase; Se – selenium; Se-Cys - Se-

cysteine; Se-Met - Se-methionine; Se^{4} – sodium selenite; Se^{6} – sodium selenate; Se^{Ye} - selenized yeast; SFA – saturated FA; SOD – superoxide dismutase; SREBPs - sterol regulatory element-binding proteins; TCh - total cholesterol; TGF-β - transforming growth factor β; TNF-α – tumor necrosis factor α; T-SFA - thrombogenic saturated fatty acids; Ts - tocopherols; UFA – unsaturated fatty acids; UFLC - ultra-fast liquid chromatography; α-T – alpha-tocopherol; α-TAc – alpha-tocopherol acetate.

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