TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2016) 40: 681-693
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doi:10.3906/vet-1509-12

Supplementation effects of seleno-compounds, carnosic acid, and fish oil on concentrations of fatty acids, tocopherols, cholesterol, and amino acids in the livers of lambs

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Received: 04.09.2015 • **Accepted/Published Online:** 13.05.2016 • **Final Version:** 15.12.2016

Abstract: Lambs were fed a diet containing 3% rapeseed oil (RO) (the RO diet), a diet with 2% RO and 1% fish oil (FO) (the FO diet), or diets with combined additions of 2% RO, 1% FO, 0.1% carnosic acid (CA) (the CA diet), and 0.35 ppm Se as selenized yeast (SeY) (the CASeY diet) or selenate (SeVI) (the CASeVI diet). The CASeVI diet most effectively increased the body mass gain of lambs, while it most efficiently decreased the liver mass. The CASeVI diet most efficiently reduced the concentration of cholesterol and the lipid peroxidation in the liver. The CA diet revealed a weaker protective effect against peroxidation damage and/or catabolism of polyunsaturated fatty acids (PUFAs) than the CASeY or CASeVI diet. Diets containing CA, especially with SeY or SeVI, stimulated the accumulation of tocopherols in the liver. Diets containing FO without/with CA, SeY, or SeVI stimulated the concentration of fatty acids in the liver, while they decreased the concentration of amino acids. Diets containing CA more efficiently stimulated the concentration of PUFAs than the RO and FO diets. The CA-supplemented diet with SeVI and especially that with SeY resulted in the highest increase in the concentration of PUFAs in the liver.

Key words: Selenized yeast, selenate, carnosic acid, fish oil, chemical composition of liver, lambs

1. Introduction

Ruminal lipid metabolism has an important impact on profiles of fatty acids (FAs) in ruminant tissues (1). Dietary esterified lipids are hydrolyzed to free FAs (i.e. nonesterified FAs) and glycerol and to small amounts of mono- and diglycerides by bacterial lipases, which are extracellular microbial enzymes assembled in small beads (1). In contrast to monogastric animals, lipid metabolism in the rumen significantly changes the dietary FA profile in ruminal digesta and ruminant tissues. Ruminal microorganisms are able to synthesize FAs de novo as well as to biohydrogenate free unsaturated FAs into corresponding free saturated configurations. Nonesterified FAs are removed from the plasma in proportion to their contents in the plasma and the rate of blood flow to the liver. Hepatic blood flow is proportional to intake of metabolizable energy; the concentration of nonesterified FAs increases with a negative energy balance. The liver is a major organ in processing energy for the body and long-chain FAs are a major source of energy. The ruminant liver is a very minor site of FAs synthesis relative to that in some monogastric animals. Although the possibilities for changing the FA profile in internal organs, muscles, or adipose tissues of ruminants are much lower compared to pigs or chickens, numerous studies focus on ways of dietary manipulation of the FA composition through various feeding strategies. Fish oil (FO) added to the diet has been shown to increase contents of polyunsaturated fatty acids (PUFAs), especially n-3 long-chain PUFAs (n-3LPUFAs) in lamb tissues (1,2). The enrichment of n-3LPUFAs in tissues can be attributed to the incorporation of small amounts of C20:5n-3 (EPA) and C22:6n-3 (DHA) in FO escaping isomerization and/or biohydrogenation in the rumen (1). A necessary step in biohydrogenation of dietary unsaturated FAs (UFAs) in the rumen is bacterial lipolysis. Thus, after ingestion, dietary lipids can be largely hydrolyzed by enzymes of ruminal microbes: *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica* (1,3). This lipolysis results in the release of free FAs from lipids to allow the biohydrogenation of free UFAs into intermediates or finally into C18:0; in fact, biohydrogenation can only happen if the carbonyl moiety is free. Thus, ruminant meat is considered to be less healthy due to its relatively low contents of PUFAs and high concentrations of saturated FAs (SFAs), which stands behind several diseases such as obesity, cancer, or cardiovascular diseases (1). Although possibilities for

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changing the FA composition in ruminant tissues are much lower compared to pigs or chickens, numerous studies focus on ways of dietary manipulation of the FA composition through various feeding strategies. In fact, increasing contents of PUFAs, and especially n-3LPUFAs, in ruminant tissues is very important in view of the generally saturated nature of FAs in ruminant meats and the negative effect this can have on human health. Fortunately, amounts and types of fat and pro- or antioxidants added to the diet can influence the biohydrogenation yield of UFAs and the FA profiles in ruminant tissues (1,4–7). Our studies indicated that feeding linseed oil or FO effectively decreased the content of SFAs in tissues and increased the content of valuable monounsaturated FAs (MUFAs) and PUFAs (4). Moreover, our studies documented that FO is able to modify the biohydrogenation yield by decreasing the enzymatic isomerization capacity of linoleic acid (LA) or α-linolenic acid (αLNA) and simultaneously elevating the *trans11*C18:1 (*t11*C18:1) concentration in ruminal bacteria and tissues (1,2,6). FO, rich in LPUFAs, inhibited the growth and activity of ruminal microorganisms (e.g., *B. fibrisolvens*) (e.g. bacterial isomerase activity). PUFAs, and especially LPUFAs, have toxic effects on cellulolytic bacteria and protozoa, acting against ruminal lactate producers and thereby favoring propionate producers (1,2). Consequently, the percentage of undesirable FAs, like atherogenic and thrombogenic SFAs (A-SFA and T-SFA) (8) and especially C18:0, in tissues decreased, whereas the percentage of UFAs, particularly n-3LPUFAs, in tissues increased (1,9). However, the balance of UFAs, especially highly unsaturated FAs, prooxidants or antioxidants (e.g., Se-compounds or tocopherols), in feed is a critical factor influencing the quality of edible carcass parts of farm animals (4,7,10,11). Numerous studies showed that diets supplemented with selenized yeast (SeY) or selenate (SeVI) change the contents of FAs, tocopherols, and malondialdehyde (MDA; a marker of the oxidative stress) in animal tissues (4,7,9,11,12). Interestingly, carnosic acid (CA; an antioxidant) is used as a preservative in food (8,13). Moreover, CA (a phenolic diterpene) can modify gut microbiota, resulting in changes of the ruminal bacterial metabolism (14).

Considering the above, we hypothesize that FO, CA, and Se (as SeY or SeVI) added to a diet can modify levels of FAs, amino acids (AAs), and tocopherols as well as modulate the oxidative stress in the lamb liver. Therefore, the first main objective of our study was to investigate effects of the FO-supplemented diet on concentrations of FAs, AAs, tocopherols, cholesterol, and MDA in the lamb liver. The second objective of our trial was to investigate effects of different chemical forms of Se (as SeY or SeVI) added to diets including CA and FO on the concentrations of FAs, AAs, tocopherols, and MDA in the lamb liver.

2. Materials and methods

2.1. Animals, housing, diets, experimental design, and sampling

Thirty male Corriedale lambs with an average body weight (BW) of 30.5 ± 2.6 kg at the beginning of the experiment were individually penned and divided into 5 groups of 6 animals. This study was conducted under the authority of the Third Local Commission of Animal Experiment Ethics at the University of Life Sciences, Warsaw, Poland.

During a 3-week preliminary period the lambs were given free access to the basal diet (the standard concentrate-hay diet with vitamins and mineral premix) (Table 1); water was offered ad libitum. The basal diet contained 120 g of crude protein, 12 g of crude fiber, and 11 MJ of metabolizable energy in 1 kg of dry mater. The basal diet was enriched with 3% rapeseed oil (RO) (the RO diet) or 2% RO and 1% odorless FO (the FO diet) (Table 2).

The first experiment (Table 2): For the next 35 days following the 3-week preliminary period the lambs were fed the RO diet (the nonsupplemented diet; Group RO) or the FO diet (Group FO).

The second experiment (Table 2): After the preliminary period, for 35 days the lambs were fed the FO diet enriched with 0.1% CA (the CA diet; Group CA), the FO diet enriched with 0.1% CA and 0.35 ppm Se as SeY (the CASeY diet; Group CASeY), or the FO diet enriched with 0.1% CA and 0.35 ppm Se as SeVI (the CASeVI diet; Group CASeVI).

All diets were adjusted weekly and supplied as two equal meals at 0730 and 1600 hours each day to ensure free access to dosed feed. Fresh drinking water was always available. At the end of the 35-day experiment the animals were killed at 0700 hours. The livers were removed, weighed, homogenized, and frozen. All livers were stored in sealed tubes at –32 °C until chromatographic analyses; each liver sample was analyzed separately.

2.2. Chemicals

HPLC-grade acetonitrile, methanol, and GC-99%-grade n-hexane were purchased from Lab-Scan (Dublin, Ireland); other reagents were of analytical grade (POCh, Gliwice, Poland). A conjugated linoleic acid (CLA) isomer mixture and other fatty acid standards, amino acid standards, *o*-phthaldialdehyde, 2,4-dinitrophenylhydrazine, 2,6-di-*tert*-butyl-*p*-cresol, sodium selenate (SeVI), 1,1,3,3-tetramethoxy propane, and 25% BF_3 in methanol were provided by Sigma (USA). Chloroform, dichloromethane, methanol, tocopherol standards, cholesterol, KOH, NaOH, Na_2SO_4, and conc. HCl were purchased from POCh (Gliwice, Poland). All other chemicals were of analytical grade and organic solvents were of HPLC grade. Carnosic acid (CA) was purchased from Hunan Geneham Biomedical Technology

Table 1. Chemical composition of the concentrate-hay diet with vitamins and mineral mixture[1] (the basal diet[2]) and rapeseed oil (RO)[3] and odorless fish oil (FO)[4] fed to lambs.

Item[5]	Meadow hay[7]	Concentrate[6]		
		Barley meal	Soybean meal	Wheat starch
Dry mass (%)	88.4	87.6	89.7	87.3
Crude protein (%)	9.50	9.94	41.8	0.90
Crude fiber (%)	27.3	2.87	4.34	-
Crude fat (%)	3.40	2.50	2.25	0.09
Ash (%)	4.85	1.84	6.16	0.12
NDF[8] (%)	59.2	18.0	18.8	-
ADF[9] (%)	32.1	4.61	6.44	-
ADL[10] (%)	4.47	1.14	1.49	-

[1] 1 kg of vitamin and mineral mixture containing: 285 g calcium, 16 g phosphorus, 56 g sodium, 42 mg cobalt as carbonate, 10 mg iodine as iodate, 1 g iron as sulfate, 6 mg Se as sodium selenite, 0.5 g copper as cupric sulfate, 5.8 g manganese as sulfate, 7.5 g zinc as sulfate; vitamins: A (500,000 IU/kg), D3 (125,000 IU/kg), and E as α-tocopherol (25,000 IU/kg).
[2] The basal diet (BD) contains: meadow hay (~36%), a mixture of soybean meal (~36%) and barley meal (~16.5%), wheat starch (~9%), and mineral-vitamin mixture (20 g/kg BD).
[3] The main fatty acids in RO (µg/g): C14:0 56, C16:0 13091, *c9*C16:1 33, C18:0 5490, *c9*C18:1 385859, *c12*C18:1 786, *c9c12*C18:2 282394, *c9c12c15*C18:3 38474, C20:0 194, *c11*C20:1 108, C22:0 430, *c15*C24:1 61.
[4] The iodine value of FO: 50-65 g/100 g FO; the acid value of FO: 20 mg KOH/g FO; the main fatty acids in FO (µg/g): C12:0 82, C14:0 12345, *c9*C14:1 215, C15:0 477, C16:0 56947, *c7*C16:1 318, *c9*C16:1 420, ΣC16:2 15586, C17:0 493, *c9*C17:1 193, C18:0 9452, *c6*C18:1 188, *c7*C18:1 842, *c9*C18:1 290592, *c12*C18:1 15834, *c14*C18:1 159, *c9c12*C18:2 114512, *c9c12c15*C18:3 20968, *c11*C20:1 24206, *c7c9c12c15*C18:4 473, *c11c14*C20:2 2270, *c8c11c14*C20:3 258, *c5c8c11c14*C20:4 304, *c8c11c14c17*C20:4 607, C22:0 139, *c13*C22:1 11036, *c11*C22:1 1704, *c5c8c11c14c17*C20:5 6792, *c13c16*C22:2 95, *c7c10c13c16*C22:4 144, *c15*C24:1 397, *c7c10c13c16c19*C22:5 1560.
[5] % in dry matter (DM).
[6] The main fatty acids in concentrate (µg/g): C14:0 104, C16:0 3189, C18:0 1425, *c9*C18:1 774, *c9c12*C18:2 29163, *c9c12c15*C18:3 1014; the gross energy (MJ per kg of dry matter (DM)): barley meal: 16.3, soybean meal: 17.8, wheat starch: 16.7.
[7] The gross energy: 17.1 MJ per kg of DM; the mean fatty acid composition of meadow hay (µg/g): C8:0 83, C12:0 142 , C14:0 239, *c9*C15:1 131, C16:0 4034, *c9*C16:1 184, C18:0 459, *c9*C18:1 1266, *c12*C18:1 72, *c9c12*C18:2 13100, *c9c12c15*C18:3 4178, C20:0 58, *c11*C20:1 74, C22:0 101, C24:0 69, *c15*C24:1 71.
[8] Neutral detergent fiber.
[9] Acid detergent fiber.
[10] Acid detergent lignin.

Ltd. (Hunan, China). Rapeseed oil (RO) and odorless fish oil (FO) were supplied by Company AGROSOL (Poland). The selenized yeast (Se-*Saccharomyces cerevisiae*) was donated by Sel-Plex (Alltech Inc., USA). About 83% of the total Se content of selenized yeast (SeY) represents Se in the form of Se-methionine (Se-Met) incorporated into the proteins of *Saccharomyces cerevisiae* (11); the chemical composition of SeY was presented in our previous publication (11).

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 degassed for 2–3 min in a vacuum with ultrasonication prior to use.

2.3. Analytical methods and chromatographic equipment

All FAs, including CLA isomers, in liver samples were saponified according to Czauderna et al. (11) followed by gentle methylations. The base- and acid-catalyzed methylations were introduced for preparation of methyl esters of fatty acids (FAMEs) in liver samples (11). FAMEs were then quantified using gas chromatography according to Czauderna et al. (11). The analyses of all FAMEs 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), a quadrupole mass selective (MS) detector (Model 5973N), and an injection port. FAME identification was validated based on electron impact ionization spectra of FAMEs and compared with authentic FAME standards and the NIST 2007 reference mass spectra library.

Table 2. The experimental scheme and the composition of the diets enriched with FO, CA, SeY, or SeVI; the relative body mass gain (BMG) of lambs; the average liver weight of lambs; and concentrations of volatile fatty acids (VFAs), CO_2, and CH_4 in the lamb rumen[1].

Group	Additives added to the basal diet (BD)	Initial body mass of lambs $m_{initial}$, kg[2]	Relative body mass gain of lambs BMG, %[3]	Liver mass		Fermentation products in the rumen			The sum of CO_2 and CH_4[7] ($\Sigma CO_2 CH_4$)
				g	g/kg body[4]	SVFAs[5]	CO_2[6]	CH_4[6]	
						mM/100 mL	dm³/kg of body mass of lamb		
The first part of the study:									
RO[8]	3% RO	30.7[a]	18.5[a]	665[a]	18.31[a]	6.72[a]	0.0170[a]	0.0099[a]	0.0269[a]
FO[9]	2% RO, 1% FO	30.6[a]	23.4[b]	669[a]	17.75[a]	5.45[b]	0.0142[a]	0.0083[a]	0.0225[a]
The second part of the study:									
CA[9]	2% RO, 1% FO, and 0.1% CA	30.6[a]	21.5[a]	660[a]	17.77[a]	5.96[a]	0.0164[a]	0.0096[a]	0.0260[a]
CASeY[9]	2% RO, 1% FO, 0.1% CA, and 0.35 ppm Se as SeY	30.3[a]	21.6[ab]	650[a]	17.67[ab]	7.51[b]	0.0236[b]	0.0138[ab]	0.0374[b]
CASeVI[9]	2% RO, 1% FO, 0.1% CA, and 0.35 ppm Se as SeVI	30.3[a]	26.8[b]	599[a]	15.58[a]	5.25[a]	0.0137[ac]	0.0080[a]	0.0217[a]

[1] Mean values in columns having different superscripts are significantly different at $^{a,b}P < 0.05$ and $^{A,B}P < 0.01$. Statistical analyses were carried out between Groups RO and FO and between Groups CA, CASeY, and CASeVI. [2] The average initial body mass (kg) of lambs after the 3-week preliminary period. [3] The relative BMG of lambs after 35 days of the experimental period; BMG, % = $[(m_{35days} - m_{initial}) \times 100\%] / m_{initial}$. [4] Derived from fresh liver mass of lambs normalized to 1 kg of a lamb's body. [5] The concentration sum of acetic, propionic, *iso*-butyric, butyric, *iso*-valeric, and valeric acid in the ruminal fluid of lambs. [6] The contents of CO_2 and CH_4 (dm³ in the normal conditions) calculated from the content of acetic, propionic, and butyric acid in the rumen fluid; the contents (dm³) of CO_2 and CH_4 per kg of body mass of lambs (dm³/kg). [7] The content sum of CO_2 and CH_4 (dm³ per kg of body mass of lambs). [8] For the 3-week preliminary period the lambs were fed the basal diet enriched with 3% RO (the RO diet). [9] For the 3-week preliminary period the lamb were fed the basal diet enriched with 2% RO and 1% FO (the FO diet).

Methylated CLA isomers in livers were determined using a Waters 625 LC system that included a controller for gradient elution and two pumps (Waters Model 515) (11). The apparatus consisted of a Waters 712 WISP autosampler, two ion-exchange columns loaded with silver ions (250 × 4.6 mm Chrompack ChromSper 5 µm Lipids, the Netherlands), and a Waters 996 photodiode array detector. The CLA isomers in livers were determined in the isocratic elution program (low rate of 1.5 mL/min) and UV detection at 234 nm (column temperature: 23 °C). The eluent for the chromatographic elutions was prepared from n-hexane and acetonitrile (99.9:0.1 v/v). All CLA isomers in livers were satisfactorily separated from all endogenous species present in livers in 65 min (7,11).

AA concentrations in liver samples were determined by reversed-phase high performance liquid chromatography (RP-HPLC) with precolumn derivatization (11). The instrument used consisted of an Alliance separation module (model 2690, Waters, USA) and a 474 fluorescence detector (Waters, USA).

Tocopherols and cholesterol in livers were quantified using RP-HPLC according to Czauderna et al. (15). The instrument used consisted of an ultrafast liquid chromatography system and a photodiode array detector (Shimadzu, Japan).

The MDA concentrations in livers were determined after saponification followed by derivatization according to Czauderna et al. (12). The chromatographic separations of derivatized MDA from endogenic species of livers were conducted using an ultrafast liquid chromatography system and a photodiode array detector (Shimadzu, Japan).

2.4. Statistical analyses

Statistical analyses were performed using the Statistica software package (StatSoft, Version 10, 2010). Statistical analyses of the effects of dietary additives (FO, CA, SeY, and SeVI) on the concentration of FAs, AAs, tocopherols, cholesterol, and MDA in livers were conducted using the nonparametric Mann–Whitney U test. The results are presented as the means of individually analyzed liver samples. Mean values in columns or rows of tables having different superscripts are significantly different at $^{a,b}P < 0.05$ and $^{A,B}P < 0.01$.

3. Results

As can be seen from the results summarized in Table 2, the CASeVI diet most efficiently increased the body mass gain (BMG) of lambs, while it most effectively decreased the liver weight. On the other hand, BMG was smaller in lambs fed the CASeY diet than in lambs fed the CASeVI diet. Lambs fed the RO diet showed the strongest effect on reducing the BMG after 35 days of the experimental period.

Our results documented that the CASeVI diet most efficiently decreased the formation of CH_4 and CO_2 as well as the content sum of these greenhouse gases (ΣCO_2CH_4) in the rumen, whereas the CASeY diet most effectively increased the contents of CO_2 and CH_4 as well as ΣCO_2CH_4. In contrary, the FO diet decreased the contents of CO_2, CH_4, and ΣCO_2CH_4 in the rumen compared with the RO diet.

3.1. Effects of the supplemented diets on the concentrations of tocopherols, cholesterol, and MDA in the liver

In the current study we could successfully investigate effects of the supplemented diets on the concentrations of δ-, γ-, and α-tocopherol (δT, γT, and αT); α-tocopheryl acetate (αTAc); cholesterol (Chol); the sum of αT and αTAc (SαT); and the sum of all assayed tocopherols (STs) in the lamb liver. The FO diet decreased the concentration of Chol, δT, γT, αT, SαT, and STs in the liver compared with the RO diet (Table 3). Similarly, the CASeVI diet reduced the concentrations of Chol, δT, γT, αT, and STs in the liver

Table 3. The concentrations of cholesterol (Chol); δ-, γ, and α-tocopherol (δT, γT, and αT); α-tocopheryl acetate (αTAc); and MDA in the livers of lambs fed the experimental diets[1].

Item	RO	FO	CA	CASeY	CASeVI
Chol, mg/g	3.62^{a}	3.44^{a}	3.18^{ab}	3.24^{a}	2.73^{b}
δT, µg/g	4.0^{a}	2.1^{b}	4.8^{a}	3.5^{ab}	2.0^{b}
γT, µg/g	6.9^{a}	4.0^{a}	4.8^{a}	7.7^{b}	6.8^{ab}
αT, µg/g	6.1^{a}	3.4^{b}	5.6^{ab}	6.7^{a}	3.8^{b}
αTAc, µg/g	6.1^{a}	7.4^{a}	8.5^{a}	11.1^{ab}	13.2^{b}
SαT[2], µg/g	12.2^{a}	10.8^{a}	14.1^{a}	17.8^{a}	17.0^{b}
STs[3],µg/g	23.1^{a}	16.9^{b}	23.7^{a}	29.0^{a}	25.8^{a}
MDA,ng/g	11.8^{a}	13.3^{a}	20.4^{a}	17.3^{a}	16.9^{a}
MDA/ΣPUFAs, ng/mg	1.30^{a}	1.50^{a}	2.16^{a}	1.64^{b}	1.71^{ab}

[1] Mean values in rows having different superscripts are significantly different at $^{a,b}P < 0.05$ and $^{A,B}P < 0.01$. Statistical analyses were carried out between Groups RO and FO and between Groups CA, CASeY, and CASeVI. [2] The concentration sum of αT and αTAc. [3] The concentration sum of δT, γT, αT, and αTAc.

compared with the lambs fed the CASeY diet. The CA diet decreased the concentration of STs in the liver compared with the CASeY and CASeVI diets.

The effects of the supplemented diets on the concentration of MDA (indicator of lipid peroxidation) and the MDA index (MDA_{index}) in the lamb liver are summarized in Table 3. Our results indicated that the FO diet more effectively numerically increased the accumulation of MDA and the value of MDA_{index} in the liver than the RO diet. On the other hand, the CASeY and CASeVI diets statistically nonsignificantly decreased the concentration of MDA and the values of MDA_{index} in the liver compared with the CA diet.

3.2. Effect of the supplemented diets on the concentrations of SFAs and MUFAs in the liver

As can be seen from our results summarized in Tables 4 and 5, all supplemented diets affected the concentrations of SFAs and MUFAs in the lamb liver. The RO diet most efficiently reduced the concentrations of C14:0, C16:0, C18:0; the sums of atherogenic (A^{SFA}) and thrombogenic (T^{SFA}) saturated fatty acids; and all assayed SFAs (ΣSFAs) in the liver. The FO diet increased values of indexes of A^{SFA} ($_{index}A^{SFA}$) and T^{SFA} ($_{index}T^{SFA}$) in the liver as compared to the RO diet. The CASeVI diet reduced the accumulation of C14:0 and values of A^{SFA} and $_{index}T^{SFA}$ in the liver in comparison with the CA and CASeY diets.

Table 4. The concentrations of C14:0, C16:0, C18:0, and CLA isomers; the sums of saturated fatty acids (ΣSFAs)[1], all FAs (ΣFAs), atherogenic[2] (A^{SFA}) and thrombogenic[3] (T^{SFA}) saturated fatty acids; indexes of A^{SFA} ($_{index}A^{SFA}$)[4] and T^{SFA} ($_{index}T^{SFA}$)[5]; and concentration ratios of selected fatty acids to ΣFAs, ΣPUFAs, and ΣSFAs in the livers of lambs fed the experimental diets[6].

Item	RO	FO	CA	CASeY	CASeVI
C14:0, μg/g	121^{A}	320^{B}	165^{ab}	176^{a}	142^{b}
C16:0, mg/g	4.63^{a}	6.18^{a}	6.16^{a}	5.96^{a}	6.72^{a}
C18:0, mg/g	1 15.3^{a}	16.7^{a}	18.1^{a}	19.1^{a}	17.6^{a}
ΣSFAs, mg/g	21.0^{a}	24.1^{a}	25.3^{a}	26.2^{a}	25.5^{a}
ΣFAs, mg/g	41.7^{a}	50.8^{a}	51.8^{a}	53.6^{a}	55.4^{a}
A^{SFA}, mg/g	4.75^{a}	6.50^{a}	6.33^{a}	6.14^{a}	6.86^{a}
T^{SFA}, mg/g	20.0^{a}	23.2^{a}	24.5^{a}	25.3^{a}	24.5^{a}
$_{index}A^{SFA}$	0.248^{a}	0.272^{b}	0.259^{a}	0.244^{ab}	0.242^{b}
$_{index}T^{SFA}$	0.581^{a}	0.624^{b}	0.629^{a}	0.588^{ab}	0.581^{b}
A^{SFA}/ΣFAs	0.114^{a}	0.126^{a}	0.122^{a}	0.115^{a}	0.121^{a}
T^{SFA}/ΣFAs	0.479^{a}	0.459^{a}	0.473^{a}	0.471^{a}	0.452^{a}
ΣSFAs/ΣFAs	0.502^{a}	0.477^{a}	0.489^{a}	0.488^{a}	0.469^{ba}
*tt*CLA[7], μg/g	142^{a}	216^{b}	296^{ab}	272^{b}	299^{a}
*t10c12*CLA, μg/g	94^{a}	64^{a}	57^{a}	44^{ab}	40^{b}
*c9t11*CLA, μg/g	26^{a}	48^{b}	94^{a}	71^{a}	95^{a}
Σ*ct/tc*CLA[8], μg/g	148^{a}	153^{a}	178^{a}	137^{b}	149^{ab}
ΣCLA[9], μg/g	382^{a}	529^{b}	666^{a}	651^{a}	630^{a}
ΣCLA/ΣSFAs[10]	1 18.2^{a}	21.9^{a}	26.3^{a}	24.9^{a}	24.5^{a}
ΣCLA/ΣFAs[11]	9.15^{a}	10.41^{a}	12.87^{a}	12.15^{a}	11.36^{a}

[1] The concentration sum of C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0, and C24:0. [2] The concentration sum of C12:0, C14:0, and C16:0. [3] The concentration sum of C14:0, C16:0, and C18:0. [4] The atherogenic index = (C12:0+4×C14:0+C16:0)/(MUFAs+n-6PUFAs+n-3PUFAs) (8). [5] The thrombogenic index = (C14:0+C16:0+C18:0)/0.5×MUFAs+0.5×n-6PUFAs+3×n-3PUFAs +n-3PUFAs/n-6PUFAs) (8). [6] Mean values in rows having different superscripts are significantly different at $^{a,b}P < 0.05$ and $^{A,B}P < 0.01$. Statistical analyses were carried out between Groups RO and FO and between Groups CA, CASeY, and CASeVI. [7] The concentration sum of *trans,trans*CLA isomers. [8] The concentration sum of *cis,trans/trans,cis*CLA isomers. [9] The concentration sum of *tt*CLA, *ct/tc*CLA, and *cc*CLA isomers. [10] The concentration ratio of ΣCLA to ΣSFAs (μg/mg). [11] The concentration ratio of ΣCLA to ΣFAs (μg/mg).

Table 5. The concentrations of *c9*C18:1, *t11*C18:1, linoleic acid (LA), α-linolenic acid (αLNA), *c5c8c11c14*C20:4 (ArAc), *c7c10c13c16c19*C22:5 (DPA), and *c4c7c10c13c16c19*C22:6 (DHA); the sums of MUFAs (ΣMUFAs), PUFAs (ΣPUFAs), n-6PUFAs (Σn-6), n-3PUFAs (Σn-3), long-chain PUFAs (ΣLPUFAs), and n-3LPUFAs (Σn-3L); values of Δ9-, Δ4-, and Δ5-desaturase indexes ($\Delta9_{index}$, $\Delta4_{index}$, and $\Delta5_{index}$); the elongase index (El_{index}); and ratios of ΣPUFAs, ΣLPUFAs, and Σn-3L to ΣSFAs or ΣFAs in the livers of lambs fed the experimental diets[1].

Item	RO	FO	CA	CASeY	CASeVI
$\Delta9_{index}$ [2]	0.392[a]	0.473[b]	0.453[a]	0.436[a]	0.477[a]
*c9*C18:1, mg/g	9.78[a]	15.28[b]	15.09[b]	14.71[a]	16.76[a]
*t11*C18:1, µg/g	2 205	- [7]	-	-	-
ΣMUFAs, mg/g	111.70[a]	17.78[b]	17.04[a]	16.84[a]	19.83[a]
LA, mg/g	2.04[a]	1.86[a]	1.85[a]	2.25[a]	2.15[a]
αLNA, µg/g	368[a]	237[b]	237[a]	272[ab]	362[b]
ArAc, mg/g	3.82[a]	3.82[a]	4.09[a]	4.43[a]	3.97[a]
DPA, mg/g	1.04[a]	0.97[a]	1.05[a]	1.24[a]	1.04[a]
DHA, mg/g	1.08[a]	1.22[a]	1.35[a]	1.53[a]	1.40[a]
ΣPUFAs, mg/g	9.04[a]	8.88[a]	9.43[a]	10.57[a]	9.84[a]
Σn-6,mg/g	6.12[a]	6.01[a]	6.30[a]	7.06[a]	6.50[a]
Σn-3, mg/g	2.85[a]	2.81[a]	3.05[a]	3.47[a]	3.26[a]
ΣLPUFAs, mg/g	6.57[a]	6.72[a]	7.26[a]	8.01[a]	7.25[a]
Σn-3L, mg/g	2.49[a]	2.57[a]	2.81[a]	3.20[a]	2.90[a]
Σn-6/Sn-3[3]	2.150[a]	2.161[a]	2.062[a]	2.068[a]	2.023[a]
$\Delta4_{index}$ [4]	0.503[a]	0.555[b]	0.567[a]	0.551[a]	0.573[a]
$\Delta5_{index}$ [5]	0.936[a]	0.919[a]	0.919[a]	0.920[a]	0.911[a]
El_{index} [6]	0.741[a]	0.720[a]	0.715[ab]	0.740[a]	0.696[b]
ΣPUFAs/ΣSFAs	0.433[a]	0.373[b]	0.371[a]	0.404[a]	0.391[a]
ΣPUFAs/ΣFAs	0.217[a]	0.178[b]	0.182[a]	0.197[a]	0.183[a]
ΣLPUFAs/ΣFAs	0.157[a]	0.132[b]	0.140[ab]	0.150[a]	0.131[b]
Σn-3L/ΣFAs	0.0596[a]	0.0506[b]	0.0544[a]	0.0597[a]	0.0522[a]

[1] Mean values in rows having different superscripts are significantly different at $^{a,b}P < 0.05$. Statistical analyses were carried out between Groups RO and FO and between Groups CA, CASeY, and CASeVI. [2] Δ9-desaturase index = *c9*C18:1/(*c9*C18:1+C18:0). [3] The concentration ratio of Σn-6PUFAs to Σn-3PUFAs. [4] The Δ4-desaturase index: $\Delta4_{index}$ = C22:6n-3/(C22:6n-3+C22:5n-3). [5] The Δ5-desaturase index: $\Delta5_{index}$ = C20:4n-6/(C20:4n-6+C20:3n-6). [6] The elongase index: El_{index} = C22:5n-3/(C22:5n-3+C20:5n-3). [7] Below the limit of quantification (L_Q).

All supplemented diets decreased ratio values of T^{SFA} and ΣSFAs to ΣFAs (T^{SFA}/ΣFAs and ΣSFAs/ΣFAs) in the liver compared with the RO diet. Similarly, all supplemented diets increased values of the Δ9-desaturase index ($\Delta9_{index}$) in the liver in comparison with the RO diet. Therefore, all supplemented diets increased concentrations of *c9*C18:1 as well as the sum of MUFAs (ΣMUFAs) in the liver compared with the RO diet. On the other hand, all supplemented diets considerably reduced the concentration of *t11*C18:1 in the liver as compared to the RO diet.

3.3. Effect of the supplemented diets on PUFA profiles in the lamb liver

All supplemented diets more efficiently stimulated the accumulation of *trans,trans*CLA (*tt*CLA), *cis9trans11*CLA (*c9t11*CLA), and all assayed CLA isomers (ΣCLA) in the liver than the RO diet (Table 4). Similarly, all supplemented diets increased values of the concentration ratios of ΣCLA to ΣSFAs and to ΣFAs (i.e. ΣCLA/ΣSFAs and ΣCLA/ΣFAs) in the liver compared with the RO diet.

As can be seen from the results summarized in Table 5, all supplemented diets fed to lambs increased the concentration of all assayed LPUFAs (ΣLPUFAs), including the sum of n-3LPUFAs (Σn-3L), in the liver in comparison with the RO diet. The CASeY diet most effectively increased the concentrations of *c5c8c11c14*C20:4 (ArAc), *c7c10c13c16c19*C22:5 (DPA), and *c4c7c10c13c16c19*C22:6 (DHA) as well as ΣLPUFAs, the sum of PUFAs (ΣPUFAs), n-6PUFAs (Σn-6), n-3PUFAs (Σn-3), and n-3LPUFAs (Σn-3L) in the liver. The diets enriched with FO and CA, irrespectively of the presence of SeY or SeVI, reduced the values of the concentration ratio of Σn-6 and Σn-3 (Σn-6/Σn-3) in the liver as compared to the RO and FO diets. The RO diet resulted in an increase in ratio values of ΣPUFAs or ΣLPUFAs to ΣSFAs or ΣFAs (i.e. ΣPUFAs/ΣSFAs, ΣPUFAs/ΣFAs, and ΣLPUFAs/ΣFAs) in the liver compared with all supplemented diets.

All supplemented diets resulted in an increase in a value of the Δ4-desaturase index ($\Delta4_{index}$) in the liver in comparison with the RO diet. On the other hand, the RO diet more effectively increased values of Δ5-desaturase ($\Delta5_{index}$) and elongation (El_{index}) indexes in the lamb liver than all supplemented diets.

3.4. Effect of the supplemented diets on the concentration of amino acids in the lamb liver

As can be seen from the results summarized in Table 6, all supplemented diets resulted in a decrease in the concentrations of cysteine, aspartic acid, glutamic acid, asparagine, histidine, serine, arginine, glycine, threonine, tyrosine, valine, phenylalanine, *iso*-leucine, and leucine

Table 6. The concentrations of individual amino acids (AAs; µg/g); the concentration sums of all assayed AAs (ΣAAs), indispensable amino acids (ΣIAAs; µg/g), and dispensable amino acids (ΣDAAs; µg/g); and the ratio of ΣIAAs to ΣDAAs (ΣIAAs/ΣDAAs) in the livers of lambs fed the experimental diets[1].

Item	RO	FO	CA	CASeY	CASeVI
Cysteine	4.04^{a}	2.87^{b}	2.98^{a}	2.84^{a}	2.56^{a}
Aspartic acid	19.3^{a}	15.1^{a}	16.3^{a}	18.5^{a}	17.3^{a}
Glutamic acid	25.1^{a}	20.1^{a}	21.5^{a}	24.3^{a}	22.5^{a}
Asparagine	2.7^{a}	2.0^{b}	2.1^{a}	1.6^{ab}	1.5^{b}
Histidine	18.8^{a}	15.1^{a}	15.7^{a}	17.7^{a}	17.0^{a}
Serine	8.3^{a}	6.6^{a}	6.9^{a}	7.2^{a}	7.9^{a}
Arginine	27.5^{a}	22.1^{a}	23.5^{a}	23.1^{a}	22.6^{a}
Glycine	5.6^{a}	4.4^{a}	4.7^{a}	4.9^{a}	4.6^{a}
Threonine	5.7^{a}	4.5^{a}	5.0^{a}	5.3^{a}	4.9^{a}
Tyrosine	24.7^{a}	19.5^{a}	21.1^{a}	20.2^{a}	11.3^{b}
Alanine	2.45^{a}	2.18^{a}	2.67^{a}	3.31^{a}	2.62^{a}
Methionine	4.76^{a}	3.68^{b}	5.43^{a}	3.65^{ab}	2.56^{b}
Valine	9.8^{a}	7.3^{a}	7.9^{a}	7.7^{a}	8.6^{a}
Phenylalanine	10.3^{a}	7.4^{a}	9.4^{a}	8.8^{a}	8.2^{a}
iso-Leucine	7.5^{a}	5.5^{a}	6.4^{a}	6.5^{a}	6.51^{a}
Leucine	18.1^{a}	14.2^{a}	15.5^{a}	17.1^{a}	16.3^{a}
homo-Cysteine	0.555^{a}	0.366^{b}	0.412^{a}	0.546^{b}	0.491^{ab}
Lysine	14.0^{a}	11.0^{a}	12.1^{a}	14.9^{a}	12.0^{a}
ΣAAs	210^{a}	164^{b}	180^{a}	189^{a}	170^{a}
ΣIAAs	70^{a}	54^{a}	62^{a}	64^{a}	59^{a}
ΣDAAs	139^{a}	110^{a}	118^{a}	124^{a}	110^{a}
ΣIAAs/ΣDAAs	0.505^{a}	0.486^{a}	0.523^{a}	0.515^{a}	0.536^{a}

[1] Mean values in rows having different superscripts are significantly different at $^{a,b}P < 0.05$. Statistical analyses were carried out between Groups RO and FO and between Groups CA, CASeY, and CASeVI.

as well as sums of all AAs (ΣAAs), indispensable amino acids (ΣIAAs), and dispensable amino acids (ΣDAAs) in the liver as compared with the RO diet. Moreover, the CA, CASeY, and CASeVI diets increased the ratio of ΣIAAs to ΣDAAs (ΣIAAs/ΣDAAs) in the liver as compared with the FO and RO diets.

The FO diet most efficiently reduced the concentration of *homo*-cysteine in the liver. Similarly, the CA and CASeVI diets decreased the concentration of *homo*-cysteine in the liver as compared to the RO diet, whereas the CASeY diet stimulated the accumulation of *homo*-cysteine in the liver as compared to other supplemented diets. All supplemented diets decreased the accumulation of sulfur amino acids (i.e. cysteine, methionine, and *homo*-cysteine) in the liver as compared to the RO diet.

4. Discussion

In our studies, neither macroscopic lesions nor pathological changes were found in the livers of lambs fed the experimental diets, irrespective of the presence of SeY or SeVI. Indeed, diets containing up to 2 mg Se per kilogram would not be toxic for animals (especially for ruminants) (16,17). On the other hand, chronic dietary inorganic Se-compounds, especially selenides or selenite, at rates of more than 5 mg Se per kilogram can be hepatotoxic and teratogenic in animals and humans (18).

Our results documented that the SeVI diet most effectively increased the BMG of lambs, whereas it most efficiently decreased the liver mass (Table 2). On the other hand, dietary SeY (rich in Se-Met) revealed negligible effect on the BMG and the liver mass (Group CASeY) compared with the CA diet. Our current study and our previous investigation documents that dietary SeVI reduced the yield of FA accumulation in the liver, heart, spleen, pancreas, and especially in muscles (9). Considering the above, we argue that the level of SeVI (the inorganic form of Se) in the diet including CA and FO (Group CASeVI) most effectively stimulated the bacterial protein synthesis, whereas it decreased the capacity of ruminal fermentation of carbohydrates into VFAs and lipogenic enzymes in the lamb body. Indeed, our results summarized in Table 2 indicated that the CASeVI diet most efficiently decreased the concentration of SVFAs and especially CH_4 and CO_2 in the lamb rumen. Taken together, our results documented that the CASeVI diet improved animal performance by reducing lamb emissions of CH_4 and CO_2 (the greenhouse gases) and so decreased the contents of CO_2 and CH_4 and ΣCO_2CH_4 in the rumen (Table 2) (19). Similarly, the FO diet decreased emissions of CH_4 and CO_2, values of ΣCO_2CH_4, and the capacity of ruminal fermentation of carbohydrates into VFAs compared with the RO diet. Therefore, the FO diet more effectively increased the BMG of lambs than the RO diet.

In contrast, the FO diet and especially the CASeVI diet reduced rumen protozoa counts and activity of methanogenic bacteria, whereas they increased the proportion of the pool of ruminal bacteria flowing to the intestines (i.e. reduced a loss of bacterial proteins through degradation in the rumen). Hence, the CASeVI diet most efficiently improved nutrient digestion. In contrast, the CASeY diet increased the rumen protozoa counts and most effectively increased the activity of methanogenic bacteria, so this diet decreased the proportion of the pool of ruminal bacteria flowing to the intestines (i.e. increased a loss of bacterial proteins through degradation in the rumen).

4.1. Impact of the supplemented diets on concentrations of cholesterol, MDA, and tocopherols in the liver

The results summarized in Tables 3–5 documented that the highest level of C14:0 in the liver resulted in the highest concentration of Chol and values of $_{index}A^{SFA}$, A^{SFA}/ΣFAs, and Σn-6/Σn-3 ratio in the livers of lambs fed the FO diet. Indeed, the cholesterol concentration in VLD, LDL, and HDL increased significantly under all dietary conditions containing high fat. As reviewed by van Heek et al. (20), the concentration of LDL cholesterol increased significantly with dietary LA (C18:2n-6) and especially C14:0. Furthermore, many epidemiological and experimental studies documented that C12:0, C16:0, and especially C14:0 possess atherogenic properties (8).

On the other hand, the lowest concentration of C14:0 in the liver correlated with the lowest concentration of Chol and values of $_{index}A^{SFA}$ and Σn-6/Σn-3 ratio in the livers of lambs fed the CASeVI diet. Thus, our current investigations are in agreement with our previous studies in which the CASeVI diet most efficiently reduced the concentration of acetic acid in the ruminal fluid of lambs (9). Indeed, as observed in other studies, VFAs differ in their physiological significance for the host; acetate is a respiratory fuel and a precursor for lipogenesis and cholesterogenesis, whereas propionate is gluconeogenic and a precursor of amino acids.

Moreover, the CASeY diet and especially the CASeVI diet decreased the values of $_{index}T^{SFA}$, T^{SFA}/ΣFAs, and the ΣSFAs/ΣFAs ratio in the liver as compared to the CA diet and the RO diet. These observations are consistent with our previous studies, in which the CASeVI diet most efficiently reduced the concentrations of T^{SFA} and A^{SFA} as well as the values of $_{index}T^{SFA}$, $_{index}A^{SFA}$, and the ΣSFAs/ΣFAs ratio in *M. longissimus dorsi* (*MLD*) and *M. biceps femoris* (*MBF*) of lambs (9). Considering the above, we argue that the CASeVI diet increased the nutritional value of the liver, *MLD*, and *MBF* of lambs. Thus, the presented results of our short-term study constitute important information for nutritionists carrying out further studies to improve the nutritional quality of feed for ruminants and humans.

Our current studies also revealed that the CA diet stimulated the peroxidation of PUFAs in the liver, as the concentrations of MDA and the value of the MDA/ΣPUFAs ratio most effectively increased in the livers of lambs fed the CA diet (Table 3). Indeed, previous studies with CA demonstrated an increase in concentrations of aldehydes (i.e. volatile compounds from lipid autoxidation) in meat of lambs fed diets supplemented with higher doses of CA (i.e. 1.2 g kg^{-1}) (8). On the contrary, the lower level of CA in diets (i.e. 0.6 g kg^{-1}) seemed to promote an antioxidant effect in lambs closer to that observed in lambs fed diets enriched with vitamin E (8). As expected, SeY or SeVI added to the diet containing CA (Groups CASeY and CASeVI) revealed antioxidative properties as the concentration of MDA and the value of the MDA/ΣPUFAs ratio in the liver were lower than in the livers of lambs fed the CA diet.

Currently, the biological activity of tocopherols (Ts) that attracts the most interest is the prevention of PUFA peroxidation. The reason for the physiological preference of αT is its specific selection of RRR-α-tocopherol (RRR-α-T) by the hepatic α-tocopherol transfer protein (αTTP) (21). On the other hand, most of the ingested βT, γT, and δT is secreted into bile or not taken up and excreted in the feces (21). Interestingly, αT is the most active tocopherol against peroxyl radicals (LOO·) and δT is the least active (i.e. αT > βT = γT > δT) (22). As can be seen from results summarized in Table 3, the CASeVI diet more efficiently decreased the concentration of αT in the liver than the CASeY diet. Concomitantly with this, the CASeVI diet more effectively reduced the concentration of MDA in the liver than the CA and CASeY diets (Table 3). Thus, the current studies support the concept that the strongest antioxidative effect of the CASeVI diet is due to the most efficient action of αT against LOO·. For this reason, we argue that the formation of oxidation products of αT (i.e. the concentration loss of αT) in the livers of SeVI- and CA-fed lambs (Group CASeVI) was higher than in Groups CA and CASeY. Moreover, we suggest that the diets containing CA (i.e. Groups CA, CASeY, and CASeVI) reduced the concentration loss of tocopherols (i.e. αT, αTAc, γT, and δT) as compared to the FO diet. Thus, we argue that CA stimulated the regeneration of tocopherol radicals (i.e. products of the antioxidant activity of tocopherols) to the original chemical form of tocopherol. The regeneration process of tocopherol radicals (·Ts) in the liver was especially intense when CA and SeY were added to the diet (Group CASeY). On the other hand, the CASeVI diet reduced the regeneration process of ·Ts radicals in the liver as compared to the CASeY diet.

As can be seen from the data summarized in Table 3, the FO diet (i.e. the diet without the antioxidant(s)) resulted in a significant decrease in the concentrations of αT, αTAc, γT, and δT as compared to the RO diet and the diets containing the antioxidant(s) (i.e. CA without/with SeY). Considering these facts, we argue that FO (rich in LPUFAs) added to the diet most efficiently reduced the yield of the regeneration process of ·Ts radicals in the livers of lambs (Group FO). Therefore, the concentrations of αT, αTAc, γT, and δT in the livers of lambs fed the FO diet were lowest (Table 3). Furthermore, an interesting finding of our studies was that dietary FO (rich in LPUFAs) increased the concentration of MDA and the value of the MDA/ΣPUFAs ratio in the liver (Group FO) as compared to lambs fed the RO diet; in fact, dietary RO is poor in LPUFAs. Thus, the current studies reinforce our recent investigations that diets enriched with LPUFAs stimulated lipid peroxidation in animal tissues (23).

4.2. Effects of the supplemented diets on concentrations of fatty acids and amino acids in the livers of lambs

FO is known to stimulate the bacterial ruminal isomerization and to inhibit complete ruminal biohydrogenation of UFAs, causing an increase in the concentrations of CLA isomers and *t*C18:1 (like *t11*C18:1) in the rumen and lamb tissues (2,6). Moreover, the FO diet and especially the CASeVI diet suppressed protozoal populations and the capacity of bacterial fermentation of carbohydrates into VFAs (9). Dietary CA modifies ruminal microbiota and hence FA metabolism, especially bacterial formation of CLA isomers and *t11*C18:1 in the rumen. CA added to the diet stimulated bacterial isomerization and reduced the final biohydrogenation to C18:0 (8,13). Therefore, all diets containing FO and especially CA, irrespective of the presence of SeY or SeVI, increased the concentration of *c9t11*CLA in the lamb rumen and the *MLD* (9) as well as the concentrations of *tt*CLA isomers, *c9t11*CLA, and values of ΣCLA/ΣSFAs and ΣCLA/ΣFAs in the liver as compared to animals of Group RO (Table 4).

Our results suggest that the effect of dietary 1% FO on biohydrogenation capacity may have been associated with a combination of factors such as larger numbers of ruminal protozoa and increased the microbial isomerization yield of *t11*C18:1 to *t10*C18:1 (24,25). Moreover, our studies imply that the diet containing FO and especially CA, SeY, and SeVI stimulated the Δ9-desaturase capacity in the liver as the concentrations of *c9t11*CLA and *c9*C18:1 increased in the livers of lambs fed the experimental diets compared with Group RO (Tables 4 and 5). All diets enriched with FO, regardless of CA, SeY, or SeVI, reduced the concentration of *t11*C18:1 (the substrate of Δ9-desaturase) in the liver and in lamb muscles (9). The current results are in agreement with our previous study, in which a diet containing linseed oil (rich in αLNA) without or with SeVI stimulated the capacity of Δ9-desaturation in the lamb liver (4).

Fortunately, in ruminants biohydrogenation of EPA and DHA appears to be limited, even when dietary FO or fish meal was not protected. In fact, in vivo studies involving dietary FO documented that EPA and DHA are biohydrogenated, although to a lesser extent than usually observed for LA or αLNA (26). However, DHA deposition is strictly metabolically regulated and cannot be substantially influenced by diet. The following reasons for this can be proposed: dietary additives, an inhibition of the enzymes (the Δ5-, Δ4-, and Δ6-desaturases and elongase and β-oxidation) by other PUFAs (like LA or ArAc) and a competition of LA, αLNA, ArAc, EPA, and DPA with DHA. Our results are in agreement with the above studies, in which dietary FO affected microbial growth, efficiency in the rumen, and the capacity of desaturases and elongases in lamb tissues (Tables 2, 4, and 5). FO added to the diet, regardless of the presence of CA, SeY, or SeVI, increased the accumulation of all assayed SFAs (ΣSFAs), MUFAs (ΣMUFAs), and FAs (ΣFAs) in the liver as compared to lambs fed the RO diet. On the other hand, all diets containing FO reduced the concentrations of all assayed AAs (ΣAAs) in the liver compared with the livers of lambs from Group RO (Table 6). Thus, it could be concluded that in the livers of lambs fed the diets containing FO without or with CA, SeY, or SeVI, the concentration of ΣFAs increased (Table 4) while the protein concentration in the liver was reduced (repartition). Thus, we suggest that these dietary additives, especially FO, stimulated hepatic gene expression of acetyl-coenzyme A carboxylase and fatty acid synthase. In contrast, these changes were not seen in muscles (*MLD* and *MBF*) of lambs from Groups FO, CA, CASeY, and CASeVI (9).

Interestingly, the PUFA deposition in the liver is metabolically regulated and cannot be substantially influenced by additives added to the diet (Table 5), although a statistically insignificant increase in the concentration of ΣPUFAs, including n-3PUFAs and n-6PUFAs, was observed in the livers of lambs fed the CASeY diet. Moreover, the CASeY diet more efficiently increased the concentration of ΣLPUFAs, including n-3LPUFAs, in the liver than the CASeVI diet. Considering the above, we suggest that the CASeY diet exerts greater protective effect against peroxidation damage and/or catabolism of PUFAs than the CASeVI diet. The SeY-supplemented diet (Group CASeY) more effectively decreased the ratio of MDA/ΣPUFAs in the liver (Group CASeY) than the SeVI-supplemented diet (Group CASeVI; Table 3). Interestingly, all diets containing CA more efficiently stimulated the accumulation of ΣPUFAs, including ΣLPUFAs, than the RO and FO diets. Supplementation of the diet with CA and Se as SeVI or especially SeY resulted in the highest increase in the concentrations of SPUFAs, including ΣLPUFAs (like DHA; Table 5). Concomitantly, the CA diet revealed weaker protective effect against peroxidation damage and/or catabolism of PUFAs than the CASeY or CASeVI diet (Table 3).

Supplementation of the diet with CA, irrespective of the presence of SeY or SeVI, increased the nutritional value of the *MLD* (9) and the liver, as the n-6PUFAs/n-3PUFAs ratio decreased and the ΣIAAs/ΣDAAs ratio increased as compared to the RO and FO diets (Tables 5 and 6). Furthermore, the CASeVI diet most effectively decreased the n-6PUFAs/n-3PUFAs ratio and most effectively increased the ΣIAAs/ΣDAAs ratio in the liver. On the other hand, the FO diet most efficiently decreased the concentration of ΣAAs and the ΣIAAs/ΣDAAs ratio in the liver. Moreover, the FO diet most effectively reduced the concentration of *homo*-cysteine in the liver, while the RO and CASeY diets most efficiently increased the concentration of *homo*-cysteine in the liver. Considering the above, we suggest that the FO diet reduced the risk of blood clots, heart attacks, and strokes, whereas the RO and CASeY diets increased the risk of appearance of these diseases. Hyperhomocysteinemia has been correlated with the occurrence of blood clots, heart attacks, strokes, and Alzheimer disease. Moreover, hyperhomocysteinemia has also been associated with early pregnancy loss and with neural tube defects (27).

4.3. Conclusion

The current studies are important to the understanding of factors that influence the concentrations of fatty acids, tocopherols, cholesterol, and amino acids in the ruminant liver. Our investigation documented that the CASeVI diet most efficiently improved animal performance by reducing lamb emissions of CH_4 and CO_2 (the greenhouse gases). Moreover, our studies indicated that this diet most effectively improved the nutritional value of the liver and muscles of lambs as the concentrations of cholesterol, atherogenic SFA, and thrombogenic SFA decreased, whereas the concentration of PUFAs increased. In addition, the CASeVI diet most efficiently reduced the lipid peroxidation in the liver. All diets containing CA, especially with SeY or SeVI, stimulated the accumulation of all tocopherols in the liver. Interestingly, all diets including FO, regardless of the addition of CA, SeY, or SeVI, stimulated the accumulation of ΣFAs, whereas they reduced the accumulation of ΣAAs in the liver.

Further studies are necessary to determine if the addition of SeY or SeVI to diets with CA and FO induces changes in the concentration of PUFAs, especially n-3LPUFAs, in other lamb internal organs. Moreover, further studies are necessary to evaluate the supplementation effect with SeY or SeVI on oxidative stress markers in other tissues

of lambs fed diets including CA and FO. Our studies provide useful information for nutritionists carrying out further investigations aimed at improving farm animal health, performance, and the nutritional quality of feed for ruminants.

Acknowledgments

The authors would like to thank the National Science Centre (Poland), which partly supported our research (Grant No. 2013/09/B/NZ9/00291). We thank Ms Grażyna Oktaba for her technical help.

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