Diets enriched in fish and rapeseed oils, carnosic acid, and different chemical forms of selenium affect fatty acid profile in the periintestinal fat and indices of nutritional properties of selected tissues of lambs

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The aim of our study was to investigate the impact of carnosic acid (CA), selenate (^{VI}Se) or selenized yeast (^{Y}Se) on concentrations of fatty acids (FA), tocopherols, cholesterol and malondialdehyde in the periintestinal fat (PIF) and muscles of lambs. Male lambs were fed the control diet containing rapeseed (RO) and fish (FO) oils, the CA diet containing RO, FO and CA, the ^{Y}Se-CA diet with RO, FO, CA and ^{Y}Se, and the ^{VI}Se-CA diet with RO, FO, CA and ^{VI}Se. The experimental diets with CA, irrespective of the presence of ^{Y}Se or ^{VI}Se, decreased sums of saturated FA (SFA) and the thrombogenic SFA in the PIF compared to the control. The experimental diets increased the Δ9-desaturation capacity in the PIF compared to the control. The experimental diets with ^{Y}Se or ^{VI}Se reduced sums of long-chain polyunsaturated FA in the PIF compared to the control and CA diets. The PIF and muscles of lambs fed the ^{VI}Se-CA diet were characterised by the highest hypocholesterolemic/hypercholesterolemic-FA ratio, and lower modified atherogenic index compared to the control.

Key words: ovine periintestinal fat, muscles, tocopherols, cholesterol, oxidative stress, modified atherogenic index

Introduction

The oxidation of polyunsaturated fatty acids (PUFA), cholesterol and proteins in tissues is one of the most prevalent modifications caused by reactive oxygen and nitrogen species (ROS and RNS) in animal organisms (Saheem et al. 2017). Numerous studies have indicated that ruminants' tissues are susceptible to oxidation of lipid unsaturated fatty acids (UFA), sulphur-amino acids in proteins or cholesterol in cell membranes (Krajewska-Bienias et al. 2017, Morán et al. 2017, Przybylski et al. 2017). Dietary antioxidants (like seleno-compounds, polyphenols, carotenoids, lycopene or tocopherols) may prevent or delay some types of oxidative damage in tissues of animals and humans (Choe and Min 2009, Rozbicka-Wieczorek et al. 2014, El-Ramady et al. 2015, Gargiulo et al. 2017). In fact, studies have shown that selenium (Se) exerts its antioxidative properties through Se-proteins/enzymes of which there may be more than 35 in mammals (Edens and Sefton 2016, Collins 2017). Recent studies have shown that Se is not only an essential part of antioxidants but also a regulator of gene expression (Juszczuk-Kubiak et al. 2016, Zhao et al. 2016). For instance, Se supplementation promotes higher levels of the gene expression of the lipoprotein lipase and apolipoprotein E, particularly in skeletal muscle and possibly in fatty acid utilisation and triacylglyceride metabolism; as a consequence, dietary Se alters lipid metabolism and protein synthesis in the tissues of mammals. Moreover, Se-enzymes suppress pro-inflammatory cell metabolisms by reducing oxidative degradation in intracellular fluid; therefore, Se-compounds have been found to improve immunity in mammals (Raman 2000, El-Ramady et al. 2015). Endogenous Se is present in the tissues and fluids of animals and humans mostly as Se-cysteine (Se-Cys), which is the crucial functional site of ~35 Se-proteins/enzymes (like thioredoxin reductases, isozymes of the glutathione-peroxidase family or iodothyronine deiodinases) or Se-methionine (Se-Met), which can be bound non-specifically to Se-Met-proteins (Collins 2017). It is worth stressing that organic chemical forms of Se (like selenized yeast) are more efficiently incorporated in rumen microbiota and tissues of ruminants compared to selenate (^{VI}Se) or selenite (^{IV}Se) (Navarro-Alarcon and Cabrera-Vique 2008, Čobanová et al. 2017, Czauderna et al. 2018). Compared to ^{VI}Se, ^{IV}Se efficiently reacts with dietary components and metabolites of ruminal microbiota (especially those with thiol groups and R-S-S-R', etc. (Czauderna and Samochocka 1981), as well as ^{IV}Se is directly reduced to elemental Se (^{o}Se) by ruminal microorganisms. Unfortunately, ^{o}Se is unreactive in the anaerobic ruminal environment (Romero-Pérez et al. 2010). Similarly, Se absorption in mammals' tissues is significantly higher from dietary ^{VI}Se than from dietary ^{IV}Se (Van Dael et al. 2001). But most importantly, concern has been raised about the potential pro-oxidative properties of ^{IV}Se and its lower stability in comparison with ^{VI}Se when added to diets (Van Dael et al. 2001).

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Recent studies have indicated that inorganic and organic Se-compounds affect microbial populations and microorganism activity (Ošt'ádalová 2012, Kišidayová et al. 2014, Čobanová et al. 2017), as well as fatty acid (FA) metabolism (like enzymatic isomerisation and biohydrogenation of UFA) in the rumen of ruminants or in in vitro incubated ruminal fluids (Czauderna et al. 2012a, 2012b, 2014, 2015, 2018, Miltko et al. 2016, Rozbicka-Wieczorek et al. 2016a, 2016b, 2016c, Białek et al. 2020). Our previous studies have indicated that dietary carnosic acid (CA), selenized yeast (^{Y}Se) or ^{VI}Se affect the concentrations of FA, total cholesterol (TCh), tocopherols and malondialdehyde (MDA, the marker of PUFA per-oxidation) in the liver, brain, muscles, blood and subcutaneous fat of lambs (Czauderna et al. 2009b, 2011, 2012a, 2012b, Rozbicka-Wieczorek et al. 2016b, 2016c). Moreover, recent studies have shown that CA (a catecholic diterpene) is used as an antioxidant and preservative in foods of animal origin (Rozbicka-Wieczorek et al. 2016b, 2016c, Morán et al. 2012a, 2012b, 2013, 2017, Ortuño et al. 2017). In fact, CA protects cellular biomolecules (like proteins, lipids, RNA and DNA) against chemical stressors, such as reactive oxygen species (ROS), paraquat (an agrochemical) or 6-hydroxydopamine. Moreover, an earlier study has reported the inhibition of pro-inflammatory mediator secretion by lipopolysaccharide-stimulated macrophages using high doses of CA (Hadad and Levy 2012). Recent studies have also shown that dietary CA can modify a rumen microorganism's profile, resulting in changes of microbiota metabolism, and the yield of biohydrogenation (BH) of UFA in the rumen (Jordán et al. 2013, Miltko et al. 2016, Rozbicka-Wieczorek et al. 2016c). For instance, CA, being rich in *Rosmarinus officinalis* L. leaves, decreased the ruminal abundance of protozoa, archaea, *Prevotella* spp., *Ruminococcus albus* and *Clostridium aminophilum*, whereas increased (though only numerically) the abundance of *Ruminococcus flavefaciens* (Cobellis et al. 2016). Interestingly, CA has the most powerful antioxidant potency among diterpenes (like carnosol, rosmanol or *iso*-rosmanol) in rosemary (Masuda et al. 2001). CA (a natural antioxidant) has a typical o-diphenol structure, so it is easily oxidised to carnosol, the product of the oxidative biotransformation of CA (Masuda et al. 2001, Johnson 2011, Ortuño et al. 2017). Considering the above, special attention should be paid to CA as a dietary supplement. We intend to examine the effect of one natural diterpene (not a mixture of diterpenes) with or without Se-compounds on FA and tocopherols assimilation in the periintestinal fat (PIF) of lambs. The PIF belongs to visceral fat that is stored within the abdominal cavity around a number of very important internal organs (such as the intestines, the pancreas and the liver). Storing higher amounts of visceral fat (like the PIF), however, is associated with increased risks of a number of health problems, much more so than subcutaneous fat. Considering all of the above, we hypothesise that the inclusion of CA (a lipophilic antioxidant) with/without Se (the essential part of Se-antioxidants) in a diet with rapeseed oil (RO) and fish oil (FO) may increase the contents of tocopherols and UFA, and especially the highly unsaturated long chain PUFA (LPUFA), in the PIF and in other selected tissues of lambs. Moreover, we hypothesise that CA and Se (as ^{Y}Se or ^{VI}Se) simultaneously added to the lambs' diet enriched in FO would reduce contents of MDA, as well as increase the values of the hypocholesterolemic/hypercholesterolemic FA ratio in the PIF and other tissues. Thus, we expected that CA and ^{Y}Se or ^{VI}Se added to a diet with RO and especially FO (rich in n-3LPUFA) would improve animal health and welfare.

Therefore, the main aim of our study is focused on animal nutrition (especially FA and tocopherols assimilation by lambs). Considering the above, we plan to investigate the effect of CA and the different chemical forms of Se (as ^{Y}Se and ^{VI}Se) added to a basal diet with RO and FO (rich in n-3LPUFA) on the contents of selected FA (especially n-3LPUFA), tocopherols, TCh and MDA, as well as on values of the hypocholesterolemic/hypercholesterolemic FA ratio, and the atherogenic and thrombogenic indices in the PIF of lambs.

Materials and methods

Animals, housing, experimental design, diets, management and sampling

Lamb welfare guidelines and all handling procedures accepted by the 3rd Local Commission of Animal Experiment Ethics at the University of Life Sciences (Warsaw, Poland) were strictly followed throughout the preliminary period and for the duration of the experiments.

Twenty-four male Corriedale lambs with an average initial body weight (BW) of 30.4±2.5 kg were individually penned as described in our previous publication (Czauderna et al. 2017). All experiments on the lambs and tissue collections were carried out at The Kielanowski Institute of Animal Physiology and Nutrition (Jabłonna, Poland). Briefly, during a 3-week preliminary period, the lambs were fed the basal diet (BD) (the standard concentrate–hay diet with mineral premix and vitamins; Table 1) (NRC 2007) enriched in 2% RO and 1% odourless FO (Table 2). The content of Se in the basal diet was 0.16 mg of Se in 1 kg of BD. After the preliminary period, the lambs were divided into 4 groups of 6 animals; a 35-day experiment was conducted, during which animals were fed the basal diet supplemented with 2% RO and 1% FO (the control diet) or with 2% RO, 1% FO and antioxidant(s) (i.e. 0.1% CA and/or 0.35 mg of Se as ^{Y}Se or ^{VI}Se in 1 kg of BD) (Table 3). About 83% of the total Se-amount of dietary ^{Y}Se is found in the chemical form of Se-Met, whereas 5% of Se is in the form of Se-Cys incorporated into the proteins of *Saccharomyces cerevisiae*.

Table 1. Chemical composition (%) of the concentrate-hay diet (the basal diet) with vitamins and mineral mixture[1] fed to lambs

Item	Meadow hay[3]	Concentrate[2]		
		Barley meal	Soybean meal	Wheat starch
Dry matter	88.4	87.6	89.7	87.3
Crude protein	9.50	9.94	41.81	0.90
Crude fibre	27.29	2.87	4.34	–
Crude fat	3.40	2.50	2.25	0.09
Ash	4.85	1.84	6.16	0.12
Neutral detergent fiber	59.17	18.02	18.81	–
Acid detergent fiber	32.08	4.61	6.44	–
Acid detergent lignin	4.47	1.14	1.49	–

[1] 1 kg of the mineral and vitamin mixture comprised: 285 g Ca, 16 g phosphorus, 56 g Na, 42 mg Co as carbonate, 10 mg iodine as iodate, 1 g Fe as sulphate, 6 mg Se as selenite, 0.5 g Cu as sulphate, 5.8 g Mn as sulphate, 7.5 g Zn as sulphate; vitamins: A (500000 IU kg^{-1}), D3 (125000 IU kg^{-1}), and E as α-tocopherol (25000 IU kg^{-1}). 20 g of the mineral and vitamin mixture was added to 1 kg of the basal diet (BD); [2] The gross energy (MJ per kg of dry matter [DM]): barley meal: 16.3, soybean meal: 17.8, wheat starch: 16.7; [3] the gross energy: 17.1 MJ per kg of DM

Table 2. The concentrations (mg kg^{-1}) of the main fatty acids in the components of the lambs' diet: concentrate, meadow hay, rapeseed oil (RO) and odourless fish oil (FO)[1]

Fatty acids	Concentrate	Meadow hay	RO	FO
C8:0	-	83	-	-
C12:0	-	142	-	82
C14:0	104	239	56	12345
C15:0	-	-	-	477
*c9*C14:1	-	131	-	215
C16:0	3189	4034	13091	56947
*c7*C16:1	-	-	-	318
*c*9C16:1	-	184	33	420
∑C16:2	-	-	-	15586
C17:0	-	-	-	493
*c9*C17:1	-	-	-	193
C18:0	1425	459	5490	9452
*c*6C18:1	-	-	6	188
*c7*C18:1	-	-	-	842
*c*9C18:1	774	1266	385859	290592
*c12*C18:1	-	72	786	15834
*c14*C18:1	-	-	-	159
C18:2n-6 (LA)	29163	13100	282394	114512
C18:3n-3 (αLNA)	1014	4178	83474	20968
*c7c9c12c15*C18:4	-	-	-	473
C20:0	-	58	430	-
*c11*C20:1	-	74	-	24206
*c11c14*C20:2	-	-	-	2270
*c8c11c14*C20:3	-	-	-	258
C20:4n-6 (AA)	-	-	-	304
*c8c11c14c17*C20:4	-	-	-	607
C20:5n-3 (EPA)	-	-	-	6792
C22:0	-	101	153	139
*c11*C22:1	-	-	-	1704
*c13*C22:1	-	-	-	11036

*c13c16*C22:2	-	-	-	95
*c7c10c13c16*C22:4	-	-	-	144
DPA	-	-	-	1560
DHA	-	-	-	26570
C24:0	-	69	-	-
*c15*C24:1	-	71	61	397

[1] The iodine value of FO: 50–65 g/100 g FO; the acid value of FO: 20 mg KOH g^{-1} FO. The energy content of FO and RO was 36.8 and 37.0 MJ kg^{-1} oil, respectively.

All details of the composition (including fatty acid compositions) of the control and experimental diets and rapeseed oil were presented in recent publications by the authors (Czauderna et al. 2017, 2018). The Se status of lambs fed the control and experimental diets has been presented in our latest papers (Czauderna et al. 2017, 2018).

The control and experimental diets were offered in two equal meals, as described previously (Czauderna et al. 2017); drinking water for animals was available *ad libitum*. The initial BW of lambs and body weight gain (BWG) are summarised in Table 3. After the 35-day experiment (i.e. at 0700–0800 h), all lambs were deprived of consciousness by intramuscular injections of xylazine (0.2–0.4 mg kg^{-1} of BW) and then slaughtered (Czauderna et al. 2017). The lambs were slaughtered in accordance with the European Union Council Regulations (EC) No 1099/2009 dated 24.09.2009 for the protection of animals at the time of slaughter in small experimental slaughterhouses. Next, tissues of PIF (78±9 g), subcutaneous fat (SCF) (92±11 g), *musculus biceps femoris* (*MBF*) (174±14 g) and *musculus longissimus dorsi* (*MLD*) (653±77 g) were immediately removed from each lamb. All samples were homogenised straight away using a tissue homogeniser (IKA®T18 basic, Ultra-Turrax®, Germany). The contents of MDA were determined in freshly homogenised PIF samples (Czauderna et al. 2011). All homogenised tissue samples were transferred into tightly sealed vessels and stored at −32 °C for further chromatographic analyses. All samples were analysed individually. The contents of FA, TCh, MDA and tocopherols in tissue samples were expressed per g of fresh matter.

Table 3. The experimental scheme, the chemical composition of the control and experimental diets, the initial body weight ($BW_{initial}$) and the body weight gain (BWG, kg) of lambs

Group [1]	Additives added to the basal diet	$BW_{initial}$ kg	BWG [2] kg
Control group [3]	2% RO and 1% FO (The control diet)	30.6±2.4	$7.2±0.3^{ab}$
CA group [3]	2% RO, 1% FO and 0.1% CA (The CA diet)	30.6±2.6	$6.6±0.3^{a}$
^{Y}Se-CA group [4]	2% RO, 1% FO, 0.1% CA and 0.35 mg of Se as ^{Y}Se in 1 kg of BD (The ^{Y}Se-CA diet)	30.3±2.7	$6.6±0.3^{a}$
^{VI}Se-CA group [4]	2% RO, 1% FO, 0.1% CA and 0.35 mg of Se as ^{VI}Se in 1 kg of BD (The ^{VI}Se-CA diet)	30.3±3.0	$8.2±0.4^{b}$

BWG = body weight gain; BD = basal diet; RO = rapeseed oil; FO = fish oil; ^{Y}Se = selenized yeast; ^{VI}Se = selenate; $BW_{initial}$ = the initial body weight of lambs after the preliminary period; [a, b] Different letters within a column indicate significant differences at $p < 0.05$; [1] For the 3-week of preliminary period lambs were fed the diet with 2% RO and 1% FO. [2] The average body weight gain (BWG, kg) of lambs fed the control or experimental diets for 35 days of the experimental period: BWG = (BW_{35days} – $BW_{initial}$), where BW_{35days} - the body weight of lambs after 35 days of experiment. The average daily diet intake was 1.08 kg per lamb. [3] The concentration of selenium in the control and CA diets was 0.16 mg of Se in 1 kg of diets. [4] The concentration of selenium in the ^{Y}Se-CA and ^{VI}Se-CA diets was 0.51 mg of Se in 1 kg of diets.

Chemicals and analytical methods

Commercial rapeseed oil and odourless fish oil (rich in highly unsaturated LPUFA) were purchased from Company AGSOL (Pacanów, Poland), CA was purchased from Hunan Geneham Biomedical Technology Ltd (Changsha Road, Changsha, Hunan, China), while selenized yeast (Se-*Saccharomyces cerevisiae*) was donated by Sel-Plex (Alltech Inc., Nicholasville, KY, USA). The vitamin and mineral premix (ID number: aPL 1405002p) was purchased from POLFAMIX OK (Grodzisk Mazowiecki, Poland).

Methanol (≥ 99.9%), HPLC-acetonitrile (≥ 99.9%) and n-hexane (≥ 99%) were purchased from Lab-Scan (Dublin, Ireland). A conjugated linoleic acid (CLA) isomer mixture, a nonadecanoic acid (as the internal standard) and other

37 fatty acid standard mixture (FAME), α-tocopherol, α-tocopheryl acetate, cholesterol, sorbic acid, 2,6-di-*tert*-butyl-*p*cresol, 25% aqueous 1,5-pentanedialdehyde solution, 2,4-dinitrophenylhydrazine (containing ~30% water), 1,1,3,3-tetramethoxypropane (99%), trichloroacetic acid and 25% BF_3 in methanol were obtained from Sigma-Aldrich (St Louis, MO, USA). KOH, NaOH, Na_2SO_4, chloroform and dichloromethane were purchased from Avantor Performance Materials (Gliwice, Poland). All other chemicals were of analytical grade.

Preparation of fatty acid methyl esters (FAME) in ovine tissues

The homogenised samples of the PIF (~10 mg), SCF (~10 mg) and muscles (40–50 mg) were saponified using a KOH solution according to methods described by Czauderna et al. (2007); nonadecanoic acid (as the internal standard) was added to each saponified biological sample. Then, mild base- and acid-catalysed methylations were introduced for the preparation of FAME in processed biological samples (Czauderna et al. 2007). Fatty acids (as FAME) in assayed biological samples were then determined using capillary-gas chromatography with mass spectrometry according to the methods presented by Rozbicka-Wieczorek et al. (2014). All analyses were performed on a Shimadzu GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica column (120 m [length] × 0.25 mm [i.d.] × 0.25 µm [film thickness]) and a quadruple mass selective detector (Model 5973 N). FAME identification was validated based on the electron impact ionisation spectra of FAME and compared to authentic FAME standards and the NIST 2007 reference mass spectra library (National Institute of Standard and Technology, Gaithersburg, MD, USA) (Rozbicka-Wieczorek et al. 2014).

Determination of tocopherols, TCh and MDA in ovine tissues

TCh, α-tocopherol (αT), δ-tocopherol (δT), γ-tocopherol (γT) and α-tocopheryl acetate (αTAC) were quantified in the homogenised samples of the PIF, SCF and muscles (*MLD* and *MBF*) using a liquid chromatographic system (SHIMADZU, Tokyo, Japan) according to methods described by Czauderna et al. (2009a). The liquid chromatographic instrument used consisted of an ultra-fast liquid chromatography system, incorporating two pumps, an autosampler, a CBM-20A communications bus module, a column oven, a Kinetex C18-column (2.6 µm; Hydro-RP, 150 mm × 2.1 mm; Phenomenex, Torrance, CA, USA) in conjunction with a guard column, a degasser and a photodiode array detector (Białek and Czauderna 2019).

The concentration of MDA in the PIF samples was determined after saponification followed by derivatisation according to methods described by Czauderna et al. (2011). The chromatographic separations of derivatised MDA from endogenic species of the processed PIF samples were conducted using an ultra-fast liquid chromatography system and a photodiode array detector (Czauderna et al. 2011).

Atherogenic and thrombogenic indices were calculated according to the equations given by Morán et al. (2013). The hypocholesterolemic/hypercholesterolemic fatty acid (h/H-Ch) ratio was calculated using the equation given by Fernández et al. (2007).

Statistical analysis

All statistical analyses of the effects of dietary additives were carried out using the Statistica 12.5 PL software package (StatSoft Inc., Tulsa, OK, USA). Differences were considered significant at $p < 0.05$. The obtained results are shown as means and SEM (standard error of mean). The influence of dietary modifications on the contents of analytes in all biological samples for variables with normal distribution was tested with one-way ANOVA and the post-hoc Honestly Significant Difference (HSD) Tukey test. For variables without normal distribution, the results were tested with Kruskal-Wallis, which is a non-parametric equivalent of one-way ANOVA, with a post-hoc multiple comparison test.

Results

Concentrations of selected saturated fatty acids (SFA) in the PIF of lambs

Experimental data reflecting the contents of SFA in the PIF of lambs fed the experimental diets are summarised in Table 4. We found that the diets enriched in CA, irrespective of the presence of ^{Y}Se or ^{VI}Se, resulted in a decrease in the contents of C8:0, C15:0, C17:0, C18:0, C20:0, C22:0 and the sum of thrombogenic SFA (T-SFA) in the PIF compared to the control diet. Moreover, the CA and ^{VI}Se-CA diets reduced the concentration of C16:0 in the PIF as compared to the control diet. All experimental diets reduced the content sum of SFA (ΣSFA) in the PIF in

comparison with the control diet. Moreover, the experimental diet with CA, irrespective of the presence of ^{Y}Se or ^{VI}Se, decreased the ratios of ΣSFA/ΣUFA and ΣSFA/ΣFA in the PIF compared to the control diet. Conversely, all experimental diets increased the contents of C21:0 and C23:0 in the PIF compared to the control diet. The diet with ^{VI}Se caused the highest increase in the content of C21:0 in the PIF compared to the control and other experimental diets. All experimental diets had no effect on the contents of C11:0, C12:0, C13:0, C14:0, the atherogenic SFA (A-SFA) and the sum of medium-chain SFA (Σ_{medium}SFA) in the PIF.

The content ratio of A-SFA to the sum of FA (A-SFA/ΣFA) in the PIF of lambs fed the ^{VI}Se-CA diet was lower than in the PIF of lambs fed the control, CA and ^{Y}Se-CA diets. The CA and ^{Y}Se-CA diets elevated the values of the A-SFA/ΣFA ratio in the PIF compared to the control diet. To the contrary, all experimental diets, especially the ^{Y}Se-CA diet, reduced the values of the T-SFA/ΣFA ratio in the PIF in comparison with the control diet.

Table 4. The concentrations (mg g^{-1} PIF) of selected individual saturated fatty acids (SFA), atherogenic-SFA[1] (A-SFA), thrombogenic-SFA[2] (T-SFA), the sums of medium-chain SFA (Σ_{medium}SFA)[3], all assayed SFA (ΣSFA)[4] and the ratios of ∑SFA to the sum of PUFA (ΣSFA/∑PUFA), UFA (∑SFA/ΣUFA) and all assayed FA (∑SFA/∑FA) in the periintestinal fat (PIF) of lambs

Item	Additive	–	CA	^{Y}Se + CA	^{VI}Se + CA	SEM	*p*-value
	Group	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
C8:0		0.071^{b}	0.041^{a}	0.039^{a}	0.040^{a}	0.002	0.03
C10:0		2.22^{b}	1.88^{a}	1.89^{a}	1.93^{ab}	0.07	0.03
C11:0		0.017	0.021	0.015	0.019	0.005	0.31
C12:0		3.16	2.97	3.38	3.23	0.6	0.09
C13:0		0.119	0.106	0.099	0.124	0.009	0.27
C14:0		31. 93	30.79	36.86	30.12	1.18	0.17
C15:0		4.71^{c}	3.35^{a}	4.09^{b}	4.18^{b}	0.08	0.03
C16:0		172^{c}	152^{ab}	164^{bc}	146^{a}	3	0.03
C17:0		10.6^{b}	8.8^{a}	9.3^{a}	9.0^{a}	0.2	0.02
C18:0		226^{c}	185^{a}	186^{a}	197^{a}	5	0.03
C20:0		0.346^{b}	0.240^{a}	0.257^{a}	0.267^{a}	0.014	0.02
C21:0		0.001^{a}	0.073^{b}	0.178^{c}	0.245^{d}	0.014	0.00
C22:0		0.270^{b}	0.199^{a}	0.204^{a}	0.211^{a}	0.012	0.00
C23:0		0.0007^{a}	0.0092^{b}	0.0081^{b}	0.0084^{b}	0.0003	0.01
A-SFA		207	186	205	180	4	0.28
A-SFA/ΣFA		0.263^{b}	0.271^{c}	0.273^{c}	0.254^{a}	0.002	0.02
T-SFA		431^{b}	368^{a}	387^{a}	373^{a}	9	0.02
T-SFA/ΣFA		0.545^{c}	0.535^{b}	0.517^{a}	0.529^{b}	0.002	0.02
Σ_{medium}SFA		37.3	35.6	42.1	35.3	0.5	0.31
ΣSFA		451^{b}	386^{a}	407^{a}	393^{a}	8	0.02
ΣSFA/ΣUFA		1.311^{d}	1.279^{c}	1.185^{a}	1.244^{b}	0.012	0.03
ΣSFA/ΣFA		0.571^{c}	0.561^{b}	0.542^{a}	0.556^{ab}	0.002	0.04

SEM = standard error of the mean; UFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids. $^{a, b}$ Different letters within a row indicate significant differences at $p < 0.05$. [1] The sum: C12:0, C14:0 and C16:0; [2] The sum: C14:0, C16:0 and C18:0; [3] The sum: C8:0, C10:0, C12:0 and C14:0; [4] The sum: C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0 and C23:0

Concentrations of unsaturated fatty acids in the PIF of lambs

The influence of the experimental diets enriched in CA with or without Se (as ^{Y}Se or ^{VI}Se) on the contents of monounsaturated fatty acids (MUFA) and PUFA are presented in Tables 5 and 6. The results of our experiments demonstrated that ^{VI}Se added to the diet with CA reduced the contents of *c7*C16:1, *c9*C16:1, *c6*C18:1 and *c11*C20:1 in comparison with the control and ^{Y}Se-CA diets (Table 5). We found that the experimental diet supplemented only with CA resulted in a decrease in the contents of *c9*C14:1, *c7*C16:1, *c9*C16:1, *c6*C18:1 and *c11*C20:1 in the PIF compared to the control diet. Conversely, no noticeable differences in the content of *t11*C18:1 (TVA) and *c9*C18:1, the sum of all assayed MUFA (ΣMUFA) and the ratio of ΣMUFA/ΣFA were found between the control and all experimental groups of lambs.

Indices of Δ9-desaturation of C18:0 ($^{C18:1}$Δ9-index) and Δ9-desaturation of C16:0 and C18:0 (ΣΔ9-index) were higher in the PIF of lambs fed the experimental diets than in the PIF of the control lambs. Moreover, the diet supplemented with ^{Y}Se most efficiently elevated the $^{C18:1}$Δ9-index and ΣΔ9-index in the PIF. Similarly, all experimental diets, particularly those supplemented with ^{Y}Se or ^{VI}Se, increased the values of the PUFA elongase index (^{Elong}index) in the PIF compared to the control diet. To the contrary, all experimental diets, especially the ^{VI}Se-CA diet, reduced the index values of Δ9-desaturation of TVA (^{CLA}Δ9-index) in the PIF in comparison with the control diet. The values of Δ4-desaturation (Δ4-index) of *c7c10c13c16c19*C22:5 (DPA) were smaller in the PIF of lambs fed the experimental diets, including extra Se (as ^{Y}Se or ^{VI}Se), than in the PIF of lambs fed the control and CA diets.

Table 5. The concentrations (mg g^{-1} PIF) of selected individual monounsaturated fatty acids (MUFA), the concentration sum of all assayed MUFA (ΣMUFA)[1] and index values of Δ9-desaturases ($^{C18:1}$Δ9-index[2], ΣΔ9-index[3] and ^{CLA}Δ9-index[4]) in the periintestinal fat (PIF) of lambs

Item	Additive	–	CA	^{Y}Se + CA	^{VI}Se + CA	SEM	*p*-value
	Group	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
*c7*C14:1		0.11	0.10	0.09	0.07	0.02	0.21
*c9*C14:1		2.15^{c}	1.57^{a}	1.83^{b}	1.64^{ab}	0.11	0.02
*iso*C15:0		0.09	0.08	0.08	0.06	0.01	0.19
*c7*C16:1		5.52^{b}	4.75^{a}	6.70^{c}	4.63^{a}	0.180	0.03
*c9*C16:1		7.45^{b}	6.66^{a}	7.58^{b}	6.01^{a}	0.20	0.04
*c9*C17:1		0.34	0.19	0.27	0.29	0.07	0.31
*t9*C18:1		0.53	0.52	0.58	0.47	0.08	0.26
*t11*C18:1 (TVA)		3.67	3.10	3.91	3.65	0.17	0.18
*c6*C18:1		25.5^{b}	16.3^{a}	16.4^{a}	12.8^{a}	1.2	0.01
*c7*C18:1		5.1	5.3	3.4	4.6	0.2	0.18
*c9*C18:1		222	201	233	213	6	0.32
*c11*C18:1		0.91	0.79	0.66	0.82	0.6	0.41
*c12*C18:1		13.2	12.0	12.0	14.4	0.5	0.27
*c11*C20:1		2.27^{c}	1.53^{b}	1.50^{b}	1.36^{a}	0.13	0.02
*c11*C22:1		0.002	0.003	0.001	0.002	0.0003	0.48
*c13*C22:1		0.009^{c}	0.008^{c}	0.003^{b}	0.001^{a}	0.001	0.01
*c15*C24:1		0.0009	0.0007	0.0006	0.0008	0.0002	0.39
ΣMUFA		291	254	290	266	7	0.33
$^{C18:1}$Δ9-index		0.497^{a}	0.521^{b}	0.556^{c}	0.519^{b}	0.004	0.02
ΣΔ9-index		0.365^{a}	0.380^{b}	0.407^{c}	0.390^{b}	0.002	0.02
^{CLA}Δ9-index (CLA/TVA)		0.398^{d}	0.310^{c}	0.298^{b}	0.269^{a}	0.004	0.00
ΣMUFA/ΣFA		0.366	0.369	0.385	0.374	0.003	0.37

SEM = standard error of the mean; CLA = conjugated linoleic acid isomers. $^{a, b}$ Different letters within a row indicate significant differences at $p < 0.05$. [1] The sum: *c7*C14:1, *c9*C14:1, *iso*C15:1, *c7*C16:1, *c9*C16:1, *c9*C17:1, *t9*C18:1, *t11*C18:1, *c6*C18:1, *c7*C18:1, *c9*C18:1, *c11*C18:1, *c12*C18:1, *c11*C20:1, *c11*C22:1, *c13*C22:1 and *c15*C24:1. [2] $^{C18:1}$Δ9-index = *c9*C18:1/(*c9*C18:1 + C18:0). [3] ΣΔ9-index = (*c9*C16:1 + *c9*C18:1)/(*c9*C16:1 + C16:0 + *c9*C18:1 + C18:0). [4] ^{CLA}Δ9-index = *c9t11*CLA/(*c9t11*CLA + TVA) (see Table 4).

As can be seen from the results in Table 6, all experimental diets affected the contents of PUFA in the PIF. Indeed, the contents of *c9t11*CLA, *c11c14*C20:2 and *c5c8c11c14c17*C20:5 (EPA) were smaller in the PIF of lambs fed the experimental diets than the control diet. Moreover, the experimental diets supplemented with ^{Y}Se or ^{VI}Se considerably reduced the contents of *c11c14*C20:2, *c5c8c11c14*C20:4, *c5c8c11c14c17*C20:5 (EPA) and *c4c7c10c13c16c19*C22:5 (DHA) in the PIF, in comparison with the control and CA diets. In contrast, the diets with ^{Y}Se or ^{VI}Se caused a strong increase in the concentration sum of *ct/tc*CLA (∑*ct/tc*CLA) in the PIFcompared with the control and CA diets.

The experimental diets, regardless of the presence of ^{Y}Se or ^{VI}Se, had no effect on the contents of *c9c12*C18:2 (LA), *c6c9c12*C18:3 (γLNA), *c6c9c12c15*C18:4, *c8c11c14c17*C20:4, *c7c10c13c16*C22:4, *c7c10c13c16c19*C22:5 (DPA), the sums of n-6PUFA (Σn-6PUFA) and PUFA (ΣPUFA), and the values of the ratio of Σn-6PUFA to Σn-3PUFA (n-6/n-3) in the PIF. It was found that the values of the content ratio of ΣPUFA to ΣFA (ΣPUFA/ΣFA) were higher in the PIF of lambs fed the ^{Y}Se-CA or ^{VI}Se-CA diets than the control and CA diets. Alternatively, the diets with ^{Y}Se or ^{VI}Se reduced the content sums of n-6LPUFA (Σn-6LPUFA), n-3LPUFA (Σn-3LPUFA) and LPUFA (ΣLPUFA) in the PIF compared to the control and CA diets. As a consequence, the experimental diets with ^{Y}Se or ^{VI}Se reduced the ratio values of Σn-3LPUFA/ΣFA and ΣLPUFA/ΣFA in the PIF compared to the control and CA diets.

Table 6. The concentrations (mg g^{-1}) of *c9t11*CLA, other *ct/ct*CLA isomers (Σ*ct/tc*CLA)[1], the sum of CLA isomers (ΣCLA)[2], selected individual PUFA, the sums of n-6PUFA (∑n-6PUFA)[3], n-3PUFA (∑n-3PUFA)[4], n-6LPUFA (∑n-6LPUFA)[5], n-3LPUFA (∑n-3LPUFA)[6], LPUFA (∑LPUFA)[7], all PUFA (∑PUFA)[8], the ratios of ∑n-6PUFA to ∑n-3PUFA (n-6/n-3), ∑PUFA to ∑SFA (∑PUFA/∑SFA), ∑PUFA to ΣFA[9] (∑PUFA/∑FA) and ∑n-3LPUFA to ∑FA (∑n-3LPUFA/∑FA)[10], and elongation and Δ4-desaturation indices in the periintestinal fat (PIF) of lambs

Item	Additive	–	CA	^{Y}Se + CA	^{VI}Se + CA	SEM	*p*-value
	Group	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
*c9t11*CLA		2.55^{c}	1.43^{a}	1.72^{b}	1.48^{a}	0.09	0.02
Σ*ct/tc*CLA		0.019^{a}	0.021^{a}	0.527^{b}	0.824^{c}	0.20	0.00
Σ*tt*CLA		0.011^{ab}	0.009^{a}	0.013^{b}	0.016^{c}	0.001	0.03
ΣCLA		2.58^{c}	1.46^{a}	2.26^{b}	2.32^{bc}	0.10	0.04
*c9c12*C18:2 (LA)		50.0	44.7	50.6	47.1	1.0	0.37
*c6c9c9*C18:3 (γLNA)		0.043	0.027	0.033	0.040	0.009	0.51
*c9c12c15*C18:3 (αLNA)		3.40^{c}	3.04^{b}	3.21^{bc}	2.69^{a}	0.09	0.02
*c6c9c12c15*C18:4		0.015	0.011	0.012	0.009	0.003	0.18
*c11c14*C20:2		0.200^{c}	0.110^{b}	0.003^{a}	0.002^{a}	0.019	0.00
*c8c11c14c17*C20:4		0.017	0.009	0.012	0.013	0.003	0.21
*c5c8c11c14*C20:4 (AA)		0.339^{b}	0.357^{b}	0.219^{a}	0.240^{a}	0.017	0.03
*c5c8c11c14c17*C20:5 (EPA)		0.130^{c}	0.109^{b}	0.021^{a}	0.012^{a}	0.011	0.02
*c7c10c13c16*C22:4		0.010	0.013	0.015	0.017	0.004	0.32
*c7c10c13c16c19*C22:5 (DPA)		0.212	0.193	0.211	0.217	0.010	0.47
*c4c7c10c13c16c19*C22:5 (DHA)		0.035^{c}	0.030^{c}	0.001^{a}	0.006^{b}	0.003	0.02
Σn-6PUFA		50.6	45.1	50.9	47.3	1.1	0.13
Σn-3PUFA		3.65^{c}	3.26^{b}	3.42^{b}	2.91^{a}	0.09	0.04
Σn-6PUFA/Σn-3PUFA (n-6/n-3)		14.4^{a}	14.5^{a}	15.2^{b}	17.0^{c}	0.3	0.03
Σn-6LPUFA		0.339^{b}	0.357^{b}	0.219^{a}	0.240^{a}	0.010	0.02
Σn-3LPUFA		0.376^{c}	0.332^{b}	0.231^{a}	0235^{a}	0.012	0.02
Σn-3LPUFA/ΣFA		0.476^{c}	0.483^{d}	0.308^{a}	0.332^{b}	0.001	0.03
ΣLPUFA		0.715^{b}	0.689^{b}	0.450^{a}	0.475^{a}	0.032	0.04
ΣPUFA		56.8	49.9	56.5	52.5	1.1	0.47
ΣLPUFA/ΣFA		0.905^{c}	1.003^{d}	0.600^{a}	0.671^{b}	0.009	0.03
∑PUFA/∑FA		0.0721^{a}	0.0726^{a}	0.0754^{c}	0.0737^{b}	0.0002	0.03
Δ4-index [11]		0.099^{c}	0.110^{d}	0.003^{a}	0.019^{b}	0.010	0.01
$^{EPA\text{-}Elong}$index [12]		0.620^{a}	0.638^{b}	0.909^{c}	0.948^{d}	0.003	0.02

SEM = standard error of the mean. $^{a, b}$ Different letters within a row indicate significant differences at $p < 0.05$. [1] The sum of *ct/tc*CLA isomers: *cis-trans*CLA: *11-13, 12-14*; *trans-cis*CLA: *7-9, 8-10, 9-11, 10-12, 11-13* and *12-14*. [2] The sum: *c9t11*CLA, *ct/tc*CLA isomers, *tt*CLA isomers (*trans-trans*: 7-7, *8-10, 9-11, 10-12, 11-13* and *12-14*) and *cc*CLA isomers (*cis-cis*: *8-10, 9-11, 10-12* and *c11-12*). [3] The sum: LA, *c6c9c12*C18:3 *c11c14*C20:2, AA and *c7c10c13c16*C22:4. [4] The sum: αLNA, *c6c9c12c15*C18:4 and Σn-3LPUFA. [5] The sum: *c11c14*C20:2, AA and *c7c10c13c16*C22:4. [6] The sum: *c8c11c14c17*C20:4, EPA, DPA and DHA. [7] The sum: Σn-6LPUFA and Σn-3LPUFA. [8] The sum: ΣCLA, Σn-3PUFA and Σn-6PUFA. [9] The sum of all fatty acids (ΣFA). [10] The concentration ratio of ∑n-3LPUFA (μg/g PIF) to ∑FA (mg/g PIF). [11] Δ4-index = *c4c7c10c13c16c19*C22:6/(*c4c7c10c13c16c19*C22:6 + *c7c10c13c16c19*C22:5). [12] $^{EPA\text{-}Elong}$index = *c7c10c13c16c19*C22:5/(*c7c10c13c16c19*C22:5 + *c5c8c11c14c17*C20:5).

Concentrations of TCh, tocopherols and MDA, and values of atherogenic, thrombogenic and hypocholesterolemic indices in selected tissues of lambs

The experimental results reflecting the concentrations of TCh, δ-tocopherol (δT), γ-tocopherol (γT), α-tocopherol (αT), α-tocopheryl acetate (αTAc) and MDA are summarised in Table 7. The diets enriched in CA with or without ^{VI}Se reduced the content of TCh in the PIF compared to the control and ^{Y}Se-CA diets. In sheep fed the diet enriched with CA and Se (as ^{Y}Se or ^{VI}Se), the contents of δT and γT in the PIF decreased in comparison with the control and CA diets. In contrast, the contents of αT and αTAc were higher in the PIF of lambs fed the diets enriched with ^{Y}Se or ^{VI}Se than in the PIF of the control lambs. The contents of αT, and the sums of αT and αTAc (∑(αT+αTac)) and all assayed tocopherols (ΣTs) were higher in the PIF of lambs fed the ^{Y}Se-CA diet than the control and ^{VI}Se-CA diets.

The experimental diets, especially the CA diet, reduced the content of MDA and the PUFA peroxidation index (MDA_{index}) in the PIF compared to the control diet. Moreover, the content of MDA was smaller in the PIF of lambs fed the diet with ^{VI}Se than in the PIF of animals fed the diet with ^{Y}Se. Interestingly, we found that the lowest level of MDA, and the value of MDA_{index} was in the PIF of lambs fed the CA diet.

Table 7. The concentrations (μg g^{-1} PIF) of total cholesterol (TCh), tocopherols and MDA (ng g^{-1} PIF)[1] and values of the PUFA peroxidation index (MDA_{index}) in the periintestinal fat (PIF) of lambs fed the control and experimental diets

Item	Additive	–	CA	^{Y}Se + CA	^{VI}Se + CA	SEM	*p*-value
	Group	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
TCh		72.3^{c}	42.7^{a}	71.8^{c}	53.9^{b}	0.2	0.04
δ–tocopherol (δT)		1.48^{b}	1.38^{b}	1.17^{a}	1.17^{a}	0.01	0.02
γ–tocopherol (γT)		1.17^{b}	1.17^{b}	0.97^{a}	0.95^{a}	0.01	0.02
α–tocopherol (αT)		2.38^{a}	2.45^{ab}	3.20^{c}	2.51^{b}	0.02	0.03
α–tocopheryl acetate (αTAc)		3.47^{a}	4.50^{c}	4.04^{b}	3.81^{b}	0.02	0.02
Σ(αT+αTAc) [2]		5.85^{a}	6.95^{c}	7.24^{c}	6.32^{b}	0.02	0.01
ΣTs [3]		8.52^{a}	9.48^{b}	9.38^{b}	8.42^{a}	0.05	0.04
MDA,		5.08^{d}	1.74^{a}	4.50^{c}	3.17^{b}	0.04	0.02
MDA_{index} [4]		0.106^{c}	0.036^{a}	0.080^{b}	0.077^{b}	0.002	0.03

SEM = standard error of the mean; MDA = malondialdehyde; $^{a, b}$ Different letters within a row indicate significant differences at $p < 0.05$. [1] Concentrations of MDA were determined immediately after the homogenization of PIF samples. [2] The sum: αT and αTAc; [3] The sum: δT, γT, αT and αTAc; [4] The concentration ratio of MDA (ng g^{-1} PIF) to ΣPUFA (mg g^{-1} PIF); MDA_{index} = MDA/ΣPUFA.

Investigation of the impact of the contents of tocopherols, A-SFA, n-6PUFA and n-3PUFA in the PIF on the atherogenic index showed that all experimental diets, especially the diet with ^{Y}Se, reduced the value of our modified atherogenic index ($_{index}A^{SFA+Toc}$) in the PIF, subcutaneous fat (SCF), *MLD* and *MBF* in comparison with the control diet (Table 8).

Table 8. The ∑PUFA/∑SFA ratio (∑PUFA/∑SFA), thrombogenic ($_{index}T^{SFA}$)[1] and atherogenic ($_{index}A^{SFA}$)[2] indices in the PIF of lambs. The hypo-cholesterolemic/hypercholesterolemic fatty acid (h/H-Ch) ratio[3] and the modified atherogenic index ($_{index}A^{SFA+Toc}$)[4] in the PIF, SCF, *musculus longissimus dorsi* (*MLD*) and *musculus biceps femoris* (*MBF*) of lambs fed the control and experimental diets

Item	Additive	–	CA	^{Y}Se + CA	^{VI}Se + CA	SEM	*p*-value
	Group	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
				the periintestinal fat (PIF)			
∑PUFA/∑SFA		0.127^{a}	0.130^{a}	0.140^{c}	0.134^{b}	0.001	0.02
h/H-Ch ratio		1.355^{a}	1.361^{a}	1.429^{b}	1.494^{c}	0.017	0.03
$_{index}T^{SFA}$		2.124^{c}	2.068^{b}	1.933^{a}	2.068^{b}	0.017	0.03
$_{index}A^{SFA}$		0.888^{b}	0.923^{c}	0.918^{c}	0.859^{a}	0.014	0.03
$_{index}A^{SFA+Toc}$		0.104^{c}	0.092^{b}	0.087^{a}	0.094^{b}	0.001	0.02
				the subcutaneous fat (SCF)			
$_{index}A^{SFA+Toc}$		0.071^{c}	0.066^{b}	0.062^{ab}	0.059^{a}	0.001	0.03
h/H-Ch ratio		1.791^{b}	1.796^{b}	1.733^{a}	1.831^{c}	0.014	0.02
				musculus longissimus dorsi (*MLD*)			
$_{index}A^{SFA+Toc}$		0.249^{c}	0.096^{ab}	0.085^{a}	0.102^{b}	0.002	0.01
h/H-Ch ratio		1.653^{a}	1.734^{b}	1.665^{a}	1.841^{c}	0.012	0.03
				musculus biceps femoris (*MBF*)			
$_{index}A^{SFA}$		0.662^{b}	0.716$^{c\ 5}$	0.719$^{c\ 5}$	0.617$^{a\ 5}$	0.011	0.03
$_{index}A^{SFA+Toc}$		0.144^{d}	0.123^{a}	0.139^{c}	0.130^{b}	0.002	0.04
h/H-Ch ratio		1.719^{b}	1.638^{a}	1.645^{a}	1.776^{c}	0.024	0.04

SEM = standard error of the mean. $^{a, b}$ Different letters within a row indicate significant differences at $p < 0.05$. [1] The thrombogenic index = (C14:0 + C16:0 + C18:0) / [(0.5 × ΣMUFA + 0.5 × Σn-6PUFA + 3 × Σn-3PUFA) / Σn-6PUFA)] (Morán et al. 2013); [2] The atherogenic index = (C12:0 + 4 × C14:0 + C16:0) / (ΣMUFA + Σn-6PUFA + Σn-3PUFA) (Morán et al. 2013); [3] h/H-Ch = (*c9*C18:1 + *c11*C18:1 + *c9c12*C18:2 + *c9c12c15*C18:3 + *c6c9c12*C18:3 + *c8c11c14*C20:3 + *c11c14c17*C20:3 + *c5c8c11c14*C20:4 + *c8c11c14c17*C20:4 + *c5c8c11c14c17*C20:5 + *c7c10c13c16*C22:4 + *c7c10c13c16c19*C22:5 + *c4c7c10c13c16c19* C22:6) / (C14:0+C16:0) (Fernández et al. 2007); [4] The modified atherogenic index ($_{index}A^{SFA+Toc}$) = $_{index}A^{SFA}$ /(1.49 x $C_{αT}$ + 1.36 x $C_{αTAc}$ + 0.15 x $C_{γT}$ + 0.05 x $C_{δT}$), where: $_{index}A^{SFA}$ - the atherogenic index ; $C_{αT}$ – the concentration of α-tocopherol; $C_{αTAc}$ - the concentration of α-tocopheryl acetate; $C_{γT}$ – the concentration of γ-tocopherol; $C_{δT}$ - the concentration of δ-tocopherol. Tocopherol concentrations - μg g^{-1}. [5] Results were published in the previous paper (Rozbicka-Wieczorek et al. 2016b).

Conversely, compared to the control diet, only the experimental diet with ^{VI}Se decreased the atherogenic ($_{index}A^{SFA}$) index (Morán et al. 2013) in the PIF (Table 8), *MLD* (Jaworska et al. 2016) and *MBF* (Table 8; Rozbicka-Wieczorek et al. 2016b), whereas in the SCF, $_{index}A^{SFA}$ was higher in lambs fed the CA and ^{Y}Se-CA diets than in the control and ^{VI}Se-CA diets (Krajewska-Bienias et al. 2017). As can be seen from the results in Table 8, all experimental diets fed to lambs decreased $_{index}T^{SFA}$ in the PIF in comparison with the control diet. It has been found that the experimental diets enriched in ^{Y}Se or ^{VI}Se increased the ΣPUFA/ΣSFA ratio in the PIF, compared to the control and CA diets. The ^{Y}Se-CA diet most effectively increased the value of the ΣPUFA/ΣSFA ratio in the PIF.

As can be seen from the current results, the ^{VI}Se-CA diet fed to lambs resulted in an increase in the h/H-Ch ratio in the PIF, SCF, *MLD* and *MBF* compared to the control and other experimental diets (Table 8). The addition of ^{Y}Se to the experimental diet increased h/H-Ch only in the PIF, in comparison with the control and CA diets.

Discussion

Our studies show that short-term dietary supplementation of CA and Se as ^{VI}Se or ^{Y}Se (rich in Se-Met) can be used to modify the concentration of FA, TCh and tocopherols in the PIF, SCF and muscles of lambs without adversely influencing performance or causing physiological disorders in the liver, blood and brain (Eun et al. 2013, Rozbicka-Wieczorek et al. 2016b, 2016c, Czauderna et al. 2017, 2018, Krajewska-Bienias et al. 2017, Przybylski et al. 2017, Białek et al. 2018). Se-compounds are metabolised to intermediates and then utilised for the biosynthesis of Se-Cys- or Se-Met-containing proteins/enzymes in the tissues of mammals (Collins 2017). The most important physiological functions of half of these Se-proteins/enzymes are to maintain the appropriate metabolism of *c5c8c11c14*C20:4, as well as low levels of free radicals or pre-oxides within cells, thus decreasing oxidative stress and peroxidative damage of UFA, DNA, cholesterol or sulphur-amino acids in proteins in the tissues of mammals (Choe and Min 2009, Čobanová et al. 2017, Czauderna et al. 2011, Saheem et al. 2017). In this context, special attention should be paid to the role of CA from *Rosmarinus officinalis*. CA is an abietane diterpenoid that is abieta-8,11,13-triene substituted by hydroxy groups at positions 11 and 12, and a carboxy group at position 20. Interestingly, CA exhibits neuroprotective, antiangiogenic, antineoplastic, antioxidant, antimicrobial and anti-HIV activity (Ibarra et al. 2011, Morán et al. 2012b, Jordán et al. 2013, Sasaki et al. 2013, Morán et al. 2017). What is more, CA added to diets has the ability to modify the microbial population in the rumen and, hence, the biosynthesis yields of volatile compounds, and UFA isomerisation and biohydrogenation in the rumen (Morán et al. 2012a, 2013, Miltko et al. 2016). Our current study and other studies have shown that CA added to diets with or without Se (as ^{Y}Se or ^{VI}Se) affected fatty acids profiles, the contents of cholesterol, its oxidation products and the accumulation of carbonyl moieties on proteins produced by oxidative stress in animal tissues (Ibarra et al. 2011, Morán et al. 2012a, Jordán et al. 2013, Rozbicka-Wieczorek et al. 2016a, 2016c). In accordance with the above, all experimental diets reduced the content of MDA, as well as the values of the PUFA peroxidation index in the PIF and SCF (Krajewska-Bienias et al. 2017), compared to the control diet.

Impact of the experimental diets on the concentrations of fatty acids in the PIF

Our results, summarised in Tables 4–6, showed that all experimental diets reduced the contents of even-long-chain SFA, *c11*C20:1, *c9t11*CLA, *c11t14*C20:2 and EPA in the PIF compared to the control, whereas the ^{Y}Se-CA and ^{VI}Se-CA diets decreased the levels of *c11t14*C20:2, *c5c8c11c14*C20:4, EPA and DHA, as well as the value of the Δ4-desaturase index in the PIF in comparison with the control and CA diets. Based on the above results, it can be argued that ^{Y}Se or ^{VI}Se added to the diet decreased the capacity of Δ6-, Δ5- and especially Δ4-desaturase required for the biosynthesis of highly unsaturated LPUFA as EPA, DPA and DHA (bio-synthesised from αLNA), and *c5c8c11c14*C20:4 and *c7c10c13c16*C22:4 (biosynthesised from LA). This is consistent with the results of a previous study (Krajewska-Bienias et al. 2017), in which the ^{Y}Se-CA and ^{VI}Se-CA diets also reduced the contents of n-6LPUFA and n-3LPUFA in the SCF in comparison with the control diet. Moreover, in an earlier study conducted on lambs, it was also found that ^{VI}Se added to a diet with linseed oil reduced the content of PUFA in the SCF and perirenal fat compared to a diet with linseed oil alone (Czauderna et al. 2012a).

As can be seen from the results summarised in Table 5, the experimental diets, especially the ^{Y}Se-CA diet, fed to lambs resulted in an increase in the capacity of Δ9 desaturation in the PIF and SCF (Krajewska-Bienias et al. 2017) compared to the control diet. Considering the above, we suggested that dietary CA and particularly ^{VI}Se added to the diet increased Δ9-desaturase capacity *via* stimulation of stearoyl-CoA desaturase mRNA expression in the PIF. Furthermore, the regulation of stearoyl-CoA desaturase activity is sensitive to diet composition, dietary supplements (like phenolic compounds, minerals or peroxisomal proliferators), as well as hormonal imbalances,

developmental processes or temperature changes (Ntambi 1999). In addition, our results reinforce the finding that Δ9-desaturation capacity depends upon the chemical form of a substrate (Rozbicka-Wieczorek et al. 2014). In fact, the experimental diets, particularly those with ^{Y}Se or ^{VI}Se, decreased the index of Δ9 desaturation of *t11*C18:1 (^{CLA}Δ9-index) in the PIF compared to the control diet (Table 5); thus, the content of *c9t11*CLA (the product of Δ9-desaturase) was lower in the PIF (Table 6) and the SCF of lambs fed the experimental diets in comparison with the control diet (Krajewska-Bienias et al. 2017).

What is more, dietary ^{Y}Se or ^{VI}Se increased the capacity of enzymatic elongation of PUFA in the PIF; in fact, the PUFA elongase index was higher in the PIF of lambs fed the experimental diets with ^{Y}Se or ^{VI}Se than the control and CA diets (Table 5). As a consequence, the content of *c11c14*C20:2 (the substrate for PUFA elongase) was considerably lower in the PIF, SCF (Krajewska-Bienias et al. 2017) and *MBF* (Table 8) of lambs fed the diets enriched in ^{Y}Se or ^{VI}Se than the control and/or CA diets. In accordance with the above, the experimental diets, especially the ^{Y}Se-CA and ^{VI}Se-CA diets, considerably increased the contents of C21:0 and C23:0 (long-chain SFA). Considering the above, we suggested that CA added to the diet increased the capacity for the enzymatic elongation of odd-chain SFA (the substrates for elongase: C15:0, C17:0, C19:0 and C21:0) in the PIF compared to the control diet. Indeed, these enzymes are regulated/affected by dietary supplements, developmental processes, hormones and chronic diseases. As a consequence, changes in elongase activities impact fatty acid profiles in mammal tissues (Jump 2009). Moreover, ^{Y}Se and especially ^{VI}Se added to the experimental diet enhanced the capacity of odd-chain fatty acid elongases compared to the diet with only CA.

Hypocholesterolemic/hypercholesterolemic FA ratio, and atherogenic and thrombogenic indices of lambs' tissues

Our current and previous studies have suggested that the experimental diets containing CA with or without ^{Y}Se stimulated atherogenic properties in the PIF and SCF (Krajewska-Bienias et al. 2017). In fact, these experimental diets increased the concentration of A-SFA in the SCF, as well as the values of $_{index}A^{SFA}$ in the PIF and SCF. Furthermore, atherogenesis, a multi-factor process, is stimulated by A-SFA (especially C14:0), and reduced by dietary n-3PUFA, as well as tocopherols (antioxidants). Recent studies have documented that diets enriched in tocopherols and plant oils rich in n-3PUFA have an antiatherogenic effect (i.e. reduced aortic cholesterol content, intimal lipid infiltration and discrete alterations to the middle layer of the arterial wall) (Saini et al. 2012, Haliga et al. 2015). In fact, "the oxidation theory" of antioxidant systems and atherosclerosis argues that oxidation of low density lipoproteins (LDL) significantly contributes to atherogenesis. Dietary antioxidants (i.e. tocopherols or tocotrienols) and coantioxidants (ascorbate and ubiquinol-10) prevent the oxidation of LDL, cholesterol and UFA in lipids, thus, delaying the atherogenesis process in animals and humans (Salvayre et al. 2016). Considering the above, we argued that the atherogenic index ($_{index}A^{SFA}$) should be calculated based on the contents of pro-atherogenic saturated fatty acids (i.e. A-SFA), MUFA, n-6PUFA and n-3PUFA, as well as the contents of anti-atherogenic tocopherols in tissues. Thus, it seems reasonable to assume that the modified atherogenic index ($_{index}A^{SFA+Toc}$) should be calculated in the following manner:

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

where: $C_{\alpha T}$, $C_{\alpha TAc}$, $C_{\gamma T}$ and $C_{\delta T}$ – the concentrations of α-tocopherol, α-tocopheryl acetate, γ-tocopherol and δ-tocopherol respectively; 1.49, 1.36, 0.15 and 0.05 are coefficients of the biological activity of tocopherols (Leth and Søndergaard 1977). In fact, all experimental diets, especially the ^{Y}Se-CA diet, reduced the values of the modified atherogenic index ($_{index}A^{SFA+Toc}$) in the PIF, SCF and *MLD* compared to the control diet. Moreover, our current studies (Table 7) and previous investigations of cholesterol biosynthesis in the liver (Rozbicka-Wieczorek et al. 2016c) documented that ^{VI}Se and/or CA added to the diet revealed anti-cholesterolemic activity.

Indeed, supplementation with ^{VI}Se or especially with selenite reduced the activity of HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) (Nassir et al. 1997). This enzyme is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that synthesizes cholesterol as well as other isoprenoids. Interestingly, HMG-CoA reductase possesses cysteine residues and is subject to regulation by thiol-disulfide exchange (Nassir et al. 1997). As a consequence, supplementation with CA (the effective antioxidant), ^{VI}Se or particularly with selenite (Czauderna and Samochocka 1981) affects thiol-disulfide exchange equilibria and disulfide bond stability of HMG-CoA reductase. Moreover, the effect of dietary CA on cholesterol reduction may be attributed to a decrease in the micellar solubilisation of cholesterol in the digestive tract, to a stimulation in bile flow, bile cholesterol and bile acid content and to a subsequent increase in the faecal excretion of steroids

(Ibarra et al. 2011, Afonso et al. 2013). Our current study and previous investigations suggest that dietary CA protects against hypercholesterolemia-induced oxidative stress, increasing the activities of antioxidant enzymes and decreasing the concentrations of reactive substances (Afonso et al. 2013, Jordán et al. 2013). Therefore, we argued that CA with or without Se (as ^{Y}Se and ^{VI}Se) reduced the concentrations of radicals (like ROS and RNS) in ovine tissues. As a consequence, the contents of MDA and the values of MDA_{index} were lower in the PIF (Table 7) and SCF (Krajewska-Bienias et al. 2017) of lambs fed the experimental diets than the control diet. Moreover, we suggested that the experimental diets enriched in antioxidants reduced the oxidative degradation of tocopherols and stimulated the regeneration of degraded tocopherols in ovine tissues (Choe and Min 2009, Morán et al. 2012a, 2012b, 2017, Jordán et al. 2013, Haliga et al. 2015, Čobanová et al. 2017). In our opinion, compared with the atherogenic index ($_{index}A^{SFA}$), the modified atherogenic index ($_{index}A^{SFA+Toc}$) provides more detailed insights into the mechanisms of atherogenesis and, thus, possesses better predictive properties. Indeed, the effect of experimental diets enriched in antioxidant(s) on the values of $_{index}A^{SFA+Toc}$ is more consistent than on the values of $_{index}A^{SFA}$ in all assayed tissues. The values of our modified atherogenic index ($_{index}A^{SFA+Toc}$) confirmed that the experimental diets including antioxidants (i.e. CA, ^{VI}Se and especially ^{Y}Se) reduced the risk of atherogenesis (Saini et al. 2012, Salvayre et al. 2016).

In this and earlier studies, we have confirmed that the CA and ^{Y}Se-CA diets reduced the thrombogenic properties in the PIF, SCF (Krajewska-Bienias et al. 2017), the rumen-surrounding fat (Białek and Czauderna 2019) and *MLD* (Jaworska et al. 2016) compared to the control. In fact, the CA and ^{Y}Se-CA diets reduced $_{index}T^{SFA}$ in these tissues more efficiently than the control diet. Similarly, compared with the control diet, the ^{VI}Se-CA diet decreased $_{index}T^{SFA}$ or T-SFA in the PIF, SCF and *MLD*, as its values were lower than in the control tissues. Our current and our recent studies have also showed that the experimental diet with ^{VI}Se increased the $\sum$PUFA/$\sum$SFA ratio in the PIF, *MLD* and *MBF*, as well as the h/H-Ch ratio in the PIF, SCF, *MLD* and *MBF* of lambs compared to the control. Considering the above, we argued that the experimental diets, especially the ^{Y}Se-CA diet, improved the health and welfare of lambs (which probably applies to other ruminants), as well as the nutritional value of lambs' meat (i.e. *MLD* and *MBF*) and the SCF for humans. Indeed, the high $\sum$PUFA/$\sum$SFA and h/H-Ch ratios and the low values of atherogenic and thrombogenic indices in *MLD* and *MBF* (Jaworska et al. 2016, Rozbicka-Wieczorek et al. 2016b) documented their suitability for healthier diets, since these diets are successful in slowing down the atherosclerosis and thrombogenesis processes. As a consequence, the meat of lambs fed the ^{Y}Se-CA diet in particular may decrease the threat of atrial fibrillation, coronary thrombosis and the risk of cardiovascular disorders in humans (Fernández et al. 2007, Haliga et al. 2015, Salvayre et al. 2016).

Conclusion

The current study is important for the understanding of the influence of dietary CA, ^{Y}Se and ^{VI}Se on the contents of FA, cholesterol and tocopherols in the adipose tissues and muscles of lambs. Alongside earlier studies performed on ruminal microbiota, the present research confirmed that dietary ^{Y}Se and ^{VI}Se, as well as CA, have the ability to modify the metabolism of FA in ruminal microorganisms, adipose tissues and muscles of lambs. Our data indicated that dietary CA combines antioxidant and hypocholesterolemic activities. The modified atherogenic index strongly suggests that the experimental diets containing CA, irrespective of the presence of ^{Y}Se or ^{VI}Se, decreased the atherogenic capacity of the PIF, SCF, *MLD* and *MBF* compared to the control diet. In our opinion, the modified atherogenic index ($_{index}A^{SFA+Toc}$) better assesses the atherogenic capacity of tissues, as it takes into consideration the contents of pro-atherogenic SFA, as well as the contents of anti-atherogenic tocopherols in the analysed tissues. Therefore, we argued that the modified atherogenic index is a better assessment tool for the atherogenic properties of tissues than the commonly used atherogenic index ($_{index}A^{SFA}$). Our studies have shown that the PIF, *MLD* and *MBF* of lambs fed the ^{VI}Se-CA diet were characterised by the highest ΣPUFA/ΣSFA and hypocholesterolemic/hypercholesterolemic FA ratios, as well as lower atherogenic and thrombogenic indices and contents of TCh and MDA compared to the control and other experimental diets. Therefore, we argued that the experimental diet with CA and ^{VI}Se in particular, improved lambs' health and welfare, as well as the dietary properties of animal products for human consumption.

Further studies are necessary to determine if diets containing CA and Se (as ^{Y}Se or ^{VI}Se) along with FO and RO induce changes in the content of FA, tocopherols, cholesterol and MDA in the perirenal fat of lambs. Moreover, new studies are necessary to determine if dietary supplementations with Se (as ^{Y}Se or ^{VI}Se) and carnosol (the oxidation product of CA) affect the bioaccumulations of FA (especially n-3LPUFA and conjugated PUFA), tocopherols, tocotrienols and peroxidation products of cholesterol and steroid hormones in adipose tissues, muscles and, in particular, in the liver, brain and heart of lambs. The current studies also provides useful knowledge for nutritionists carrying out further studies aimed at improving the health and welfare of farm animals, as well as the pro-healthy properties of animal products for human consumption.

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