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Original Research Article

Dietary carnosic acid and seleno-compounds change concentrations of fatty acids, cholesterol, tocopherols and malondialdehyde in fat and heart of lambs

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ABSTRACT

The aim of the current study was to evaluate the impact of carnosic acid (CA), selenised yeast (^{Y}Se) and selenate (^{VI}Se) supplemented to diets, including fish oil (FO) and rapeseed oil (RO), on the content of fatty acids, total cholesterol (TCh), tocopherols and malondialdehyde in the fat located between the thigh muscles and the heart in lambs. Twenty-four male Corriedale lambs were divided into 4 groups of 6 animals. Animals were fed a diet with FO and RO (the control diet) or experimental diets containing RO, FO and CA with/without Se (as ^{Y}Se or ^{VI}Se). The experimental diets without/with ^{Y}Se or ^{VI}Se changed concentrations of fatty acids in the fat and heart compared to the control. All experimental diets increased the levels of c11c14C20:2, c5c8c11c14C20:4, c5c8c11c14c17C20:5 and the sums of long-chain polyunsaturated fatty acids (LPUFA) and conjugated linoleic acid isomers in the fat compared to the control. The experimental diet containing ^{Y}Se or ^{VI}Se increased the content of Se, TCh, c11c14C20:2, c8c11c14C20:3, c5c8c11c14C20:4, c5c8c11c14c17C20:5, c7c10c13c16c19C22:5, c4c7c10c13c16c19C22:6 and the concentration sum of n-3LPUFA, n-6LPUFA and tocopherols in the heart in comparison with the control diet and the diet containing only CA. Experimental diets reduced the concentration of malondialdehyde in the fat and heart in comparison with the control diet. Our dietary intervention has great potential for future practical and commercial implementations.

1. Introduction

It is well known that adipose tissues (like intermuscular fat [IMF]) and the liver play an essential role in fatty acid metabolism, whereas fatty acids (FA) significantly modulate the physiological functions of the heart and spleen, as well as the muscles (Addison et al., 2014; Ahmed et al., 2018; Peter et al., 2013). N-3 polyunsaturated fatty acids (n-3PUFA) have been proven to have a beneficial effect on lipid profiles, oxidant-anti-oxidant balance, cytokine cascade, nitric oxide synthesis, as well as the parasympathetic and sympathetic tone in the heart tissue (Peter et al., 2013). Therefore, in current studies, special attention has been paid to the heart and the visible storage of lipids in adipocytes (literally intermuscular fat, Addison et al., 2014) located between the thigh muscles of experimental lambs. Importantly, IMF is a strong predictor of reduced exercise capacity in heart failure patients. Numerous studies have indicated that dietary fish and vegetable oils can influence the profile of FA in adipose tissues, including IMF, as well as in other tissues and internal organs of animals (Addison et al., 2014; Ahmed et al., 2018; Białek and Czauderna, 2019). Nowadays, the purpose of modifying overall diet patterns for farm animals is to produce high-quality animal products meeting dietary recommendations for increased intake of antioxidants, phenolics and oils (particularly rich in n-3 long-chain PUFA [LPUFA]), and decreased intake of thrombogenic and atherogenic saturated FA (T-SFA and A-SFA). Conversely, at present, the “more natural” composition of grazing lambs can also be recommended (Mozaffarian, 2016).

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Experimental and epidemiological investigations (Attia et al., 2015; Morán et al., 2013) have shown that T-SFA and A-SFA possessed thrombogenic and atherogenic activity, whereas α-linolenic acid (c9c12c15C18:3; αLNA) and its products of desaturation and elongation (i.e. n-3LPUFA) improved anti-inflammatory status and immune response, and benefited the cardiovascular system by reducing platelet aggregation, cholesterol and serum triglycerides (Byelashov et al., 2015; Calder, 2013, 2017; Micha et al., 2017; Mozaffarian, 2016). Conversely, recent studies documented that vegetable oils (rich in PUFA) or fish oil (FO, rich in n-3LPUFA) added to diets stimulated oxidative deterioration in tissues of monogastric animals and ruminants (Białek and Czauderna, 2019; Czauderna et al., 2017). Considering the above, an adequate content of unsaturated FA (UFA) in dietary oils together with antioxidants, like seleno-compounds, tocopherols, phenolic compounds (e.g. carnosic acid) is essential for the good health of farm animals and humans (Białek and Czauderna, 2019; Micha et al., 2017; Mozaffarian, 2016). Diverse inorganic chemical forms of Se (e.g. selenite [^{IV}Se] or selenate [^{VI}Se]) or selenised yeast (^{Y}Se) are used as a nutritional source of Se. ^{IV}Se and ^{VI}Se are mainly metabolised to intermediates and then utilised for the biosynthesis of Se-cysteine (Se-Cys) ^{SeCys}tRNA (transfer-RNA for Se-Cys) to pair with the codon of Se-Cys for the formation of enzymes containing Se-Cys (like the glutathione peroxidase (GPx) family, thioredoxin reductase and selenoprotein P). The most important physiological role of half of Se-Cys enzymes is to maintain the proper metabolism of arachidonic acid and low levels of free radicals or reactive oxygen species within cells, thus reducing oxidative stress in mammal organisms (Rayman, 2008; Raymond et al., 2014). GPx acts synergistically with tocopherols in the regulation of lipid peroxidation; in particular, phospholipid hydroperoxide GPx interacted more directly than cytosolic and mitochondrial GPx in protecting UFA from peroxidation damage (Fairweather-Tait et al., 2011). Positive correlations were observed between the contents of UFA and Se in diets (Fairweather-Tait et al., 2011; Yu et al., 2008). To the contrary, Se-methionine originating from dietary ^{Y}Se is mainly incorporated into proteins of the animal and human body in the place of Met (Navarro-Alarcon and Cabrera-Vique, 2008).

Recent studies have also shown a positive effect of carnosic acid (CA), a phenolic diterpene isolated from rosemary (*Rosmarinus officinalis*), in increasing the protein expression of antioxidative enzymes (Jordan et al., 2013; Morán et al., 2013; Wu et al., 2015); dietary CA supplementation stimulated the protein expression of the γ-glutamate-cysteine ligase catalytic subunit, the γ-glutamate-cysteine ligase modifier subunit, superoxide dismutase and glutathione reductase. As a consequence, CA (the effective antioxidant) protects UFA, particularly highly unsaturated LPUFA, as well as stimulating the reduction of oxidised glutathione (GS–GS) (Wu et al., 2015). Moreover, recent studies documented that CA and Se-compounds (like ^{VI}Se and ^{Y}Se) affected ruminal microbial population and profiles of volatile FA and FA in the rumen (Białek et al., 2018; Białek and Czauderna, 2019; Del Razo-Rodriguez et al., 2013; Miltko et al., 2016; Morán et al., 2012). Our previous investigations have shown that experimental diets containing CA with/without ^{VI}Se or ^{Y}Se affected concentrations of UFA, cholesterol, tocopherols, and malondialdehyde (MDA, a marker of oxidative stress (Czauderna et al., 2011)) in the liver, brain, blood, muscles, subcutaneous fat, rumen surrounding fat, and rumen in lambs (Białek et al., 2018; Białek and Czauderna, 2019; Czauderna et al., 2017; Miltko et al., 2016). Considering the above, we hypothesised that Se-compounds (as ^{VI}Se or ^{Y}Se) and CA added to a diet enriched in FO (rich in LPUFA) and rapeseed oil (RO) would also modify the contents of FA, and modulate oxidative stress in the IMF and heart of lambs. Therefore, the purpose of the present study was to investigate the effect of different chemical forms of Se (as ^{VI}Se or ^{Y}Se) and CA added to a diet enriched in RO (rich in n-6PUFA) and odourless FO (rich in n-3LPUFA) on the content of FA, total cholesterol (TCh), tocopherols and MDA in the IMF and heart of lambs.

2. Materials and methods

The present studies were conducted under the authority of the III Local Commission of Animal Experiment Ethics at the University of Life Sciences (02–786 Warsaw, Ciszewskiego 8, Poland).

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

Twenty-four male Corriedale lambs with an average body weight (BW) of 30.4 ± 2.5 kg at the beginning of the experiments were individually penned; animals were divided into 4 groups of 6 lambs. Studies carried out on lambs have been described in detail in a previous publication (Białek and Czauderna, 2019). Briefly, during a 3-week preliminary period, lambs were given free access to water and basal diet (BD) enriched in 10 g FO/kg BD and 20 g RO/kg BD (Tables 1 to 3). The BD consists of the following components: a mixture of soybean (360 g/kg BD) and barley (165 g/kg BD) meals, wheat starch (95 g/kg BD), meadow hay (360 g/kg BD) and mineral–vitamin premix (20 g/kg BD). The BD analysis was performed in The Kielanowski Institute of Animal Physiology and Nutrition, PAS (Jabłonna, Poland); dry matter (934.01), crude protein (984.13), crude fibre (978.10), crude fat (920.39), ash (942.05), neutral detergent fibre (2002.04; Mertens, 2002), acid detergent fibre (973.13) and acid detergent lignin (973.13) in BD were analysed according to AOAC (2005). All AOAC methods for analysing the nutritional composition for BD were presented in the supplementary materials associated with this paper. The composition of the ingredients in BD is summarised in Tables 1 to 3. Lambs were given the control and experimental diets as hay and concentrate in a different way. RO, FO, as well as the supplements (CA, ^{Y}Se and ^{VI}Se), were added to the concentrate; the control and all experimental diets along with the supplement(s) were prepared daily. After the 3 wk preliminary period, for 35 d the animals, housed in individual pens, were assigned to 4 treatments: the control lambs were fed the control diet prescribed in the preliminary period and three experimental groups received the control diet enriched with antioxidants (i.e. CA, ^{VI}Se and ^{Y}Se) (Tables 1 to 3). The amount of the diets given to lambs during the preliminary and experimental periods was adjusted weekly to both the body weight of animals and their nutritional requirements (Strzetelski et al., 2014). The feeding standard was based on the recommendation of Strzetelski et al. (2014). Technical professional staff prepared the control and all experimental diets daily; all diets were administered to animals twice a day (07:30 and 16:00) in equal amounts. As can be seen from the data summarised in Tables 1 to 3, the control and experimental diets were formulated to be isoproteinous and isoenergetic; therefore, both control and experimental lambs were given the same amount of food. The doses were wholly consumed by the sheep. The average daily diet intake per lamb was 1.08 kg (i.e. 0.378 kg of hay and 0.702 kg of concentrate enriched with 2% RO and 1% FO with or without the antioxidants – Tables 1 to 3). This amount of the control and experimental diets provided to each lamb closely met 100% of the animal's nutritional needs (Strzetelski et al., 2014). Water for the lambs was available ad libitum. At the end of the experimental period, the lambs were deprived of consciousness by intramuscular injections of xylazine (2 to 4 mg per 10 kg of BW) and then slaughtered. Immediately after slaughter, the heart was removed from each lamb. The IMF located between the muscles of the thighs was carefully carved out from the muscles. The collected hearts and fat tissues were homogenised and then

Table 1
Chemical composition of the ingredients in the basal diet (the concentrate-hay diet with vitamins and mineral mixture[1]) (% of dry matter).

Item	Meadow hay[2]	Concentrate[3]		
		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	–
Gross energy, MJ/kg of DM	17.1	16.3	17.8	16.7

[1] One kilogram of the mineral and vitamin mixture comprised: 285 g Ca, 16 g P, 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 500,000 IU/kg, vitamin D_3 (125,000 IU/kg), and vitamin E as α-tocopherol (25,000 IU/kg).
[2] Main fatty acids in the meadow hay (mg/kg): C8:0 83, C12:0 142, C14:0 239, c9C15:1 131, C16:0 4,034, c9C16:1 184, C18:0 459, c9C18:1 1,266, c12C18:1 72, LA 13,100, α-linolenic acid (αLNA) 4,178, C20:0 58, c11C20:1 74, C22:0 101, C24:0 69, c15C24:1 71.
[3] Main fatty acids in concentrate (mg/kg): C14:0 104, C16:0 3,189, C18:0 1,425, c9C18:1 774, linoleic acid (LA) 29,163, αLNA 1,014.

stored in sealed tubes at − 32 °C until further analysis. Each lamb's collected tissue was analysed separately. The contents of FA, TCh, tocopherols and MDA in the IMF and heart were expressed from fresh matter (i.e. on 1 g of fresh tissue of fat or heart).

2.2. Reagents and chemicals

Methanol (≥99.9%), n-hexane (≥99%) and HPLC-acetonitrile (≥99.9%) were obtained from Lab-Scan (Dublin, Ireland). Nonadecanoic acid, conjugated linoleic acid (CLA) isomers, and an FA standard mixture (37 FA methyl esters [FAME] mix), α-tocopherol (αT), α-tocopheryl acetate (αTAc), cholesterol, 2,6-di-tert-butyl-pcresol, 25% aqueous 1,5-pentanedialdehyde solution, 1,1,3,3-tetramethoxy-propane (99%), sorbic acid, 2,4-dinitrophenylhydrazine (containing approximately 30% water), trichloroacetic acid and 25% BF_3 in methanol were purchased from Sigma Aldrich (St Louis, MO, USA). Chloroform, dichloromethane KOH, NaOH and Na_2SO_4, were obtained from Avantor Performance Materials (Gliwice, Poland). All other chemicals were of analytical grade. The vitamin and mineral premix (ID number: aPL 1405002p) was obtained from POLFAMIX OK (Poland). CA was purchased from Hunan Geneham Biomedical Technology Ltd (Changsha Road, Changsha, Hunan, China). The ^{Y}Se (selenised-*Saccharomyces cerevisiae*) was obtained from Sel-Plex (Alltech In., Nicholasville, KY, USA). Odourless FO and RO were supplied by Company AGSOL (Pacanów, Poland). FO and RO were stored in tightly sealed containers at 4 °C in a dark place. The FA profiles in RO and FO were presented in our previous publication (Czauderna et al., 2017).

Table 2
Chemical composition of the basal diet (BD)[1,2,3].

Item	Content
DM, g/kg	884.3
Crude protein, g/kg DM	201.9
Crude fibre, g/kg DM	118.6
Crude fat[4], g/kg	21.7
Total crude fat[5], g/kg	51.7
Ash, g/kg DM	42.8
Neutral detergent fiber, g/kg DM	310.5
Acid detergent fiber, g/kg DM	146.3
Acid detergent lignin, g/kg DM	23.3
Metabolized energy[6], MJ/kg DM	13.50

BD = basal diet.
[1] The control diet composed by the BD enriched in 20 g RO/kg BD and 10 g FO/kg BD. Se content in 1 kg of the control diet: 0.16 mg.
[2] The experimental diets composed by the control diet enriched in antioxidants: the carnosic acid (CA) diet (1 g of CA/kg BD), the selenised yeast (^{Y}Se)-CA diet (1 g CA/kg BD and 0.35 mg Se as ^{Y}Se/kg BD) and the selenate (^{VI}Se)-CA diet (1 g CA/kg BD and 0.35 mg Se as ^{VI}Se/kg BD). Se contents in the CA, ^{Y}Se-CA and ^{VI}Se-CA diets (mg/kg diet): 0.16, 0.51 and 0.51, respectively.
[3] The contents of toxic elements in the BD, mg/kg: As, 1.39 ± 0.03; Cd, 0.068 ± 0.001; Sb, 0.0155 ± 0.0015 and Pb, 0.514 ± 0.003.
[4] Crude fat originating from BD (i.e. the meadow hay and concentrate).
[5] Total crude fat originating from the BD and added oils (i.e. RO and FO). The energy content of FO and RO was 36.8 and 37.0 MJ/kg oil, respectively.
[6] Metabolized energy (MJ) in 1 kg of dry matter (Regadas Filho et al., 2011).

2.3. Chemical methods and chromatographic configuration

2.3.1. Preparation of FAME

The homogenised IMF (approximately 15 mg) and heart tissues (approximately 50 mg) from lambs were saponified according to methods described by Czauderna et al. (2007). Nonadecanoic acid (as the internal standard) was added to each saponified tissue sample. Then, base- and acid-catalysed methylations were used for the preparation of FAME in assayed biological samples (Czauderna et al., 2007). FA (as FAME) in analysed tissue samples was then studied using gas chromatography (GC) with mass spectrometry (MS) according to the methods presented by Rozbicka-Wieczorek et al. (2014). FAME analyses were conducted on a Shimadzu GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica column (120 m × 0.25 mm i.d. × 0.25 μm film thickness) and a mass 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 (NIST, 2007). Determination of FAME concentrations in tissue samples was based on total ion current chromatograms and/or selected ion monitoring chromatograms (Rozbicka-Wieczorek et al., 2014).

A-SFA are the concentration sum of C12:0, C14:0 and C16:0, whereas T-SFA are the concentration sum of C14:0, C16:0 and C18:0 (Morán et al., 2013). Atherogenic and thrombogenic indices were calculated according to the equations given by Morán et al. (2013). The hypocholesterolemic/hypercholesterolemic FA (h/H-Ch) ratio was calculated using the equation given by Fernández et al. (2007).

2.3.2. Analysis of CLA isomers in lamb tissues using the silver chromatographic method

Prior to silver chromatographic analyses, lambs' tissues were saponified according to the method of Czauderna et al. (2007). Chromatographic analyses of CLA (C18:2) isomers (as free FA) were performed on 4 ion-exchange columns loaded with silver ions

Table 3
The experimental scheme and weight change of lambs.

Group	Supplements added to 1 kg of the BD	Initial live weight ($m_{initial}$)[1], kg	Final live weight (m_{35days})[2], kg	Average daily live weight gain ($\Delta m/35$)[3], kg
Control	20 g RO and 10 g FO	30.567[a]	37.798[ab]	0.206[b]
CA	20 g RO, 10 g FO and 1 g CA	30.603[a]	37.167[a]	0.189[a]
^{Y}Se-CA	20 g RO, 10 g FO, 1 g CA and 0.35 mg Se as ^{Y}Se	30.267[a]	36.885[a]	0.189[a]
^{VI}Se-CA	20 g RO, 10 g FO, 1 g CA and 0.35 mg Se as ^{VI}Se	30.333[a]	38.448[b]	0.232[c]

BD = basal diet; RO = rapeseed oil; FO = fish oil; CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate.
[a, b] the mean values in columns bearing different superscripts are significantly different at $P \leq 0.05$.
[1] The average initial live weight ($m_{initial}$, kg) of lambs after the 3 wk preliminary period.
[2] The average live weight (m_{35days}, kg) of lambs after 35 d of the experimental period.
[3] Average daily live weight gain ($\Delta m/35$, kg) of lambs, $\Delta m/35 = (m_{35days} - m_{initial})/35$.

(Ag^{+}) (Chrom-pack ChromSpher, 5 μm, Lipids; 250 mm × 4.6 mm; Varian, the Netherlands) and a Waters HPLC 625LC chromatograph equipped with a photodiode detector (DAD) (Milford, MA, USA). Identifications of CLA isomers were based on the UV spectra of CLA isomer standards and retention times; sorbic acid (c2c4C6:2) was used as the internal standard (Białek et al., 2018).

2.3.3. Analysis of tocopherols, TCh and MDA in the IMF and heart

Concentrations of TCh, γ-tocopherol (γT), δ-tocopherol (δT), αT and αTAc were analysed in the IMF and heart tissues using a reversed-phase (C_{18}) liquid chromatographic system (SHIMADZU, Tokyo, Japan) according to methods described by Czauderna et al. (2009). The liquid chromatographic instrument used consisted of an ultra-fast liquid chromatography system (UFLC-DAD), incorporating two LC-20ADXP pumps (UFLCXR), a SIL-20ACXR autosampler (LFLCXR), a CBM-20A communications bus module, a CTO-20A column oven, a DGU-20A5 degasser and a SPD photodiode array detector (DAD) (Czauderna et al., 2009). Concentrations of MDA in the IMF and heart samples were determined after saponification, followed by derivatisation according to methods described by Czauderna et al. (2011). The separations of derivatised MDA from endogenic species of the IMF and heart samples were conducted using UFLC-DAD (Czauderna et al., 2011).

2.4. Statistical analyses

Statistical analyses of the impact of the experimental diets were performed using the Statistica 12.5 PL software package (StatSoft Inc., Tulsa, OK, USA). Significance was determined at a P value ≤ 0.05. Data are shown as mean values and SEM (standard error of mean). The effects of the experimental diets on the analyte concentrations in the IMF and heart tissues for variables with normal distribution were tested with one-way ANOVA and the post-hoc RIR Tuckey test. For variables without normal distribution, results were tested with Kruskal–Wallis, which is a non-parametric equivalent of one-way ANOVA, and the post-hoc multiple comparison test.

3. Results

In our study, the ^{VI}Se-CA diet increased ($P \leq 0.05$) the average daily live weight gain ($\Delta m/35$, kg) in comparison to the control, CA and ^{Y}Se-CA diets, whereas the CA and ^{Y}Se-CA diets reduced ($P \leq 0.05$) the value of $\Delta m/35$ compared to the control diet. Similarly, the final live weight of lambs fed the ^{VI}Se-CA diet was higher ($P \leq 0.05$) than that of the CA and ^{Y}Se-CA diets, whereas no difference ($P > 0.05$) in the final live weight of lambs was noted between the control, CA and ^{Y}Se-CA diets.

3.1. Concentrations of selected saturated FA (SFA) and monounsaturated FA (MUFA) in the IMF and heart

As shown in Table 4, dietary CA and ^{Y}Se inclusion decreased ($P \leq 0.05$) the concentrations of C10:0, C17:0 and C18:0, as well as the ratios of the content sum of all assayed SFA (ΣSFA) to the sum of all assayed UFA (ΣUFA) (ΣSFA/ΣUFA), and to the sum of all assayed FA (ΣFA) (ΣSFA/ΣFA) in the IMF compared to the control and ^{VI}Se-CA diets. Also, the experimental diet containing only CA reduced the concentration of ΣSFA in the IMF compared to the control and ^{VI}Se-CA diets. The ^{Y}Se-CA diet most efficiently increased ($P \leq 0.05$) the concentration ratio of A-SFA to ΣFA (A-SFA/ΣFA) in the IMF compared to the control, CA and ^{VI}Se-CA diets.

As shown in Table 5, the concentrations of C16:0, C18:0, A-SFA and the content ratio of ΣSFA/ΣUFA in the heart of lambs fed the CA and ^{VI}Se-CA diets were higher ($P \leq 0.05$) than those for the control diet. The ^{VI}Se-CA diet most efficiently reduced ($P \leq 0.05$) the concentrations of C14:0 and C17:0 in the heart compared to the control, CA and ^{Y}Se-CA diets. The experimental diets supplemented with ^{Y}Se or ^{VI}Se decreased ($P \leq 0.05$) the content ratio of ΣSFA to the sum of all assayed PUFA (ΣSFA/ΣPUFA), whereas increased ($P \leq 0.05$) the content ratio of ΣSFA/ΣFA compared to the control and CA diets.

In Table 6, it can be seen that CA and ^{VI}Se-CA diets decreased ($P \leq 0.05$) the concentrations of c9C14:1, c7C16:1, c7C18:1 and

Table 4
The concentrations (mg/g fat) of SFA in the IMF.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
C10:0	1.85[c]	1.68[ab]	1.55[a]	1.73[bc]	0.05	0.03
C12:0	3.24	3.02	3.56	3.13	0.14	0.11
C14:0	32.91	31.53	38.52	32.51	1.09	0.13
C15:0	3.25	2.93	3.47	3.65	0.06	0.07
C16:0	154	147	158	152	8	0.19
C17:0	10.0[b]	8.6[ab]	7.2[a]	10.3[b]	0.3	0.04
C18:0	173[bc]	162[ab]	155[a]	182[c]	4	0.03
A-SFA	190	182	201	188	5	0.31
A-SFA/ΣFA	0.255[b]	0.257[b]	0.270[c]	0.250[a]	0.002	0.04
T-SFA	361	340	352	367	7	0.35
T-SFA/ΣFA	0.482	0.477	0.476	0.490	0.005	0.19
ΣSFA	379[bc]	357[a]	368[ab]	386[c]	6	0.04
ΣPUFA/ΣSFA	0.1422	0.1445	0.1514	0.1441	0.0005	0.08
ΣSFA/ΣUFA	1.029[c]	1.005[b]	0.989[a]	1.057[d]	0.004	0.02
ΣSFA/ΣFA	0.507[b]	0.500[a]	0.497[a]	0.515[c]	0.002	0.03

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; SFA = saturated fatty acids; IMF = fat located between the thigh muscles; SEM = standard error of the mean; A-SFA = atherogenic-SFA (the concentration sum of C12:0, C14:0 and C16:0); T-SFA = thrombogenic-SFA (the concentration sum of C14:0, C16:0 and C18:0); ΣSFA = all assayed SFA (the concentration sum of 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, C23:0 and C24:0); ΣPUFA/∑SFA = the ratio of the sum of polyunsaturated fatty acids to ∑SFA, ∑SFA/ΣUFA = the ratio of ∑SFA to the sum of unsaturated fatty acids; ∑SFA/∑FA = the ratio of ∑SFA to all assayed fatty acids.
[a, b, c, d] Different letters within a row indicate significant differences at $P \leq 0.05$.

Table 5
The concentrations (μg/g heart) of SFA in the heart.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
C10:0	18.3	11.2	11.0	18.1	1	0.28
C12:0	11.4	17.9	18.8	20.2	0.9	0.07
C14:0	79^{b}	284^{c}	88^{b}	15^{a}	5	0.01
C16:0	2,992^{a}	4,172^{c}	3,406^{b}	3,985^{c}	38	0.04
C17:0	104^{b}	176^{c}	83^{b}	4^{a}	0.3	0.03
C18:0	5,413^{a}	6,534^{b}	5,506^{a}	6,805^{b}	49	0.04
A-SFA	3,083^{a}	4,473^{c}	3,513^{ab}	4,020^{bc}	57	0.04
A-SFA/ΣFA	0.153	0.170	0.172	0.165	0.002	0.09
T-SFA	8,484	10,990	9,000	10,805	73	0.23
T-SFA/ΣFA	0.423	0.426	0.439	0.444	0.005	0.17
ΣPUFA/ΣSFA	0.709^{b}	0.564^{a}	0.765^{d}	0.738^{c}	0.003	0.03
ΣSFA/ΣUFA	0.750^{a}	0.778^{b}	0.800^{c}	0.799^{c}	0.003	0.03
ΣSFA/ΣFA	0.430^{a}	0.433^{a}	0.445^{b}	0.446^{b}	0.002	0.04

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; SFA = saturated fatty acids; SEM = standard error of the mean; A-SFA = atherogenic-SFA (the concentration sum of C12:0, C14:0 and C16:0); T-SFA = thrombogenic-SFA (the concentration sum of C14:0, C16:0 and C18:0); ΣSFA = all assayed SFA (the concentration sum of 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, C23:0 and C24:0); ΣPUFA/∑SFA = the ratio of the sum of polyunsaturated fatty acids to ∑SFA, ∑SFA/ΣUFA = the ratio of ∑SFA to the sum of unsaturated fatty acids; ∑SFA/∑FA = the ratio of ∑SFA to all assayed fatty acids.
[a, b, c] Different letters within a row indicate significant differences at $P \leq 0.05$.

c14C18:1 in the IMF compared to the control diet. In contrast, the concentrations of c11C20:1 and c13C22:1 in the IMF of lambs fed all experimental diets enriched in ^{Y}Se or ^{VI}Se were higher ($P \leq 0.05$) than those of the control diet. Moreover, in lambs fed the experimental diets, a value of t11C18:1 (TVA) desaturase index (^{CLA}Δ9-index; CLAΔ9-index = c9t11CLA/[c9t11CLA + TVA]) in the IMF decreased ($P \leq 0.05$) compared to the control diet. Regarding the Δ9-desaturase index ($^{C18:1}$Δ9-index; $^{18:1}$Δ9-index = c9C18:1/[c9C18:1 + C18:0]), when CA was added to the experimental diet, irrespective of the presence of ^{Y}Se, an increase in the value of $^{C18:1}$Δ9-index in the IMF was observed ($P \leq 0.05$) compared to the control and ^{VI}Se-CA diets.

Our study showed that the CA diet led to an increase ($P \leq 0.05$) in the concentrations of c9C14:1, c7C16:1, c9C16:1, c7C18:1 and c11C20:1, as well as the ratios of concentration sum of MUFA (∑MUFA) to ∑PUFA (∑MUFA/∑PUFA) and ∑MUFA/∑FA in the heart compared to the control, ^{Y}Se-CA and ^{VI}Se-CA diets (Table 7). Conversely, when ^{VI}Se was added to the experimental diet, a significant decrease in the concentrations of c9C14:1, c7C16:1, c9C16:1, c7C18:1, c11C20:1, c15C24:1 and the content ratios of ∑MUFA/∑PUFA and ∑MUFA/∑FA was observed compared to the control and CA diets (Table 7).

3.2. Concentrations of PUFA in the IMF and heart

Our study demonstrated that all experimental diets significantly increased ($P \leq 0.05$) the contents of concentration sums of cis,trans/trans, cisCLA isomers (Σct/tcCLA) and all assayed CLA isomers (ΣCLA) in the IMF compared to the control diet (Table 8). Similarly, the experimental diets enriched in ^{Y}Se or ^{VI}Se elevated ($P \leq 0.05$) the level of c9t11CLA in the IMF compared to the control and CA diets. Also, all experimental diets increased ($P \leq 0.05$) the concentrations of c11c14C20:2, c5c8c11c14C20:4 (AA) and c5c8c11c14c17C20:5 (EPA), the content sums of all assayed n-6LPUFA (Σn-6LPUFA) and LPUFA (ΣLPUFA), as well as the content ratios of Σn-6LPUFA/Σn-3LPUFA, Σn-3LPUFA/ΣFA and ΣLPUFA/ΣFA, and the values of the index of elongases (Σ^{Elong}index; Σ^{Elong}index = [c11c14C20:2 + c7c10c13c16c19C22:5]/[c11c14C20:2 + c7c10c13c16c19C22:5 + c5c8c11c14c17C20:5 + c9-c12C18:2]) in the IMF compared to the control diet. The diet supplemented with CA and ^{Y}Se decreased the Σn-6PUFA/Σn-3PUFA ratio in the IMF compared to the control and other experimental diets.

In the current study, the concentrations of c9t11CLA and ΣCLA in the heart of lambs fed the CA and ^{VI}Se-CA diets were higher ($P \leq 0.05$) than those of the control and ^{Y}Se-CA diets (Table 9). Similarly, the experimental diet supplemented with ^{VI}Se increased ($P \leq 0.05$) the concentrations of c9c12c15C18:3 (αALA), c11c14C20:2, c8c11c14C20:3, AA, EPA, c7c10c13c16c19C22:5 (DPA), c4c7c10c13c16c19C22:5 (DHA), Σn-6LPUFA and Σn-3LPUFA, as well

Table 6
The concentrations (mg/g fat) of MUFA in the IMF.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
c9C14:1	2.83^{c}	2.30^{a}	2.34^{a}	2.69^{b}	0.01	0.02
c7C16:1	8.75^{c}	5.51^{b}	7.80^{c}	2.79^{a}	0.03	0.03
c9C16:1	13.37	12.32	13.08	13.14	0.17	0.13
t11C18:1 (TVA)	5.32	5.05	5.081	6.91	0.15	0.16
c7C18:1	12.72^{b}	9.13^{a}	9.45^{a}	9.03^{a}	0.08	0.03
c9C18:1	240	241	249	245	5	0.62
c12C18:1	22.24	19.79	20.56	25.78	0.24	0.33
c14C18:1	8.25^{c}	7.40^{b}	8.26^{c}	2.53^{a}	0.07	0.02
c11C20:1	0.843^{a}	0.826^{a}	1.003^{b}	1.319^{c}	0.011	0.03
c13C22:1	0.046^{a}	0.088^{b}	0.088^{b}	0.128^{c}	0.004	0.02
ΣMUFA	314	303	317	309	8	0.33
C18:1Δ9-index[1]	0.582^{a}	0.603^{b}	0.617^{b}	0.572^{a}	0.005	0.03
CLAΔ9-index[2]	0.155^{a}	0.164^{b}	0.178^{c}	0.169^{b}	0.002	0.02
ΣMUFA/ΣFA	0.421	0.428	0.428	0.411	0.004	0.29

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; MUFA = monounsaturated fatty acids; IMF = fat located between the thigh muscles; SEM = standard error of the mean; CLA = conjugated linoleic acid (C18:2); c = cis; t = trans; ΣMUFA = all assayed MUFA (the concentration sum of c7C14:1, c9C14:1, isoC15:1, c7C16:1, c9C16:1, c9C17:1, t9C18:1, t11C18:1, c6C18:1, c7C18:1, c9C18:1, c11C18:1, c12C18:1, c11C20:1, c11C22:1, c13C22:1 and c15C24:1); ΣMUFA/ΣFA = the ratio of ∑MUFA to all assayed fatty acids.
[a, b, c] Different letters within a row indicate significant differences at $P \leq 0.05$.
[1] The index values of Δ9-desaturases, $^{C18:1}$Δ9-index = c9C18:1/(c9C18:1 + C18:0).
[2] The index values of Δ9-desaturases, CLAΔ9-index = c9t11CLA/(c9t11CLA + TVA).

Table 7
The concentrations (μg/g heart) of MUFA in the heart.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
c9C14:1	32^{c}	218^{d}	14^{b}	2^{a}	1	0.01
c7C16:1	69^{c}	137^{d}	35^{b}	3^{a}	2	0.01
c9C16:1	69^{c}	101^{d}	29^{b}	3^{a}	3	0.03
t11C18:1 (TVA)	1.02	1.13	0.83	1.41	0.09	0.31
c7C18:1	172^{c}	274^{d}	32^{b}	6^{a}	4	0.03
c9C18:1	3,942	6,450	3,702	4,539	59	0.37
c12C18:1	1,063	846	601	1,038	48	0.24
c11C20:1	23.0^{b}	34.4^{c}	5.8^{a}	6.6^{a}	0.9	0.03
c15C24:1	12.4^{c}	13.8^{c}	3.6^{b}	0.2^{a}	0.4	0.04
ΣMUFA/ΣPUFA	0.882^{c}	1.278^{d}	0.634^{a}	0.700^{b}	0.004	0.03
$^{C18:1}$Δ9-index[1]	0.417	0.486	0.403	0.399	0.016	0.29
^{CLA}Δ9-index[2]	0.970	0.981	0.975	0.976	0.006	0.34
ΣMUFA/ΣFA	0.267^{b}	0.310^{c}	0.218^{a}	0.230^{a}	0.003	0.03

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; MUFA = monounsaturated fatty acids; SEM = standard error of the mean; CLA = conjugated linoleic acid (C18:2); c = cis; t = trans; ΣMUFA = all assayed MUFA (the concentration sum of c7C14:1, c9C14:1, isoC15:1, c7C16:1, c9C16:1, c9C17:1, t9C18:1, t11C18:1, c6C18:1, c7C18:1, c9C18:1, c11C18:1, c12C18:1, c11C20:1, c11C22:1, c13C22:1 and c15C24:1); ΣMUFA/ΣFA = the ratio of ∑MUFA to all assayed fatty acids.
[a, b, c, d] Different letters within a row indicate significant differences at $P \leq 0.05$.
[1] The index values of Δ9-desaturases, $^{C18:1}$Δ9-index = c9C18:1/(c9C18:1 + C18:0)].
[2] The index values of Δ9-desaturases, CLAΔ9-index = c9t11CLA/(c9t11CLA + TVA).

Table 8
The concentrations (mg/g fat) of PUFA in the IMF.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
c9t11CLA	1.03^{a}	1.02^{a}	1.12^{b}	1.42^{c}	0.03	0.04
Σct/tcCLA[1]	0.411^{a}	0.813^{c}	1.034^{d}	0.599^{b}	0.012	0.01
ΣCLA[2]	1.44^{a}	1.83^{b}	2.15^{c}	2.02^{bc}	0.04	0.04
c9c12C18:2 (LA)	50.4	47.3	50.4	51.4	1.0	0.43
c9c12c15C18:3 (αLNA)	1.80	1.96	2.54	1.67	0.09	0.06
c11c14C20:2	0.001^{a}	0.065^{b}	0.067^{b}	0.136^{c}	0.005	0.01
c5c8c11c14C20:4 (AA)	0.067^{a}	0.083^{b}	0.111^{c}	0.123^{c}	0.004	0.02
c5c8c11c14c17C20:5 (EPA)	0.016^{a}	0.057^{b}	0.060^{b}	0.066^{b}	0.003	0.02
c7c10c13c16c19C22:5 (DPA)	0.114^{b}	0.086^{a}	0.095^{ab}	0.145^{c}	0.003	0.03
Σn-6PUFA[3]	50.5	47.4	50.5	51.5	1.0	0.18
Σn-3PUFA[4]	1.93^{a}	2.11^{b}	2.69^{c}	1.88^{a}	0.03	0.03
ΣPUFA[5]	53.8	51.4	55.4	55.6	1.1	0.19
Σn-6PUFA/Σn-3PUFA	26.1^{c}	22.5^{b}	18.8^{a}	27.4^{c}	0.4	0.04
Σn-6LPUFA[6]	0.067^{a}	0.083^{b}	0.110^{c}	0.123^{c}	0.003	0.03
Σn-3LPUFA[7]	0.129^{a}	0.143^{ab}	0.155^{b}	0.210^{c}	0.004	0.02
ΣLPUFA[8]	0.197^{a}	0.226^{b}	0.266^{c}	0.333^{d}	0.006	0.02
Σn-6LPUFA/Σn-3LPUFA	0.520^{a}	0.583^{b}	0.710^{c}	0.586^{b}	0.004	0.02
Σn-3LPUFA/ΣFA[9]	0.170^{a}	0.204^{b}	0.215^{b}	0.295^{c}	0.004	0.04
ΣLPUFA/ΣFA[10]	0.254^{a}	0.330^{b}	0.363^{c}	0.478^{d}	0.005	0.03
ΣPUFA/ΣFA[11]	0.072	0.072	0.075	0.074	0.001	0.29
$^{Σ Elong}$index[12]	0.00225^{a}	0.00317^{b}	0.00321^{b}	0.0542^{c}	0.00004	0.02

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; PUFA = polyunsaturated fatty acids; IMF = fat located between the thigh muscles; SEM = standard error of the mean; CLA = conjugated linoleic acid (C18:2); c = cis; t = trans; LPUFA = long-chain polyunsaturated fatty acids; FA = fatty acids; Σ ^{Elong}index = index of elongases.
$^{a, b, c, d}$ Different letters within a row indicate significant differences at $P \leq 0.05$.
[1] The concentration sum of ct/tcCLA isomers: cis-transCLA: 11-13, 12-14; trans-cisCLA: 7-9, 8-10, 9-11, 10-12, 11-13 and 12-14.
[2] The concentration sum: c9t11CLA, ct/tcCLA isomers, ttCLA isomers (trans−trans: 7-7, 8-10, 9-11, 10-12, 11-13 and 12-14) and ccCLA isomers (cis−cis: 8-10, 9-11, 10-12 and c11-12).
[3] The concentration sum: LA, c6c9c12C18:3 c11c14C20:2, AA and c7c10c13c16C22:4.
[4] The concentration sum: αLNA, c6c9c12c15C18:4 and Σn-3LPUFA.
[5] The concentration sum: ΣCLA, Σn-3PUFA and Σn-6PUFA.
[6] The concentration sum: c11c14C20:2, AA and c7c10c13c16C22:4.
[7] The concentration sum: c8c11c14c17C20:4, EPA, DPA and DHA.
[8] The concentration sum: Σn-6LPUFA and Σn-3LPUFA.
[9] The concentration ratio of Σn-3LPUFA, μg/g IMF to ΣFA (mg/g IMF).
[10] The concentration ratio of Σn-3LPUFA, μg/g IMF to ΣFA (mg/g IMF).
[11] The concentration sum of all FA (ΣFA).
[12] Σ ^{Elong}index = (c11c14C20:2 + c7c10c13c16c19C22:5)/(c11c14C20:2 + c7c10c13c16c19C22:5 + c5c8c11c14c17C20:5 + c9c12C18:2).

as the values of the $^{Σ Elong}$index and the ratios of Σn-3LPUFA/ΣFA, ΣLPUFA/ΣFA and ΣPUFA/ΣFA in the heart compared to the control and CA diets. Moreover, ^{VI}Se added to the experimental diet significantly increased ($P \leq 0.05$) the value of $^{Σ Elong}$index and the concentrations of c9t11CLA, ΣCLA, αLNA, c11c14C20:2, c8c11c14C20:3, DPA and Σn-3LPUFA, and numerically ($P > 0.05$) increased the contents of LA, AA, EPA, DHA, Σn-6PUFA, Σn-3PUFA and Σn-6LPUFA in the heart compared to the experimental diet enriched in ^{Y}Se. All experimental diets, particularly those enriched in ^{Y}Se or ^{VI}Se, decreased ($P \leq 0.05$) the values of the Σn-6PUFA/Σn-3PUFA and Σn-6LPUFA/Σn-3LPUFA ratios in the heart in comparison with the control diet. The experimental diets enriched in ^{VI}Se, and especially in ^{Y}Se, increased ($P \leq 0.05$) the concentration ratio of ΣPUFA/ΣSFA in the heart compared to the control and CA diets. Compared to the control diet, the ratio of ΣPUFA/ΣSFA was lower in the heart of lambs fed the CA diet.

3.3. Concentrations of TCh, tocopherols and MDA in the IMF and heart

The current study demonstrated that adding ^{VI}Se to the experimental diet increased ($P \leq 0.05$) the concentrations of TCh and αT, and numerically ($P > 0.05$) elevated the concentration sum of αT and αTAc (Σ[αT+αTAc]) in the IMF compared to the control, CA and ^{Y}Se-CA diets (Table 10). Similarly, the ^{Y}Se-CA diet stimulated ($P \leq 0.05$) the accumulation of TCh in the IMF in comparison to the control and CA diets. Conversely, the experimental diet supplemented with ^{Y}Se or ^{VI}Se decreased ($P \leq 0.05$) the concentration of MDA and the value of the PUFA peroxidation index (MDA_{index}; MDA_{index} = MDA/ΣPUFA) in the IMF compared to the control and CA diets. Moreover, in the IMF of lambs fed the CA diet the concentration of MDA and values of MDA_{index} and $^{new}MDA_{index}$ ($^{new}MDA_{index}$ = MDA/[ΣPUFA + 0.5 × ΣMUFA]) decreased ($P \leq 0.05$) in comparison to the control diet.

It has also been shown that the experimental diets supplemented with ^{Y}Se or ^{VI}Se increased ($P \leq 0.05$) the concentrations of TCh, γT, αTAc and Σ(αT+αTAc) in the heart compared to the control and CA diets (Table 10). When the diet was supplemented only with CA, the concentrations of αT and Σ(αT+αTAc) in the heart were higher ($P \leq 0.05$) than those of the control diet. In contrast, all experimental diets decreased ($P \leq 0.05$) the concentration of MDA and the value of $^{new}MDA_{index}$ in the heart compared to the control diet.

The effects of the experimental diets on the h/H-Ch ratio, and the thrombogenic ($_{index}T^{SFA}$), atherogenic (indexASFA) and modified atherogenic ($_{index}A^{SFA + Toc}$) indices in the IMF and heart of lambs are presented in Table 11. The values of the h/H-Ch ratio and $_{index}T^{SFA}$ in the IMF were higher ($P \leq 0.05$) for lambs on the CA and ^{VI}Se-CA diets compared with lambs fed the control and ^{Y}Se-CA diets. In contrast, when ^{Y}Se was added to the experimental diet, the values of the h/H-Ch ratio and $_{index}T^{SFA}$ in the IMF decreased most efficiently compared to the control, CA and ^{VI}Se-CA diets. The value of $_{index}A^{SFA + Toc}$ in the IMF of lambs fed the ^{VI}Se-CA diet was lower ($P \leq 0.05$) than that of the control, CA and ^{Y}Se-CA diets. The ^{VI}Se-CA diet most efficiently decreased ($P \leq 0.05$) the value of $_{index}A^{SFA + Toc}$

Table 9
The concentrations (μg/g heart) of in PUFA the heart.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
c9t11CLA	33^{a}	58^{b}	33^{a}	58^{b}	2	0.03
Σct/tcCLA[1]	5.6	5.0	5.5	5.1	0.2	0.06
ΣCLA[2]	38.6^{a}	63.1^{b}	38.5^{a}	63.1^{b}	0.02	0.04
c9c12C18:2 (LA)	3,579	3,730	3,766	4,184	96	0.51
c9c12c15C18:3 (αLNA)	253^{a}	311^{b}	320^{b}	388^{c}	7	0.03
c11c14C20:2	80^{a}	77^{a}	107^{b}	168^{c}	3	0.02
c8c11c14C20:3	53^{a}	57^{a}	105^{b}	142^{c}	3	0.03
c5c8c11c14C20:4 (AA)	1,810^{a}	1,755^{a}	2,133^{c}	2,469^{c}	43	0.03
c5c8c11c14c17C20:5 (EPA)	87^{a}	92^{a}	157^{b}	167^{b}	4	0.04
c7c10c13c16c19C22:5 (DPA)	115^{a}	132^{a}	170^{b}	209^{c}	4	0.02
c4c7c10c13c16c19C22:6 (DHA)	91^{a}	102^{a}	177^{b}	208^{b}	4	0.03
Σn-6PUFA[3]	5,442	5,542	6,003	6,795	99	0.23
Σn-3PUFA[4]	546^{a}	636^{ab}	824^{b}	972^{b}	27	0.04
ΣPUFA[5]	6,107	6,317	6,972	7,998	101	0.22
Σn-6PUFA/Σn-3PUFA	10.0^{c}	8.7^{b}	7.3^{a}	7.0^{a}	0.2	0.03
Σn-6LPUFA[6]	1,943^{a}	1,888^{a}	2,344^{b}	2,779^{b}	31	0.03
Σn-3LPUFA[7]	292^{a}	325^{a}	504^{b}	584^{c}	11	0.03
Σn-6LPUFA/Σn-3LPUFA	6.65^{c}	5.81^{b}	4.65^{a}	4.76^{a}	0.03	0.01
Σn-3LPUFA/ΣFA[8]	0.0147^{a}	0.0136^{a}	0.0248^{b}	0.0239^{b}	0.0006	0.03
ΣLPUFA/ΣFA[9]	0.107^{a}	0.084^{a}	0.135^{b}	0.132^{b}	0.004	0.04
ΣPUFA/ΣFA[10]	0.306^{b}	0.265^{a}	0.343^{c}	0.331^{c}	0.001	0.03
Σ^{Elong}index[11]	0.0504^{a}	0.0517^{a}	0.0659^{b}	0.0798^{c}	0.0006	0.03

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; PUFA = polyunsaturated fatty acids; SEM = standard error of the mean; CLA = conjugated linoleic acid (C18:2); c = cis; t = trans; LPUFA = long-chain polyunsaturated fatty acids; FA = fatty acids; Σ ^{Elong}index = index of elongases.
$^{a, b, c}$ Different letters within a row indicate significant differences at $P \leq 0.05$.
[1] The sum of ct/tcCLA isomers: cis-transCLA: 11-13, 12-14; trans-cisCLA: 7-9, 8-10, 9-11, 10-12, 11-13 and 12-14.
[2] The sum: c9t11CLA, ct/tcCLA isomers, ttCLA isomers (trans–trans: 7-7, 8-10, 9-11, 10-12, 11-13 and 12-14) and ccCLA isomers (cis–cis: 8-10, 9-11, 10-12 and c11-12).
[3] The concentration sum: LA, c6c9c12C18:3 c11c14C20:2, AA and c7c10c13c16C22:4.
[4] The concentration sum: αLNA, c6c9c12c15C18:4 and Σn-3LPUFA.
[5] The concentration sum: ΣCLA, Σn-3PUFA and Σn-6PUFA.
[6] The concentration sum: c11c14C20:2, c8c11c14C20:3, AA and c7c10c13c16C22:4.
[7] The concentration sum: c8c11c14c17C20:4, EPA, DPA and DHA.
[8] The concentration ratio of Σn-3LPUFA, μg/g heart to ΣFA (mg/g heart).
[9] The concentration ratio of ΣLPUFA, μg/g heart to ΣFA (mg/g heart).
[10] The concentration sum of all FA (ΣFA).
[11] Σ ^{Elong}index = (c11c14C20:2 + c7c10c13c16c19C22:5)/(c11c14C20:2 + c7c10c13c16c19C22:5 + c5c8c11c14c17C20:5 + c9c12C18:2).

Table 10
The concentrations of total cholesterol, tocopherols and MDA and values of the PUFA peroxidation indices in the IMF and heart.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
IMF						
Total cholesterol, μg/g tissue	49.1^{c}	42.1^{a}	47.5^{c}	62.5^{b}	1.1	0.03
α-Tocopherol (αT), μg/g tissue	2.10^{a}	2.67^{ab}	2.35^{a}	3.36^{c}	0.02	0.03
α-Tocopheryl acetate (αTAc), μg/g tissue	5.56^{c}	5.07^{ab}	5.28^{bc}	4.85^{a}	0.04	0.02
Σ(αT+αTAc)[1], μg/g tissue	7.66	7.74	7.64	8.22	0.07	0.17
MDA[2], ng/g tissue	1.64^{c}	1.14^{b}	0.72^{a}	0.74^{a}	0.02	0.03
MDA$_{index}$[3]	0.031^{c}	0.022^{b}	0.013^{a}	0.013^{a}	0.001	0.03
^{new}MDA$_{index}$[4], × 10^{-3}	7.77^{c}	5.61^{b}	3.37^{a}	3.52^{a}	0.06	0.02
The heart						
Total cholesterol, μg/g tissue	126^{a}	153^{a}	210^{b}	226^{b}	3	0.03
δ-Tocopherol, μg/g tissue	1.84	1.80	2.77	1.77	0.9	0.12
γ-Tocopherol, μg/g tissue	3.58^{b}	3.07^{a}	5.43^{d}	4.08^{c}	0.08	0.02
αT, μg/g tissue	1.96^{a}	3.54^{c}	2.47^{b}	4.21^{d}	0.10	0.04
αTAc, μg/g tissue	1.58^{a}	1.53^{a}	3.71^{b}	3.63^{b}	0.03	0.03
Σ(αT+αTAc), μg/g tissue	3.54^{a}	5.07^{b}	6.18^{c}	7.83^{d}	0.07	0.02
Σall-Ts[5], μg/g tissue	8.96^{a}	9.94^{a}	14.38^{b}	13.68^{b}	0.08	0.03
Se, μg/g	0.799^{a}	0.871^{b}	1.134^{c}	1.138^{c}	0.011	0.03
MDA[2], ng/g tissue	15.0^{b}	11.6^{a}	11.5^{a}	10.6^{a}	0.3	0.03
MDA$_{index}$[3]	2.45^{c}	1.84^{b}	1.65^{b}	1.33^{a}	0.01	0.02
^{new}MDA$_{index}$[4]	1.705^{c}	1.120^{b}	1.252^{b}	0.982^{a}	0.012	0.02

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; MDA = malondialdehyde; PUFA = polyunsaturated fatty acids; IMF = fat located between the thigh muscles.
$^{a, b, c, d}$ Different letters within a row indicate significant differences at $P \leq 0.05$.
[1] The concentration sum: αT and αTAc.
[2] Concentrations of MDA in tissues were determined immediately after the homogenization of the IMF and heart samples.
[3] The concentration ratio of MDA (ng/g tissue) to ΣPUFA (mg/g tissue), MDA$_{index}$ = MDA/ΣPUFA.
[4] The concentration ratio of MDA (ng/g tissue) to ΣPUFA and MUFA (mg/g tissue), ^{new}MDA$_{index}$ = MDA/(ΣPUFA + 0.5 × ΣMUFA).
[5] The concentration sum of all tocopherols, Σall-Ts = δT + γT + αT + αTAc.

Table 11
The hypocholesterolemic/hypercholesterolemic FA (h/H-Ch) ratio and the thrombogenic ($_{index}T^{SFA}$), atherogenic (indexASFA) and modified atherogenic ($_{index}A^{SFA + Toc}$) indices in the IMF and the heart of lambs fed the control and experimental diets.

Item	Group				SEM	*P*-value
	Control	CA	^{Y}Se-CA	^{VI}Se-CA		
IMF						
h/H-Ch ratio[1]	1.806^{b}	1.844^{c}	1.746^{a}	1.834^{c}	0.008	0.02
$_{index}T^{SFA}$[2]	1.804^{c}	1.749^{b}	1.696^{a}	1.871^{d}	0.007	0.03
$_{index}A^{SFA}$[3]	0.790	0.787	0.854	0.785	0.007	0.27
$_{index}A^{SFA + Toc}$[4]	0.0739^{b}	0.0723^{b}	0.0799^{c}	0.0676^{a}	0.0006	0.02
The heart						
h/H-Ch ratio[1]	3.741^{c}	3.230^{a}	3.261^{a}	3.398^{b}	0.009	0.04
$_{index}T^{SFA}$[2]	0.822^{c}	0.851^{d}	0.715^{a}	0.735^{b}	0.002	0.03
$_{index}A^{SFA}$ [3]	0.291^{a}	0.345^{c}	0.338^{c}	0.304^{b}	0.001	0.04
$_{index}A^{SFA + Toc}$ [4]	0.0511^{d}	0.0436^{c}	0.0349^{b}	0.0255^{a}	0.0003	0.01

CA = carnosic acid; ^{Y}Se = selenised yeast; ^{VI}Se = selenate; IMF = fat located between the thigh muscles; SEM = standard error of the mean; c = cis.
[a, b, c, d] Different letters within a row indicate significant differences at $P \leq 0.05$.
[1] h/H-Ch ratio = (c7C18:1 + c9C18:1 + c12C18:1 + c14C18:1 + c11C20:1 + 13C22:1 + c9c12C18:2 + c9c12c15C18:3 + c6c9c12C18:3 + c5c8c11c14C20:4 + c11c14C20:2 + c5c8c11c14c17C20:5 + c7c10c13c16C22:4 + c7c10c13c16c19C22:5)/(C14:0 + C16:0) (Fernández et al., 2007).
[2] The thrombogenic index, indexTSFA = (C14:0 + C16:0 + C18:0)/[(0.5 × ΣMUFA + 0.5 × Σn-6PUFA + 3 × Σn-3PUFA)/Σn-6PUFA)] (Morán et al., 2013).
[3] The atherogenic index, indexASFA = (C12:0 + 4 × C14:0 + C16:0)/(ΣMUFA + Σn-6PUFA + Σn-3PUFA) (Morán et al., 2013).
[4] The modified atherogenic index, $_{index}A^{SFA + Toc} = {}_{index}A^{SFA}/(1.49 \times C_{\alpha T} + 1.36 \times C_{\alpha TAc} + 0.15 \times C_{\gamma T} + 0.05 \times C_{\delta T})$, where: $_{index}A^{SFA}$ - the atherogenic index; $C_{\alpha T}$ - the concentration of α-tocopherol (αT); $C_{\alpha TAc}$ - the concentration of α-tocopheryl acetate (αTAc); $C_{\gamma T}$ - the concentration of γ-tocopherol (γT); $C_{\delta T}$ - the concentration of δ-tocopherol (δT). Tocopherol concentrations, μg/g tissue.

in the IMF compared to the control and other experimental diets, whereas no difference ($P > 0.05$) in the value of $_{index}A^{SFA + Toc}$ was noted between the CA and the control diets.

In our study, a significant decrease ($P \leq 0.05$) in the values of the h/H-Ch ratio and $_{index}A^{SFA + Toc}$ was observed in the heart of lambs fed the experimental diets compared to the control diet (Table 11). Moreover, the experimental diets containing CA, irrespective of the presence of ^{Y}Se, increased ($P \leq 0.05$) the values of $_{index}A^{SFA}$ and $_{index}A^{SFA + Toc}$ in the heart compared to the experimental diet supplemented with ^{VI}Se. All experimental diets increased ($P \leq 0.05$) the value of $_{index}A^{SFA}$ in the heart compared to the control diet. The value of $_{index}T^{SFA}$ in the heart of lambs fed the experimental diets supplemented with ^{Y}Se or ^{VI}Se was lower ($P \leq 0.05$) than that of the control and CA diets; the CA diet most efficiently increased the value of $_{index}T^{SFA}$.

4. Discussion

The results of our studies documented that CA enriched in FO and RO supplemented to the diet does not cause malicious or harmful symptoms (e.g. diarrhoea and vomiting) to lambs. Furthermore, no noticeable pathological changes, acute toxic effects of Se-compounds and toxic changes of all diets enriched in CA, ^{Y}Se or ^{VI}Se were observed in the heart, adipose tissues, muscles, as well as in any other internal organ of the lambs (Białek et al., 2018; Białek and Czauderna, 2019; Czauderna et al., 2017; Miltko et al., 2016; Rozbicka-Wieczorek et al., 2016a, 2016b). Thus, our most recent studies, including the present one, are consistent with earlier research (Fairweather-Tait et al., 2011; Navarro-Alarcon and Cabrera-Vique, 2008; Rayman, 2008; Raymond et al., 2014; Yu et al., 2008), which have indicated that ^{VI}Se and SeY rich in Se-Met (in contrast to ^{IV}Se and especially selenide) are less reactive, and because $tRNA_{Met}$ does not distinguish between Se-Met and Met, Se-Met is incorporated into proteins in the place of Met (Navarro-Alarcon and Cabrera-Vique, 2008). The fate of supplemental Se-Met depends on whether Se released from Se-Met by microbial metabolism in the rumen is further degraded to the inorganic chemical form of Se or bio-incorporated into the proteins of ruminal microorganisms, as Se-Cys or Se-Met (Navarro-Alarcon and Cabrera-Vique, 2008).

In our study, ^{VI}Se added to the diet enriched in CA most effectively increased the average daily live weight gain of lambs compared to the control, CA and ^{Y}Se-CA diets. Indeed, the ^{VI}Se-CA diet decreased ruminal microbial fermentation rate (Miltko et al., 2016). As a consequence, this experimental diet most effectively increased bacterial protein biosynthesis, which is in agreement with our earlier studies, as the ^{VI}Se-CA diet is characterised by the highest level of protein content in the lamb muscle (Jaworska et al., 2016). Furthermore, the ^{VI}Se-CA diet decreased the yield of the ruminal fermentation of carbohydrates into volatile FA (like acetic, propionic, butyric and valeric acids) and lipogenic enzymes in the tissues of lambs (acetate is a precursor for lipogenesis). Thus, the ^{VI}Se-CA diet most efficiently decreased the synthesis of CH_4 and CO_2 in the rumen of lambs (Miltko et al., 2016). CH_4 is a high-energy compound and its elimination as a waste product causes the loss of approximately 8% of the total digestible energy of the diet (Wolin, 1979). Furthermore, dietary ^{VI}Se can be used for the synthesis of Se-Cys, which is inserted into the Se-enzyme's primary structure (Raymond et al., 2014). These Se-Cys containing enzymes stimulate thyroid hormone synthesis, which regulates important biochemical reactions, particularly protein synthesis and enzymatic capacity, accompanied by an increase in metabolic rate. Considering the above, we argue that ^{VI}Se added to the diet stimulates anabolic processes in the lambs' tissues. In contrast, the experimental diet with ^{Y}Se (rich in Se-Met) decreased the average daily live weight gain of lambs compared to the experimental diet with ^{VI}Se. Indeed, Se-Met derived from SeY is mainly incorporated into body proteins in the place of Met; these Se-proteins are not considered Se-enzymes (Raymond et al., 2014). In contrast to Se-Cys containing enzymes, Se-Met containing proteins revealed a negligible effect on the biosynthesis of the thyroid hormone regulating important biochemical reactions, particularly protein biosynthesis. Moreover, the ^{Y}Se-CA diet most efficiently increased the synthesis of CH_4 and CO_2 in the rumen of lambs (Miltko et al., 2016; Rozbicka-Wieczorek et al., 2016b).

4.1. Effects of the experimental diets on concentrations of FA and tocopherols in the heart and IMF

In ruminant tissues, the quality of fat is as important as its quantity. Currently, health professionals emphasise the association between nutrition and a number of non-communicable diseases in

humans (especially cancer and cardiovascular diseases), and have focused scientific research on the role of physiologically important FA. Dietary advice recommends an increase in the level of PUFA (especially n-3LPUFA) in human diets, a decrease in the concentrations of T-SFA and A-SFA, the maintenance of the ratio of PUFA to SFA at approximately 0.45 or higher, and an increase in the intake of n-3PUFA relative to n-6PUFA, such that the n-6PUFA/n-3PUFA ratio is less than 4 (Ahmed et al., 2018; Attia et al., 2015; Byelashov et al., 2015; Calder, 2013, 2017; Peter et al., 2013). In previous studies, diets enriched with linseed oil rich in PUFA (especially αLNA) maintained the ratio of PUFA to SFA at approximately 0.5 or higher in the liver, pancreas, kidneys, adipose tissues, blood plasma and the muscles of lambs (Krajewska et al., 2012; Niedźwiedzka et al., 2008). In our current studies, the diets were supplemented with 2% RO rich in PUFA (especially in linoleic acid [LA]) and 1% FO rich in LPUFA (particularly in n-3LPUFA). Ruminal microbiota, however, efficiently reduced concentrations of dietary PUFA through isomerisation and biohydrogenation reactions (Białek et al., 2018; Białek and Czauderna, 2019; Del Razo-Rodriguez et al., 2013). Dietary FO, rich in highly unsaturated LPUFA, reduced ruminal bacterial isomerase activity and the bacterial biohydrogenation of UFA to C18:0, causing the accumulation of a number of UFA intermediates in ruminants' tissues (Białek et al., 2018; Del Razo-Rodriguez et al., 2013). Similarly, dietary CA supplementation also modifies the ruminal microbiota population, reducing the capacity of the bacterial isomerisation of UFA and the biohydrogenation of UFA to C18:0 and, hence, the ruminal biosynthesis of volatile FA and FA compositions in ruminants' tissues (Białek and Czauderna, 2019; Del Razo-Rodriguez et al., 2013; Jordan et al., 2013; Morán et al., 2013). Our studies documented that all experimental diets resulted in lower values of the ΣPUFA/ΣSFA ratio in the IMF (Table 4) than the recommended ratio (i.e. 0.1422 to 0.1514 versus 0.45 or higher) (Reinagel, 2012).

Moreover, as a consequence of the high concentration of LA (approximately 28.2%) in dietary RO, the Σn-6PUFA/Σn-3PUFA ratio in the IMF (>20; Table 8) and in the heart (>7; Table 9) was very high for all groups, compared to the literature (the recommended ratio is ≤ 4) (Byelashov et al., 2015; Calder, 2013, 2017). The current results are consistent with our previous studies, in which the Σn-6PUFA/Σn-3PUFA ratio was also high in the subcutaneous fat (10.7 to 12.1) and whole blood (10.6 to 17.8) of lambs fed with diets enriched in RO, FO, CA and Se (as ^{Y}Se or ^{VI}Se) (Czauderna et al., 2017; Krajewska-Bienias et al., 2017). Notably, the experimental diets, especially those supplemented with ^{Y}Se or ^{VI}Se, as well as the control diet, resulted in higher values of the ΣPUFA/ΣSFA ratio in the heart (Table 5) than the minimum value of the recommended ratio (i.e. 0.45). Thus, our results indicated that preferences in bioaccumulations of SFA, n-3PUFA and n-6PUFA are different in the heart and IMF. Interestingly, n-3LPUFA, and especially n-6LPUFA, are more efficiently incorporated in the heart than in the IMF (Tables 8 and 9). In fact, the membrane phospholipids of the heart, liver or brain respond to PUFA concentrations in diets in a similarly biphasic manner (Abbott et al., 2012). The membrane lipids of these internal organs, especially the heart, are highly responsive to PUFA concentrations in diets. Moreover, dietary PUFA affects membrane FA composition to a much greater extent than dietary SFA and MUFA. Indeed, the presence of PUFA influences membrane fluidity, an essential parameter determining the efficiency of interactions between membrane-bound small molecules and membrane proteins: the more double bonds a PUFA contains, the more "fluid" the membranes of cells (Valentine and Valentine, 2004).

Our current studies are consistent with the results of recent investigations in which dietary CA supplementation affects FA profiles in the rumen and tissues of ruminants. In fact, compared to the control diet, the experimental diet enriched in CA modified FA profiles in the IMF and heart (Tables 4 to 9), as well as in other adipose tissues, muscles and internal organs of lambs (Białek et al., 2018; Białek and Czauderna, 2019; Krajewska-Bienias et al., 2017; Miltko et al., 2016; Rozbicka-Wieczorek et al., 2016a). Moreover, our studies suggest that CA added to the diet, regardless of the presence of ^{VI}Se, increased the accumulation of the bacterial isomerisation products of PUFA (i.e. c9t11CLA) in the heart compared to the control and ^{Y}Se-CA diets. Similarly, CA supplemented to the diet, regardless of the presence of ^{Y}Se or ^{VI}Se, stimulated the incorporation of ct/tcCLA isomers (PUFA isomerisation products; Table 8) and the index value of Δ9-desaturation of TVA (^{CLA}Δ9-index) (Table 6) in the IMF compared to the control diet. Thus, our current results are in agreement with our recent investigations in which the CA, ^{Y}Se-CA and ^{VI}Se-CA diets also increased Δ9-desaturation of C18:0 and TVA in the subcutaneous adipose tissue and the rumen-surrounding fat compared to the control diet (Białek and Czauderna, 2019; Krajewska-Bienias et al., 2017).

Our studies documented that, compared to the control diet, the diets supplemented with CA, irrespective of the presence of ^{Y}Se or ^{VI}Se (the antioxidants) significantly increased the capacity of fatty acid elongases (Σ^{Elong}index) in the IMF (0.00225 versus 0.00317 to 0.00542; Table 8) and significantly decreased the concentration of MDA in the IMF (1.64 versus 0.74 to 1.14) and heart (15.0 versus 10.6 to 11.6; Table 10). Similarly, compared to the control diet, all experimental diets reduced the values of MDA_{index} in the IMF (0.031 versus 0.013 to 0.022) and heart (2.45 versus 1.33 to 1.84; Table 10).

Moreover, our results showed that ^{VI}Se added to the experimental diet with CA intensifies the increase in the capacity of fatty acid elongases in the IMF compared to the control, CA and ^{Y}Se-CA diets. Conversely, compared to the control diet, the capacity of fatty acid elongases statistically increased in the heart of lambs fed the experimental diets containing ^{Y}Se and especially ^{VI}Se (Table 9). Thus, our current results are in agreement with the findings of Stiuso et al. (2014), in which a diet supplemented with the derivative Realsil (the active antioxidant) stimulated fatty acid elongase activity. Moreover, previous studies (Jump, 2009; Kihara, 2012) documented that fatty acid elongases are expressed differently in various mammal tissues. These enzymes are also regulated by diet composition, hormones and during organism development. As a consequence, changes in elongase capacity particularly affect the profiles of LPUFA in the analysed tissues. In fact, our results indicated (Tables 8 and 9) that ^{Y}Se and ^{VI}Se added to the experimental diet considerably increased the capacity of fatty acid elongases, as the values of Σ^{Elong}index and the concentrations of Σn-6LPUFA and, especially, Σn-3LPUFA statistically or numerically increased in the IMF and particularly in the heart, compared to the control and CA diets.

4.2. Effects of the experimental diets on concentrations of TCh, tocopherols and MDA in the heart and IMF

As the key component of GPx, which possesses antioxidative properties, Se has been considered to play an important role in lipid metabolism. The association of serum Se contents with lipid levels and dyslipidaemia, however, is still controversial (González-Estecha et al., 2017; Ju et al., 2018). Se levels in human or animal diets positively correlate with concentrations of serum lipids. Recent evidence comes from selenium-replete populations, such as that of the United States, which have shown that high Se-status increases the risk of diabetes and hyperlipidaemia. The potential mechanisms that might explain the consistent associations of Se are still unclear, although a number of pathways involving Se-compounds or Se-proteins are known to interact strongly with both lipids and lipoproteins (Stranges et al., 2011).

Therefore, enhanced levels of dietary Se (especially as ^{IV}Se or ^{VI}Se) are positively associated with increased TCh in some tissues of animals and humans. Indeed, ^{IV}Se or ^{VI}Se added to diets stimulated the biosynthesis of Se-Cys that can be incorporated into Se-Cys-enzymes. In contrast, Se-Met derived from SeY is non-specifically incorporated into Se-Met proteins (i.e. non-enzymatic proteins). In fact, our current investigation and previous studies on lambs have also documented that, especially, ^{VI}Se added to the diet increased the concentrations of TCh in the heart and IMF, as well as in the rumen-surrounding fat (Białek and Czauderna, 2019) and subcutaneous fat (Krajewska-Bienias et al., 2017) compared to diets without supplemented Se. These findings are also confirmed by the fact that the ^{Y}Se or ^{VI}Se introduced to the experimental diets decreased the h/H-Ch ratio and $_{index}A^{SFA}$. Moreover, the experimental diet enriched with ^{VI}Se increased the concentration of A-SFA in the heart compared to the control diet.

In the current study, we found that the concentration of tocopherols in the heart, in particular, was significantly influenced by ^{Y}Se and ^{VI}Se supplementation, which is fully consistent with previous studies (Białek and Czauderna, 2019; Czauderna et al., 2017, 2018; Helzlsouer et al., 2000; Leskovec et al., 2018). Compared to adipose tissues (including IMF), Se supplemented to diets is efficiently accumulated in the heart, liver, muscles or blood (Czauderna et al., 2017, 2018). Studies have shown that the role of Se in improving the effectiveness of tocopherols is (at least in part) due to the fact that dietary Se stimulates the bioaccumulation of αT. Tocopherols may be carried by Se-lipoprotein fractions associated with serum γ-globulin. Thus, one physiological role of Se appears to be related to Se-compounds, which act as a carrier of tocopherols and which may function in the bioaccumulation, retention, prevention of destruction of tocopherols, as well as the transfer of αT across cell membranes. From the results of studies applying organic or inorganic forms of Se, it can be seen that part of dietary Se in the liver, heart, muscles or blood is metabolised into antioxidative enzymes containing Se-Cys (Fairweather-Tait et al., 2011; Navarro-Alarcon and Cabrera-Vique, 2008; Rayman, 2008; Raymond et al., 2014; Yu et al., 2008). These Se-enzymes protect cell components against oxidation; therefore, dietary Se also has an important role in sparing tocopherols in the tissues of animals and humans. These findings are in keeping with our current and previous studies showing that dietary supplementation of ^{Y}Se or ^{VI}Se resulted in an increase in the concentrations of tocopherols, especially αT and αTAc, in the heart (Table 10), as well as in the liver and muscles (Czauderna et al., 2018) compared to the control diet. At the same time, the experimental diets enriched in ^{Y}Se or ^{VI}Se decreased oxidative stress in the IMF, heart (Table 10), subcutaneous adipose tissues (Krajewska-Bienias et al., 2017), rumen-surrounding fat (Czauderna et al., 2018) and musculus longissimus dorsi (Czauderna et al., 2018), as concentrations of MDA and the values of the PUFA peroxidation indices decreased compared to the control diet. Interestingly, the addition of only 1% CA to the experimental diets revealed a similar impact on oxidative stress in these tissues in diets supplemented by ^{Y}Se or ^{VI}Se.

In our opinion, the proposed $_{index}A^{SFA\ +\ Toc}$ better assesses the atherogenic capacity of assayed tissues, as our modified index takes into consideration the concentrations of pro-atherogenic SFA, as well as the concentrations of anti-atherogenic tocopherols in tissues.

Our current study, as well as other studies (Mozaffarian, 2016; Micha et al., 2017), documented that there is a critical need to better understand how specific aspects of dietary diversity, composition and supplements may influence food choices, optimal consumption of healthy dietary ingredients and energy intake, especially in the long term. Understanding these mechanisms is particularly important, because it helps in promoting healthy products of animal origin with appropriate energy contents in both normal-weight and overweight adults (Regadas Filho et al., 2011; Mozaffarian, 2016; Micha et al., 2017; De Oliveira Otto et al., 2018).

5. Conclusions

In summary, the experimental diets enriched in ^{Y}Se or ^{VI}Se resulted in the successful enrichment of IMF and, especially, the heart with pro-healthy n-3LPUFA (EPA and DHA in particular). Thus, our present results are in agreement with our previous studies in which the diets enriched simultaneously in CA and Se (as ^{Y}Se or ^{VI}Se) stimulated the accumulation of PUFA (including n-3LPUFA) in the musculus biceps femoris (MBF) and the musculus longissimus dorsi (MLD) of lambs. Furthermore, compared to the control diet, the experimental diets enriched with ^{Y}Se or ^{VI}Se stimulated the bioaccumulation of γT, αT, αTAc and TCh, and decreased the values of the h/H-Ch ratio in the heart. Our modified atherogenic index strongly suggests that the experimental diets enriched in ^{Y}Se, and especially in ^{VI}Se, decreased the atherogenic capacity of the heart tissue. Moreover, ^{Y}Se or ^{VI}Se added in the experimental diet reduced the thrombogenic capacity of the heart tissue. Our current and previous studies documented that all experimental diets, especially those enriched in ^{VI}Se, reduced oxidative stress in the IMF and the heart, as well as in the MBF and MLD. Thus, our investigations provide important insights for nutritionists conducting studies to improve the nutritional quality of feed for ruminants, as well as the welfare of livestock. The experimental diet enriched in ^{VI}Se is especially useful and convenient in attempts to modify the composition of ruminant meat and IMF. We argued that the dietary intervention presented herein has great potential for future practical and commercial implementations. Furthermore, the research model used may constitute a solid base on which to perform further research, e.g. to establish the influence of the dietary supplementation of tocopherols (αT in particular) and lycopene added in a diet with CA, RO and FO on the chemical composition and oxidative stress in the heart, muscles and adipose tissues of lambs.

It is worth noting, however, that currently the “more natural” composition of grazing lambs can also be recommended.

Author contributions

Małgorzata Białek: Visualization, Investigation, Data curation, Formal analysis, Software; Validation. **Marian Czauderna**: Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Kamil Zaworski**: Validation, Software, Writing – review. **Katarzyna Krajewska**: Formal analysis, Validation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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