Original Research Article

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

Małgorzata Białeć, Marian Czauderna*, Kamil Zaworski, Katarzyna Krajewska

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland

A R T I C L E   I N F O

Article history:
Received 18 April 2020
Received in revised form 4 November 2020
Accepted 22 November 2020
Available online 22 April 2021

Keywords:
Selenium
Carnosic acid
Fatty acid
Tocopherol
Cholesterol
Lamb

A B S T R A C T

The aim of the current study was to evaluate the impact of carnosic acid (CA), selenised yeast (YSe) and selenate (VISe) 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 YSe or VISe). The experimental diets without/with 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 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.

* Corresponding author.
E-mail address: m.czauderna@ifzz.pl (M. Czauderna).

© 2021 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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; Białeć 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 [LPUSA]), 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).

* Corresponding author.
E-mail address: m.czauderna@ifzz.pl (M. Czauderna).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.
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; ωLNA) 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 (Byelaszov 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 (Biatek 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 (Biatek and Czauderna, 2019; Micha et al., 2017; Mozaffarian, 2016). Diverse inorganic chemical forms of Se (e.g. selenite [IVSe] or selenate [VISe] or selenised yeast (YSe)) are used as a nutritional source of Se. VISe and VSe are mainly metabolised to intermediates and then utilised for the biosynthesis of Se-cysteine (Se-Cys) selenocysteine tRNA (transfer-RNA for Se-Cys) to pair with the codon of Se-Cys for the formation of Se-Cysteine (Cys). The Se-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 tocopherols in the regulation of lipid peroxidation (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 VSe 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 \( ^{75} \text{Se} \) and \( ^{77} \text{Se} \)) affected ruminal microbial population and profile of volatile FA and FA in the rumen (Biatek et al., 2018; Biatek and Czauderna, 2019; Del Razo-Rodriguez et al., 2013; Miltiko et al., 2016; Morán et al., 2012). Our previous investigations have shown that experimental diets containing CA with/without \( ^{75} \text{Se} \) or \( ^{77} \text{Se} \) 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 (Biatek et al., 2018; Biatek and Czauderna, 2019; Czauderna et al., 2017; Miltiko et al., 2016). Considering the above, we hypothesised that Se-compounds (as \( ^{75} \text{Se} \) or \( ^{77} \text{Se} \)) 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 \( ^{75} \text{Se} \) or \( ^{77} \text{Se} \)) 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 (Biatek 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, corn 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 (984.05), 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, \( ^{75} \text{Se} \) and \( ^{77} \text{Se} \)), 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, \( ^{75} \text{Se} \) and \( ^{77} \text{Se} \)) (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...
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-p-cresol, 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% BF3 in methanol were purchased from Sigma Aldrich (St Louis, MO, USA). Chemicals were of analytical grade. The vitamin and mineral premix (ID number: aPL 1405002p) was obtained from POLFAMIX OK (Poland). 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

| Item               | Content          |
|--------------------|------------------|
| DM, g/kg           | 884.3            |
| Crude protein, g/kg DM | 201.9             |
| Crude fibre, g/kg | 118.6            |
| Crude fat, g/kg   | 21.7             |
| Total crude fat, g/kg | 51.7            |
| Ash, g/kg DM      | 42.8             |
| Neutral detergent fibre, g/kg DM | 310.5             |
| Acid detergent fibre, g/kg DM | 146.3             |
| Acid detergent lignin, g/kg DM | 23.3             |
| Metabolized energy, 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 (Se)-CA diet (1 g CA/kg BD and 0.35 mg Se as SeSO4/kg BD) and the selenium (Se)-CA diet (1 g CA/kg BD and 0.35 mg Se as SeSO4/kg BD). Se contents in the CA, Se-CA and SeSe-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; Pb, 0.0155 ± 0.0015 and Pb, 0.014 ± 0.0033.
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.
2.3.3. Analysis of tocopherols, TCh and MDA in the IMF and heart

Concentrations of TCh, γ-tocopherol (γT), δ-tocopherol (δT) and α-tocopherol (αT) in IMF and heart tissues were determined after saponification, followed by derivatisation according to methods described by Czauderna et al. (2009). The liquid chromatographic instrument used consisted of a reversed-phase (C18) liquid chromatographic system (SHIMADZU, Tokyo, Japan) according to methods described by 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 the 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 Tukey 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 VSe-CA diet increased (P < 0.05) the average daily live weight gain (Δm/35, kg) in comparison to the control, CA and YSe-CA diets, whereas the CA and VSe-CA diets reduced (P < 0.05) the value of Δm/35 compared to the control diet. Similarly, the final live weight of lambs fed the VSe-CA diet was higher (P < 0.05) than that of the CA and YSe-CA diets, whereas no difference (P > 0.05) in the final live weight of lambs was noted between the control, CA and YSe-CA diets.

### Table 3

| Group | Supplements added to 1 kg of the BD | Initial live weight (m_initial), kg | Final live weight (m_final), kg | Average daily live weight gain (Δm/35), kg |
|-------|-----------------------------------|----------------------------------|-------------------------------|-------------------------------------|
| Control | 20 g RO and 10 g FO | 30.567<sup>a</sup> | 37.798<sup>b</sup> | 0.206<sup>b</sup> |
| CA | 20 g RO, 10 g FO and 1 g CA | 30.603<sup>a</sup> | 37.167<sup>c</sup> | 0.189<sup>c</sup> |
| 5Se-CA | 20 g RO, 10 g FO and 0.35 mg Se as 5Se | 30.267<sup>a</sup> | 36.885<sup>c</sup> | 0.189<sup>c</sup> |
| 5Se-CA | 20 g RO, 10 g FO, 1 g CA and 0.35 mg Se as 5Se | 30.333<sup>a</sup> | 38.448<sup>c</sup> | 0.232<sup>c</sup> |

BD = basal diet; RO = rapeseed oil; FO = fish oil; CA = carnosic acid; 5Se = selenised yeast; 3Se = selenate.

<sup>a, b, c, d</sup> Different letters within a row indicate significant differences at P ≤ 0.05.

### Table 4

| Item | Control | CA | 5Se-CA | 3Se-CA | SEM | P-value |
|------|---------|----|--------|--------|-----|---------|
| C10:0 | 1.85<sup>c</sup> | 7.48<sup>c</sup> | 1.55<sup>a</sup> | 1.73<sup>bc</sup> | 0.05 | 0.03 |
| C12:0 | 3.24<sup>c</sup> | 3.02<sup>c</sup> | 3.56<sup>c</sup> | 3.13<sup>c</sup> | 0.14 | 0.11 |
| C14:0 | 32.91<sup>c</sup> | 31.53<sup>c</sup> | 38.52<sup>c</sup> | 32.51<sup>c</sup> | 1.09 | 0.14 |
| C15:0 | 3.25<sup>c</sup> | 2.93<sup>c</sup> | 3.47<sup>c</sup> | 3.65<sup>c</sup> | 0.06 | 0.07 |
| C16:0 | 154<sup>a</sup> | 147<sup>a</sup> | 158<sup>a</sup> | 152<sup>a</sup> | 8 | 0.19 |
| C17:0 | 10.0<sup>c</sup> | 8.6<sup>c</sup> | 7.2<sup>c</sup> | 10.3<sup>b</sup> | 0.3 | 0.04 |
| C18:0 | 173<sup>c</sup> | 162<sup>c</sup> | 155<sup>a</sup> | 182<sup>c</sup> | 4 | 0.03 |
| A-FA | 190<sup>a</sup> | 182<sup>a</sup> | 201<sup>a</sup> | 188<sup>a</sup> | 5 | 0.31 |
| A-FA/2FA | 0.255<sup>b</sup> | 0.257<sup>b</sup> | 0.270<sup>c</sup> | 0.250<sup>c</sup> | 0.002 | 0.04 |
| T-FA | 361<sup>c</sup> | 340<sup>c</sup> | 352<sup>c</sup> | 367<sup>c</sup> | 7 | 0.35 |
| T-FA/2FA | 0.482<sup>c</sup> | 0.477<sup>c</sup> | 0.476<sup>c</sup> | 0.490<sup>c</sup> | 0.005 | 0.19 |
| 2FA | 379<sup>c</sup> | 375<sup>c</sup> | 368<sup>c</sup> | 389<sup>c</sup> | 6 | 0.04 |
| 2FA/2FA | 0.1422<sup>c</sup> | 0.1445<sup>c</sup> | 0.1514<sup>c</sup> | 0.1441<sup>c</sup> | 0.0005 | 0.08 |
| 2FA/2FA | 1.029<sup>b</sup> | 1.005<sup>b</sup> | 0.989<sup>b</sup> | 1.057<sup>d</sup> | 0.004 | 0.02 |
| 2FA/2FA | 0.507<sup>b</sup> | 0.500<sup>d</sup> | 0.497<sup>c</sup> | 0.515<sup>c</sup> | 0.002 | 0.03 |

CA = carnosic acid; 5Se = selenised yeast; 3Se = selenate; SFA = saturated fatty acids; IMF = fat located between the thigh muscles; SEM = standard error of the mean. 

<sup>a, b, c, d</sup> Different letters within a row indicate significant differences at P ≤ 0.05.
Our study showed that the CA diet led to an increased (P < 0.05) in the concentrations of c9C14:1, c7C16:1, c9C16:1, and c11C20:1, as well as the ratios of concentration sum of MUFA (ΣMUFA) to ΣPUFA (ΣMUFA/ΣPUFA) and ΣMUFA/ΣFA in the heart compared to the control, ySe-CA and ySe-CA diets (Table 7). Conversely, when ySe was added to the experimental diet, a significant decrease in the concentrations of c9C14:1, c7C16:1, c9C16:1, c11C20:1, c15C24:1 and the content ratios of ΣMUFA/ΣPUFA and ΣMUFA/ΣFA 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 < 0.05) the contents of concentration sums of cis/trans, trans, cisCLA isomers (Σcis/tcCLA) and all assayed CLA isomers (ΣCLA) in the IMF compared to the control diet (Table 8). Similarly, the experimental diets enriched in ySe or ySe elevated the level of c9t11CLA in the IMF compared to the control and CA diets. Also, all experimental diets increased (P < 0.05) the concentrations of c11C42:0, c5c8c11c14c20:4 (AA) and c5c8c11c14c17c20:5 (EPA), the content sums of all assayed n-6PUFA (Σn-6PUFA) and LPUFA (ΣLPUFA), as well as the content sums of Σn-6PUFA/Σn-3PUFA, Σn-3PUFA/ΣFA and ΣLPUFA/ΣFA, and the values of the index of elongases (ΣElongindex; ΣElongindex = [c11c14c20:2 + c7c10c13c16c19c22:5]/[c11c14c20:2 + c7c10c13c16c19c22:5 + c5c8c11c14c17c20:5 + c9c12c18:2]) in the IMF compared to the control diet. The diet supplemented with CA and ySe decreased the sum-6PUFA/ΣPUFA ratio in the IMF compared to the control and other experimental diets.

In the current study, the concentrations of c9t11CLA and 2CLA in the heart of lambs fed the CA and ySe-CA diets were higher (P < 0.05) than those of the control and ySe-CA diets (Table 9). Similarly, the experimental diet supplemented with ySe increased (P < 0.05) the concentrations of c9c12c15c18:3 (ζALA), c11c14c20:2, c8c11c14c20:3, AA, EPA, c7c10c13c16c19c22:5 (DPA), c4c7c10c13c16c19c22:5 (DHA), Σn-6PUFA and Σn-3PUFA, as well as the values of the index of elongases (ΣElongindex; ΣElongindex = [c11c14c20:2 + c7c10c13c16c19c22:5]/[c11c14c20:2 + c7c10c13c16c19c22:5 + c5c8c11c14c17c20:5 + c9c12c18:2]) in the IMF compared to the control and other experimental diets.
as the values of the $\Sigma E_{\text{Elong}}$ index and the ratios of $\Sigma n$-3PUFA/$\Sigma FAs$, $\Sigma LPUFA/\Sigma FAs$ and $\Sigma PUFA/\Sigma FAs$ in the heart compared to the control and CA diets. Moreover, $^{76}$Se added to the experimental diet significantly increased ($P < 0.05$) the value of $\Sigma E_{\text{Elong}}$ index and the concentrations of $c9t11c12C18:3$, $c9c12C18:2$ and $c9c12C18:4$ in the heart compared to the control diet and CA diets. The $c5c8c11c14c17C20:5$ was lower in the heart of lambs fed the CA diet compared to the control diet.

Table 8

| Item | Group | Control | CA | $^{76}$Se-CA | $^{76}$Se-CA | SEM | P-value |
|------|-------|---------|----|--------------|--------------|-----|---------|
| $c9t11C18:2$ | 1.07$^a$ | 1.02$^a$ | 1.12$^b$ | 1.42$^c$ | 0.03 | 0.04 |
| $c5c8c11c14c17C20:5$ | 0.06$^d$ | 0.08$^d$ | 0.11$^d$ | 0.12$^d$ | 0.04 | 0.04 |
| $c5c8c11c14c17C20:5$ | 0.016$^a$ | 0.057$^b$ | 0.066$^b$ | 0.006 | 0.03 | 0.02 |
| $c7c10c13c16c19c22:5$ | 0.114$^a$ | 0.086$^a$ | 0.095$^ab$ | 0.145$^c$ | 0.03 | 0.03 |
| $\Sigma n$-6PUFA | 50.5 | 47.4 | 50.5 | 51.5 | 1.0 | 0.18 |
| $\Sigma n$-3PUFA | 1.93$^a$ | 2.11$^b$ | 2.68$^b$ | 1.88$^c$ | 0.03 | 0.03 |
| $\Sigma PUFA$ | 58.8 | 51.4 | 55.4 | 55.6 | 1.1 | 0.19 |
| $\Sigma 6$-PUFA/$\Sigma 3$-PUFA | 26.1$^c$ | 22.5$^d$ | 18.8$^e$ | 27.4$^c$ | 0.4 | 0.04 |
| $\Sigma 6$-PUFA/$\Sigma 3$-PUFA | 0.067$^a$ | 0.038$^b$ | 0.110$^c$ | 0.123$^d$ | 0.03 | 0.03 |
| $\Sigma 3$-PUFA | 0.129$^a$ | 0.143$^b$ | 0.155$^c$ | 0.210$^d$ | 0.04 | 0.02 |
| $\Sigma a$-CLA | 0.197$^a$ | 0.226$^b$ | 0.266$^c$ | 0.333$^d$ | 0.06 | 0.02 |
| $\Sigma 6$-PUFA/$\Sigma 3$-PUFA | 0.520$^a$ | 0.583$^b$ | 0.710$^c$ | 0.586$^d$ | 0.04 | 0.02 |
| $\Sigma 3$-PUFA | 0.170$^a$ | 0.200$^b$ | 0.215$^c$ | 0.295$^d$ | 0.04 | 0.04 |
| $\Sigma 6$-PUFA/$\Sigma 3$-PUFA (µg MUFA/IMF) | 0.0225$^a$ | 0.00317$^b$ | 0.00321$^b$ | 0.0542$^c$ | 0.00004 | 0.02 |

$^{76}$Se or $^{76}$Se decreased ($P < 0.05$) the concentration of MDA and the value of the $\Sigma FAs$ peroxidation index ($\Sigma MDA_{\text{index}}/\Sigma MDA_{\text{index}}$) 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 $\Sigma MDA_{\text{index}}$ and newMDA$_{\text{index}}$ decreased ($P < 0.05$) in comparison to the control diet.

It has also been shown that the experimental diets supplemented with $^{76}$Se or $^{76}$Se increased ($P < 0.05$) the concentrations of TCh, $\gamma$T and $\alpha$TaC in the heart compared to the control and CA diets (Table 10). When the diet was supplemented only with CA, the concentrations of $\gamma$T and $\Sigma (S \alpha T + \alpha Tac)$ in the heart were higher ($P < 0.05$) than those of the control diet. In contrast, all experimental diets decreased ($P < 0.05$) the concentration of MDA and the value of newMDA$_{\text{index}}$ in the heart compared to the control diet.

The effects of the experimental diets on the h/H-Ch ratio, and the thrombogenic (indexT$^{\text{Thromb}}$), atherogenic (indexASFA) and modified atherogenic (indexASMFA, indexASMFA) indices in the IMF and heart of lambs are presented in Table 11. The values of the h/H-Ch ratio and indexASMFA in the IMF were higher ($P < 0.05$) for lambs on the CA and $^{76}$Se-CA diets compared with lambs fed the control and $^{76}$Se-CA diets. In contrast, when $^{76}$Se was added to the experimental diet, the values of the h/H-Ch ratio and indexASMFA in the IMF decreased most efficiently compared to the control, CA and $^{76}$Se-CA diets. The value of indexASMFA/Tor in the IMF of lambs fed the $^{76}$Se-CA diet was lower ($P < 0.05$) than that of the control, CA and $^{76}$Se-CA diets. The $^{76}$Se-CA diet most efficiently decreased ($P < 0.05$) the value of indexASMFA/Tor.

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

The current study demonstrated that adding $^{76}$Se to the experimental diet increased ($P < 0.05$) the concentrations of TCh and $\alpha$TaC and numerically ($P > 0.05$) elevated the concentration sum of $\alpha$T and $\alpha$TaC ($\alpha$T + $\alpha$TaC) in the IMF compared to the control, CA and $^{76}$Se-CA diets (Table 10). Similarly, the $^{76}$Se-CA diet stimulated ($P < 0.05$) the accumulation of TCh in the IMF in comparison to the control and CA diets. Conversely, the experimental diet supplemented with $^{76}$Se or $^{76}$Se decreased ($P < 0.05$) the concentration of MDA and the value of the $\Sigma FAs$ peroxidation index ($\Sigma MDA_{\text{index}}/\Sigma MDA_{\text{index}}$) 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 $\Sigma MDA_{\text{index}}$ and newMDA$_{\text{index}}$ ($\Sigma MDA_{\text{index}} + 0.5 \times \Sigma MUFA$) decreased ($P < 0.05$) in comparison to the control diet.
Table 9
The concentrations (μg/g heart) of in PUFA the heart.

| Item Group | SEM | P-value |
|------------|-----|---------|
| Control    | CA  | VSe-CA  | VSe-CA |
| c9Δ11CLA   | 33a | 58b     | 58b    | 2     | 0.03 |
| c10Δ12CLA  | 5.6 | 5.0     | 5.5    | 5.1   | 0.2  | 0.06 |
| VSe-CA     | 0.2 | 0.02    | 0.02   | 0.02  | 0.02 |
| c9Δ11C12Δ18.2 (LA) | 3.579 | 3.730 | 3.766 | 4.184 | 96   | 0.51 |
| c9Δ11C15Δ18.3 (αLNA) | 253a | 311b | 320b | 388c | 7    | 0.03 |
| c11Δ14C20.2 | 80b | 77b | 107b | 168c | 3    | 0.02 |
| c8Δ11C14C20.3 | 53a | 57a | 105b | 142c | 3    | 0.03 |
| c5Δ8C11Δ14C20.4 (AA) | 1.810a | 1.755a | 2.133c | 2.469d | 43   | 0.03 |
| c5Δ8C11Δ14C20.5 (EPA) | 87a | 92a | 157b | 167b | 4    | 0.04 |
| c7Δ10C13Δ16C19Δ22.5 (DPA) | 115a | 132a | 170b | 209b | 4    | 0.02 |
| c4Δ7Δ10C13Δ16C19Δ22.6 (DHA) | 91a | 102a | 177b | 208b | 4    | 0.03 |
| 2n-6PUFA   | 5.442 | 5.542 | 6.003 | 6.795 | 99   | 0.23 |
| 2n-3PUFA   | 546a | 636ab | 824a | 972b | 27   | 0.04 |
| 2PUFA      | 6107 | 6317 | 6972 | 7998 | 101  | 0.22 |
| 2n-6PUFA/2n-3PUFA | 10.0 | 8.7 | 7.3 | 7.0 | 0.2 | 0.03 |
| 2n-6PUFA/2n-3PUFA | 1.943 | 1.888 | 2.344b | 2.779b | 31 | 0.03 |
| 2n-3PUFA   | 292a | 325a | 504b | 584b | 11   | 0.03 |
| 2n-6PUFA/2n-3PUFA | 6.65c | 5.81b | 4.65c | 4.76c | 0.03 | 0.01 |
| 2PUFA/2FA  | 0.0147a | 0.0136a | 0.0248b | 0.0239b | 0.0006 | 0.03 |
| 2n-6PUFA/2FA | 0.107a | 0.084a | 0.135b | 0.132c | 0.004 | 0.04 |
| 2FA        | 0.306b | 0.265a | 0.327b | 0.331c | 0.001 | 0.03 |
| 2n-3PUFA/2FA | 0.0504a | 0.0517b | 0.0659b | 0.0708b | 0.0006 | 0.03 |

CA = carnosic acid; VSe = seleneised yeast; MDA = malondialdehyde; PUFA = polyunsaturated fatty acids; SEM = standard error of the mean; CLA = conjugated linoleic acid (C18:2); – c = cis; t = trans; PUFA = long-chain polyunsaturated fatty acids; FA = fatty acids; Σ Elin index = index of elongases.

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

| Item Group | SEM | P-value |
|------------|-----|---------|
| Control    | CA  | VSe-CA  | VSe-CA |
| IMF        |     |         |       |
| Total cholesterol, μg/g tissue | 49.1c | 42.1a | 47.5c | 62.5b | 1.1 | 0.03 |
| 10-Cis-CLA | 2.10c | 2.67b | 2.35a | 3.36a | 0.02 | 0.03 |
| 10-Tocopheryl (10T), μg/g tissue | 5.56c | 5.07b | 5.28a | 4.85b | 0.04 | 0.02 |
| Σ (10T + 10Tc), μg/g tissue | 7.66c | 7.74c | 7.64c | 8.22c | 0.07 | 0.17 |
| MDA, ng/g tissue | 1.64c | 1.14a | 0.72a | 0.74b | 0.02 | 0.03 |
| MDA/IMF index | 0.031c | 0.022b | 0.001a | 0.003b | 0.001 | 0.03 |
| n-3PUFA/IMF index | 0.637c | 0.561c | 0.334a | 0.352b | 0.06 | 0.02 |
| The heart   |     |         |       |
| Total cholesterol, μg/g tissue | 126a | 153a | 210b | 226a | 3   | 0.03 |
| 10-Tocopheryl (10T), μg/g tissue | 1.84c | 1.80c | 2.77c | 1.77c | 0.9 | 0.12 |
| MDA, ng/g tissue | 3.58c | 3.07b | 5.43d | 4.08c | 0.08 | 0.02 |
| n-3PUFA/IMF index | 1.58a | 1.53a | 3.71b | 3.63a | 0.03 | 0.03 |
| n-3PUFA/IMF index | 3.54c | 5.07b | 6.18c | 7.83c | 0.07 | 0.02 |
| MDA/IMF index | 8.96c | 9.94c | 14.38b | 13.68b | 0.08 | 0.03 |
| Se, μg/g | 0.799a | 0.871b | 1.134c | 1.138c | 0.011 | 0.03 |
| MDA, ng/g tissue | 15.0b | 11.6b | 11.5a | 10.6a | 0.3 | 0.03 |
| Se/MDA index | 4.25c | 1.84a | 1.65b | 1.33a | 0.01 | 0.02 |
| n-3PUFA/Se/MDA index | 1.70a | 1.12b | 1.25b | 0.982b | 0.012 | 0.02 |

CA = carnosic acid; VSe = seleneised yeast; MDA = malondialdehyde; PUFA = polyunsaturated fatty acids; IMF = fat located between the thigh muscles.

References and footnotes:

1. Different letters within a row indicate significant differences at P < 0.05.
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 PUFA (ng/g tissue), MDA/PUFA.
4. The concentration ratio of MDA (ng/g tissue) to MUFA (ng/g tissue), MDA/PUFA.
5. The concentration ratio of MDA (ng/g tissue) to SFA (ng/g heart), MDA/PUFA.
6. The concentration ratio of MDA (ng/g heart) to SFA (mg/g heart).
7. The concentration ratio of MDA (ng/g heart) to SFA (mg/g heart).
8. The concentration sum of all FA (2FA).
9. Σ Elin index = (11C14C20.2 + c7C10c13c16c19c22.5)/(c11C14C20.2 + c7C10c13c16c19c22.5 + c5c8c11c14c17C20.5 + c9c12C18.2).
10. The concentration sum of all FA (2FA).
11. Σ Elin index = (c11C14C20.2 + c7C10c13c16c19c22.5)/(c11C14C20.2 + c7C10c13c16c19c22.5 + c5c8c11c14c17C20.5 + c9c12C18.2).
in the IMF compared to the control and other experimental diets, whereas no difference \((P > 0.05)\) in the value of \(\text{indexASFA} + \text{Toc}\) was noted between the CA and the control diets.

In our study, a significant decrease \((P < 0.05)\) in the values of the h/H-Ch ratio and \(\text{indexASFA} + \text{Toc}\) 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 \(^{35}\)Se, increased \((P < 0.05)\) the values of \(\text{indexASFA}\) and \(\text{indexASFA} + \text{Toc}\) in the heart compared to the experimental diet supplemented with \(^{35}\)Se. All experimental diets increased \((P < 0.05)\) the value of \(\text{indexASFA}\) in the heart compared to the control diet. The value of \(\text{indexASFA}\) in the heart of lambs fed the experimental diets supplemented with \(^{35}\)Se or \(^{35}\)Se was lower \((P < 0.05)\) than that of the control and CA diets; the CA diet most efficiently increased the value of \(\text{indexASFA}\).

### 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, \(^{35}\)Se or \(^{35}\)Se were observed in the heart, adipose tissues, muscles, as well as in any other internal organ of the lambs (Bliek et al., 2018; Bliek and Czauderna, 2019; Czauderna et al., 2017; Miltko et al., 2016; Rozbicka-Wiezorek 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 \(^{35}\)Se and SeY rich in Se-Met (in contrast to \(^{35}\)Se and especially selenide) are less reactive, and because tRNAMet 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, \(^{35}\)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 \(^{35}\)Se-CA diets. Indeed, the \(^{35}\)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 \(^{35}\)Se-CA diet is characterised by the highest level of protein content in the lamb muscle (Jaworska et al., 2016). Furthermore, the \(^{35}\)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 \(^{35}\)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 \(^{35}\)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 \(^{35}\)Se added to the diet stimulates anabolic processes in the lambs’ tissues. In contrast, the experimental diet with \(^{35}\)Se (rich in Se-Met) decreased the average daily live weight gain of lambs compared to the experimental diet with \(^{35}\)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 \(^{35}\)Se-CA diet most efficiently increased the synthesis of CH\(_4\) and CO\(_2\) in the rumen of lambs (Miltko et al., 2016; Rozbicka-Wiezorek 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

---

**Table 11**

The hypcholesterolemic/hypercholesterolemic FA (h/H-Ch) ratio and the thrombogenic (indexTSFA), atherogenic (indexASFA) and modified atherogenic (indexASFA + Toc) indices in the IMF and the heart of lambs fed the control and experimental diets.

| Item | Group | Control | CA | \(^{35}\)Se-CA | \(^{35}\)Se-CA |
|------|-------|---------|----|-------------|-------------|
| IMF  | h/H-Ch ratio\(^{1}\) | 1.806\(^{b}\) | 1.844\(^{a}\) | 1.746\(^{a}\) | 1.834\(^{a}\) |
|      | indexTSFA | 1.804\(^{a}\) | 1.749\(^{b}\) | 1.696\(^{a}\) | 1.871\(^{a}\) |
|      | indexASFA | 0.790 | 0.787 | 0.854 | 0.785 |
|      | indexASFA + Toc | 0.0735\(^{b}\) | 0.0773\(^{b}\) | 0.0799\(^{a}\) | 0.0676\(^{a}\) |
| The heart | h/H-Ch ratio\(^{1}\) | 3.741\(^{a}\) | 3.230\(^{b}\) | 3.261\(^{a}\) | 3.398\(^{b}\) |
|      | indexTSFA | 0.822\(^{d}\) | 0.851\(^{d}\) | 0.715\(^{a}\) | 0.735\(^{b}\) |
|      | indexASFA | 0.291\(^{a}\) | 0.345\(^{a}\) | 0.338\(^{a}\) | 0.394\(^{b}\) |
|      | indexASFA + Toc | 0.0511\(^{a}\) | 0.0436\(^{c}\) | 0.0340\(^{b}\) | 0.0255\(^{a}\) |

\(^{a}\), \(^{b}\), \(^{c}\), \(^{d}\) Different letters within a row indicate significant differences at \(P < 0.05\).

\(^{1}\) h/H-Ch ratio = [(C7:18 + e9C18 + c12C18 + c14C18 + c11C20 + 13C22 + c9C12C18:2 + c9C12C15:18:3 + c6C9C12C18:3 + c5C8C11C14C20:4 + c11C14C20:2 + c5C8C11C14C17C20:5 + c7C10C13C16C22:4 + c7C10C13C16C19C22:5)/[(C14:0 + C16:0 + C18:0)] (Fernández et al., 2007).

\(^2\) The thrombogenic index, indexTSFA = [(1.36 × C14:0 + 0.5 × n-6PUFA + 3 × n-3PUFA)/2n-6PUFA] (Fernández et al., 2007).

\(^3\) The atherogenic index, indexASFA = [(1.36 × C14:0 + 0.5 × n-6PUFA + 3 × n-3PUFA)/2n-6PUFA] (Morán et al., 2013).

\(^4\) The modified atherogenic index, indexASFA + Toc = indexASFA(1.49 × C71 + 1.36 × C12:0 + 0.15 × C17 + 0.05 × C19), where: indexASFA - the atherogenic index; C71 - the concentration of α-tocopheryl acetate (αTAc); C17 - the concentration of γ-tocopherol (γT); C19 - the concentration of δ-tocopherol (δT). Tocopherol concentrations, μg/g tissue.
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; Niedzwiedzka 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 (Biatek et al., 2018; Biatek 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 UFAs to C18:0, causing the accumulation of a number of UFA intermediates in ruminants’ tissues (Bialkowski et al., 2020; Del Razo-Rodriguez et al., 2013). Similarly, dietary CA supplementation also modifies the ruminal microbiota population, reducing the capacity of the bacterial isomerisation of UFAs and the biohydrogenation of UFAs to C18:0 and, hence, the biosynthesis of volatile FA and FA compositions in ruminants’ tissues (Biatek 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 2PUFA/2SFA 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 Σ-6PUFA/Σ-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 Σ-6PUFA/Σ-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 diets enriched in RO, FO, CA and Se (as 3Se or 3Se) (Czauderna et al., 2017; Krajewska-Bienias et al., 2017). Notably, the experimental diets, especially those supplemented with 3Se or 5Se, 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 (Valentines and Valentines, 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 (Biatek et al., 2018; Biatek 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 3Se or 5Se, increased the accumulation of the bacterial isomerisation products of PUFA (i.e. c9t11CLA) in the heart compared to the control and 3Se-CA diets. Similarly, CA supplemented to the diet, regardless of the presence of 3Se or 5Se, stimulated the incorporation of ct/tcCLA isomers (PUFA isomerisation products; Table 8) and the index value of Δ9-desaturation of TVA (CLΔ9-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, 3Se-CA and 5Se-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 (Biatek 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 3Se or 5Se (the antioxidants) significantly increased the capacity of fatty acid elongases (CLΔ2-index) in the IMF (0.00225 versus 0.00317 to 0.00542; Table 8) and significantly decreased the concentration of MDAs in the IMF (1.70 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 MDAindex 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 5Se 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 3Se-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 3Se and especially 5Se (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 Realisil (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 3Se and 5Se added to the experimental diet considerably increased the capacity of fatty acid elongases, as the values of CLΔ2-index and the concentrations of Σ-6LPUFA and, especially, Σ-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 antioxidantive properties, Se has been considered to play an important role in lipid metabolism. The association of serum Se contents with lipid levels and dyslipidemia, 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 VSe or VISe) are positively associated with increased TCh in some tissues of animals and humans. Indeed, VISe or VISe added to diets stimulated the biosynthesis of Se-Cys that can be incorporated into Se-Cys-enzymes. In contrast, Se-Met derived from SeV 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, VISe added to the diet increased the concentrations of TCh in the heart and IMF, as well as in the rumen-surrounding fat (Bialek 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 SeV or VISe introduced to the experimental diets decreased the h/H-Ch ratio and index A-SFA. Moreover, the experimental diet enriched with VISe 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 ViSe and ViSe supplementation, which is fully consistent with previous studies (Bialek and Czauderna, 2019; Czauderna et al., 2017, 2018; Helzlsoeur et al. 2000; Leskovec et al., 2018). Compared to adipose tissues (including IMF), Se supplemented to diets 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 hT. 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 hT 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 heart, liver, muscles or blood is metabolised into antioxidant 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 5Se or 5Se resulted in an increase in the concentrations of tocophorols, especially hT and TAc, 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 5Se or 5Se 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 SISe or VISe.

In our opinion, the proposed index A-SFA (αToc 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 ViSe or ViSe 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 ViSe or ViSe) 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 VISe or VISe stimulated the bioaccumulation of γT, αT, δTAc 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 5Se, and especially in VISe, decreased the atherogenic capacity of the heart tissue. Moreover, 5Se or VISe 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 VISe, 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 VISe 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 Bialek: 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.

Acknowledgement

This study was in part supported by the National Science Centre (NCN), Poland: Grant No. 2013/09/B/NZ9/00291 (ID: 217066) and by the statutory funds from The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland.
