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Enrichment of lamb rations with carnosic acid and seleno-compounds affects the content of selected lipids and tocopherols in the pancreas

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ABSTRACT. The aim of this study was to evaluate changes in the content of fatty acids (FA), tocopherols, total cholesterol (T-Ch) and malondialdehyde (MDA) in the pancreas of lambs fed supplemented diets. Corriedale lambs were fed a diet with 2% rapeseed oil (RO) and 1% fish oil (FO) (control diet) or experimental diets with the addition of 2% RO, 1% FO, 0.1% carnosic acid (CAc) (CAc diet) and 0.35 ppm Se in the form of selenized yeast (SeYe) (CAcSeYe diet) or selenate (SeVI) (CAcSeVI diet). An increase in the content of total saturated FA (SFA), thrombogenic SFA and all FA was observed in the pancreas of lambs fed the CAc diet compared to control and CAcSeYe diets. All experimental diets increased the ratio of monounsaturated FA (ΣMUFA) to ΣFA and desaturation indices in the pancreas compared to the control diet. Conjugated linoleic acid isomer contents and the hypocholesterolemic/hypercholesterolemic FA ratio in the pancreas of lambs fed all experimental diets was higher than in those fed the control diet. The CAcSeVI diet decreased the content of T-Ch in the pancreas compared to the control, CAc and CAcSeYe diets. Lambs fed the CAc diet had a higher content of tocopherols in the pancreas than the lambs fed the control, CAcSeYe and CAcSeVI diets. The CAcSeVI diets decreased the content of T-Ch and tocopherols in the pancreas compared to the CAcSeYe diet. The CAc diet increased modified PUFA peroxidation indices in the pancreas compared to the control, CAcSeYe and CAcSeVI diets.

Introduction

The pancreas is one of the most important internal organs in humans and animals. It has endocrine and exocrine functions the latter involve the secretion of digestive enzymes, ions and water into the gastrointestinal tract. Digestive enzymes are necessary to convert food into molecules that can be absorbed through the surface lining of the gastrointestinal tract into the body. Previous studies have shown that essential fatty acids (FA) have a significant impact on pancreatic endocrine and exocrine functions (Biden et al., 2004; Heller et al., 2004). Significant inverse correlations were found between FA bioaccumulation and pancreatic and gallbladder responses to FA, suggesting a relationship between the length of intestine exposed to FA and the amount of cholecystokinin (and/or other neuro-hormonal factors) released, which increases pancreatic secretion and gallbladder contraction (Yang and Li, 2012). The pancreatic FA profile reflected FA composition and specific peptide contents in the test diets. The magnitude of pancreatic FA changes was an important factor influencing pancreatic endocrine and exocrine functions (Acosta-Montaño and García-González, 2018; Baynes et al., 2018; Kapica et al., 2018; Oh et al., 2018). Effects of pancreatic FA on insulin secretion and pancreatic β-cell survival are related to the degree

of FA saturation and carbon chain length (Oh et al., 2018). Saturated FA (SFA) with a chain length of 16 carbon atoms (C16:0) or greater (palmitate or stearate) induced cytotoxicity, while a reduction of carbon chain length to C14:0 or C12:0 was less toxic to β-cells (Newsholme et al., 2007). However, mono- (MUFA) and poly-unsaturated FA (PUFA) did not induce pancreatic β-cell apoptosis and this effect was independent of chain length. Treating β-cells with unsaturated FA (UFA) (e.g. arachidonic acid, AA) was shown to induce glucose-stimulated insulin secretion and pancreatic β-cell proliferation. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) also prevented cytokine-induced cell death in pancreatic islets and increased insulin secretion (Baynes et al., 2018). Controlled supplementation of long-chain n-3PUFA (n-3LPUFA) was shown to decrease triglyceride and cholesterol levels in the pancreas and intensify insulin secretion. Dietary n-3PUFA deficiency was accompanied by basal hyperinsulinemia and hypo-glycemia, as well as excessive insulin response to glucose. However, the pancreas with a higher UFA content (especially n-3LPUFA) in its lipid fraction is sensitive to oxidative damage because it contains high quantities of efficiently oxidising PUFA (particularly highly unsaturated LPUFA). Oxidative damage caused by PUFA leads to the formation of very reactive and toxic species (such as aldehydic end-products, including malondialdehyde (MDA), 4-hydroxy-2,3-nonenal or 4-hydroxy-2,3-alkenals of different chain lengths) (Sultana et al., 2013). Unfortunately, pancreatic β-cells and insulin-sensitive cells are extremely susceptible to oxidative stress due to high endogenous production of reactive oxygen (ROS) and nitrogen (RNS) species and a low expression of antioxidant enzymes (Yang and Li, 2012; Eguchi et al., 2021). Oxidative stress plays a critical role during pancreatic β-cell neogenesis, proliferation and development of type 2 diabetes (T2D). In fact, the primary function of unique β-cells in the pancreas is to produce and release hormones (insulin and amylin) that lower blood glucose levels through various mechanisms.

Considering the above, an adequate amount of UFA (particularly n-3LPUFA) in diets together with antioxidants, like phenolic compounds (e.g. carnosic acid), seleno-compounds (Se-compounds), flavonoids (e.g. quercetin), tocopherols, lycopene or β-carotene is essential for the proper health of farm animals and humans (Angst et al., 2013; Čobanová et al., 2017; Czauderna et al., 2017). Indeed, some antioxidants, when added to diets, stimulated proliferation rates of pancreatic β-cells, which not only confirmed the detrimental effects of oxidative stress on β-cell regeneration, but also documented the potential benefits of applying certain antioxidants as therapeutics for T2D patients to improve β-cell regeneration (Eguchi et al., 2021). Fortunately, previous studies have demonstrated that carnosic acid (CAc), a phenolic diterpene, has strong antioxidant and anticarcinogenic properties (Jordán et al., 2013; Morán et al., 2013, 2017). CAc efficiently protects PUFA in lipids against oxidative damages, as CAc (similar to Se-compounds or tocopherols) scavenges RNS and ROS. During this process, CAc added to diets, especially prevented oxidation of highly unsaturated PUFA in mammalian tissues (Jordán et al., 2013; Morán et al., 2013). Conversely, higher doses of CAc (≥ 1.2 g/kg diet) appeared to be detrimental for mammals (Afonso et al., 2013; Raes et al., 2015). In fact, CAc could also act as a substrate for the peroxidase system and stimulate DNA damage (Aruoma et al., 1992).

The physiological role of Se-compounds among antioxidants should also be emphasized (Čobanová et al., 2017; Merkord et al., 2017; Ibrahim et al., 2020). Se in inorganic chemical forms (like selenite (SeIV) or selenate (SeVI)) is less effectively accumulated in mammalian tissues compared to its organic forms (e.g. selenized yeast (SeYe), Se-methionine (Se-Met) or Se-cysteine (Se-Cys)) (Czauderna et al., 2009c, 2017; Suganthi et al., 2019). SeIV or SeVI are metabolized to intermediates (like selenides) and subsequently partially eliminated primarily in urine and faeces or anabolized mainly to Se-Cys containing enzymes (Navarro-Alarcon and Cabrera-Vique, 2008; Gebreeyessus and Zewge, 2019). On the other hand, dietary SeYe was shown to significantly stimulate the bioaccumulation of Se-Met-containing proteins in mammalian tissues (Navarro-Alarcon and Cabrera-Vique, 2008). When required, these organic Se forms can be metabolized to Se-Cys-containing enzymes. The essential physiological role of half of the Se-Cys-containing enzymes is to maintain low levels of free radicals or pre-oxides within cells, thereby decreasing oxidative stress and peroxidative damage of UFA (LPUFA in particular) and cholesterol in mammalian tissues (Mehdi and Dufrasne, 2016; Čobanová et al., 2017). These Se-Cys-containing enzymes act in concert with tocopherols to protect mammalian cell membranes (Rooke et al., 2005). The nutritional requirements of ruminants for Se are estimated at ~0.1 mg/kg dry matter, although feed containing Se at 0.5 mg/kg diet would not be toxic for ruminants (such as sheep, cows or goats) (Navarro-Alarcon and Cabrera-Vique, 2008; Raymond et al., 2014; Čobanová et al., 2017).

Based on these findings, we hypothesized that CAc and Se (as SeYe or SeVI) added to the ovine ration enriched in 1% fish oil (FO) (rich in n-3LPUFA) and 2% rapeseed oil (RO) (rich in n-6PUFA) could significantly modify the levels of selected lipid compounds and modulate oxidative stress in the pancreas of lambs. A higher content of RO, and especially FO (rich in LPUFA), would stimulate oxidative stress in animal tissues and adversely affect ruminal microbiota (Wąsowska et al., 2006; Białek et al., 2020). Therefore, the main objective of our study was to investigate the effects of CAc, SeYe and SeVI addition to the diet containing 1% FO and 2% RO on the levels of FA, tocopherols, total cholesterol (T-Ch) and MDA in the pancreas of lambs.

Material and methods

Lambs, housing, diets, experimental design and sampling

Twenty-four male Corriedale lambs were selected from a flock of lambs (110 male lambs) according to their age (82–90 days) and body weight (BW) (23.3 ± 2.1 kg). All experiments on lambs were carried out at The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences (PAS) (Jabłonna, Poland). Animal welfare guidelines and handling procedures recommended by the 3rd Local Ethics Committee for Animal Experimentation at the Warsaw University of Life Sciences (Warsaw, Poland) (approval number: 41/2013) were strictly followed throughout the preliminary and experimental period. During a 3-week preliminary period, the lambs were individually penned; the length, width and height of the pen were (cm) 170, 130 and 150, respectively. All animals were fed a basal diet (BD) enriched with FO and RO (i.e. the control diet). The animals had free access to the diet; water was offered *ad libitum*. The BD was offered at 7:30 am and 4:00 pm every day. The BD consisted of the following ingredients: meadow hay (360 g/kg BD), a mixture of barley (165 g/kg BD) and soybean (360 g/kg BD) meals, wheat starch (90 g/kg BD) and a mineral-vitamin mixture (20 g/kg BD); the ingredients in dry matter (DM) ration were as follows: meadow hay, barley meal, soybean meal and wheat starch (Table 1). Chemical composition of the BD and its ingredients was conducted at The Kielanowski Institute of Animal Physiology and Nutrition, PAS (Jabłonna, Poland) using AOAC methods (AOAC International, 2005). The chemical composition of BD components is presented in Table 1. After a 3-week preliminary period, animals with an average BW of 30.4 ± 2.5 kg were divided into 4 groups (6 lambs each) with a similar average initial body weight. A 35-day dietary experiment was conducted during which lambs were fed the BD enriched in 2% RO and 1% FO (control diet) or three experimental diets with 2% RO, 1% FO and antioxidants (0.1% CAc and/or 0.35 ppm Se as SeYe or SeVI) (Table 2). The control and three experimental diets were administered to animals twice daily (7:30 am and 4:00 pm) in equal amounts; drinking water was available

Table 1. Chemical composition of the ingredients (%) in the basal diet (BD), [1] concentrate-hay diet with a mixture of vitamins and minerals; [2] % of dry matter (DM) (AOAC International, 2005)

Specification	Meadow hay[3]	Concentrate[4]		
		wheat starch	barley meal	soybean meal
DM, % BD	88.41	87.57	89.73	87.28
CP	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
NDF	59.17	18.02	18.81	-
ADF	32.08	4.61	6.44	-
ADL	4.47	1.14	1.49	-

Specification	Chemical composition of the BD
DM, g/kg BD	884.32
CP, g/kg DM	201.87
Crude fibre, g/kg DM	118.64
Crude fat[5], g/kg BD	21.71
TCF[6], g/kg BD	51.66
Ash, g/kg DM	42.80
NDF, g/kg DM	310.47
ADF, g/kg DM	146.33
ADL, g/kg DM	23.32
GE, MJ/kg DM	17.88
Se concentration, mg/kg BD	0.16

CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; TCF – total c rude fat; GE – gross energy; [1] 1 kg of BD contains 20 g of mineral and vitamin mixture (premix); 1 kg of mineral and vitamin mixture contained: g: Ca 285, P 16, Na 56 , Fe 1 (as sulphate), Cu 0.5 (as sulphate), Mn 5.8 (as sulphate), Zn 7.5 (as sulphate); mg: Co 42 Co (as carbonate), I 10 (as iodate), Se 6 (as sodium selenite); IU: vit. A 500 000, vit. D_3 125 000 and vit. E 25 000 (as α-tocopherol); [2] gross energy (MJ/kg of DM): meadow hay 17.1, barley meal 16.3, soybean meal 17.8, wheat starch 16.7; toxic element contents in BD: mg/kg: as 1.39 ± 0.03, Cd 0.068 ± 0.001, Sb 0.0155 ± 0.0015 and Pb 0.514 ± 0.003; [3] main FA contents in meadow hay: mg/kg: C8:0 83, C12:0 142, C14:0 239, *c9*C15:1 131, C16:0 4034, *c9*C16:1 184, C18:0 459, *c9*C18:1 1266, *c12*C18:1 72, *c9c12*C18:2 (LA) 13100, *c9c12c15*C18:3 (αLNA) 4178, C20:0 58, *c11*C20:1 74, C22:0 101, C24:0 69, *c15*C24:1 71; [4] main FA contents in concentrate: mg/kg: C14:0 104, C16:0 3189, C18:0 1425, *c9*C18:1 774, LA 29163, αLNA 1014; [5] crude fat from BD (i.e. the meadow hay and concentrate); [6] total crude fat from BD and added oils (i.e. RO and FO); total gross energy of BD enriched in RO and FO without or with antioxidant(s) (i.e. CAc, SeYe or SeVI)

Table 2. Experimental design and composition of the control and experimental diets, live weight (LW), body weight gain (BWG), pancreas weight and feed conversion efficiency (FCE) of lambs

Experimental design		Live weight			Pancreas weight		FCE[5], kg/kg
Group/Diet:	Supplements added to 1 kg of basal diet (BD)	Initial LW, kg[1]	Final LW, kg[2]	BWG, kg	g[3]	g/kg LW[4]	
Control[6]	20 g RO and 10 g FO	30.56 ± 2.43	37.67 ± 2.08^{ab}	7.08 ± 0.41^{ab}	58.13 ± 3.49^{c}	1.54^{c}	0.19^{ab}
CAc[7]	20 g RO, 10 g FO and 1 g CAc	30.61 ± 2.59	37.16 ± 2.26^{b}	6.63 ± 0.39^{b}	42.04 ± 3.87^{a}	1.13^{a}	0.17^{b}
CAcSeYe[7]	20 g RO, 10 g FO, 1 g CAc and 0.35 mg Se as SeYe	30.34 ± 2.68	36.83 ± 2.69^{b}	6.46 ± 0.38^{b}	51.28 ± 3.81^{b}	1.39^{b}	0.17^{b}
CAcSeVI[7]	20 g RO, 10 g FO, 1 g CAc and 0.35 mg Se as SeVI	30.32 ± 2.97	38.52 ± 3.06^{a}	8.23 ± 0.40^{a}	51.16 ± 2.04^{b}	1.33^{b}	0.22^{a}

RO – rapeseed oil; FO – fish oil; CAc – carnosic acid; SeYe – organic Se as selenised yeast (*Saccharomyces cerevisiae*); SeVI – inorganic Se as sodium selenite. BWG = final LW−Initial LW; [1] average initial body weight (mean ± SD) of lambs after 3 weeks of the preliminary period; during the 3-week preliminary period lambs were fed the control diet; [2] average body weight (mean ± SD) of lambs fed the diets for 35 days of the experimental period; [3] average weight of pancreas; [4] relative weight of pancreas (g/kg) = pancreas weight (g) / final LW of lambs (kg); [5] FCE for 35 days of the experimental period; FCE = (kg body weight gain (BWG)) / (kg diet intake); [6] Se content in 1 kg control diet: mg: 0.16; [7] Se contents in the CAc, CAcSeYe and CAcSeVI diets (mg Se/kg diet): 0.16, 0.51 and 0.51, respectively; Se content in 1 kg meadow hay, soybean meal and barley meal were: mg: 0.003, 0.020 and 0.016, respectively; Se content in wheat starch was below the detection limit; ^{abc} – mean values with different superscripts in the same column are significantly different at $P \leq 0.05$

ad libitum. The control and experimental diets were formulated to be *iso*-energetic and *iso*-proteinous. The quantities of the control and experimental diets were weekly adjusted to both body weight of the animals and their nutritional requirements, according to Strzetelski et al. (2014), to avoid feed refusals. The average daily diet intake was 1.08 kg per lamb; during the experimental period, each lamb consumed 37.8 kg of the control or experimental diet. At the end of the 35-day experiment, all animals were anaesthetized with intramuscular xylazine injections (2–4 mg per 10 kg BW) and subsequently slaughtered. The animals were slaughtered in accordance with the Council Regulation (EC) No 1099/2009 of 24.09.2009 on the protection of animals at the time of killing. The pancreas was collected from each lamb directly after slaughter. Homogenised pancreas samples were stored in sealed tubes at −32 °C until further analytical procedures. The content of all analytes in the pancreas samples was expressed as fresh matter.

Chemicals and reagents

Methanol, acetonitrile and n-hexane of HPLC-grade were purchased from Lab-Scan (Dublin, Ireland). Sodium selenate (SeVI), a mixture of conjugated linoleic acid (CLA) isomers and a standard mixture (FAME) of other 37 fatty acids (FA), 25% BF_3 in methanol, α-tocopheryl acetate, α-tocopherol, sorbic acid, cholesterol, 2,6-di-*tert*-butyl-*p*-cresol, 25% aqueous 1,5-pentanedialdehyde solution, 2,4-dinitro phenylhydrazine, 1,1,3,3-tetramethoxy-propane (99%) and trichloroacetic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Dichloro-methane, NaOH, KOH and HCl were purchased from Avantor Performance Materials (Gliwice, Poland).

Other chemicals were of analytical grade. Highly selenised yeast *Saccharomyces cerevisiae* (SeYe) was obtained from Sel-Plex (Alltech In., Nicholasville, KY, USA). Approximately 83% of the total Se-content of SeYe is in the form of Se-Met, while 5% of Se is in the form of Se-Cys incorporated into the proteins of selenised *S. cerevisiae*. CAc was obtained from Hunan Geneham Biomedical Technology Ltd (Changsha, Hunan, China). A Polfamix O-K vitamin and mineral mixture was purchased from Trouw Nutrition Polska Sp. z o. o. (Grodzisk Mazowiecki, Poland). Odourless FO and RO were supplied by AGSOL (Pacanów, Poland). The energy content of FO and RO was 36.8 and 37.0 MJ/kg oil, respectively. Odourless FO contained the following main FA (mg/kg FO): C12:0 82, C14:0 12 345, *cis9*C14:1 (*c9*C14:1) 215, C15:0 477, C16:0 56 947, *c7*C16:1 318, *c9*C16:1 420, ΣC16:2 15 586, C17:0 493, *c9*C17:1 193, C18:0 9452, *c6*C18:1 188, *c7*C18:1 842, *c9*C18:1 290 592, *c12*C18:1 15 834, *c14*C18:1 159, *c9c12*C18:2 (LA) 114 512, *c9c12c*15C18:2 (αLNA) 20 968, *c11*C20:1 24 206, *c7c9c12c15*C18:4 473, *c11c14*C20:2 2270, *c8c11c14*C20:3 258, *c5c8c11c14*C20:4 (AA) 304, *c8c11c14c17*C20:4 607, C22:0 139, *c13*C22:1 11 036, *c11*C22:1 1704, *c5c8c11c14c17*C20:5 (EPA) 6792, *c13c16*C22:2 95, *c7c10c13c16*C22:4 144, *c15*C24:1 397, *c7c-10c13c16c19*C22:5 (DPA) 1560 and *c4c7c10c-13c16c19*C22:5 (DHA) 26 570. RO included the following main FA (mg/kg RO): C14:0 56, C16:0

13 091, *c9*C16:1 33, C18:0 5490, *c9*C18:1 385 859, *c12*C18:1 786, LA 282 394, αLNA 38 474, C20:0 194, *c11*C20:1 108, C22:0 430 and *c15*C24:1 61.

Analytical methods and chromatographic equipment

Fatty acid determination. Homogenized pancreatic samples (50–70 mg) were saponificated using KOH solutions according to methods previously described by Czauderna et al. (2009a). Subsequently, base- and acid-catalysed methylations were introduced to prepare FA methyl esters (FAME) in the processed pancreas samples (Czauderna et al., 2009a). FAME were then quantified in biological samples using capillary gas chromatography (GC) with mass spectrometry (MS) according to methods previously described by Czauderna et al. (2009a). FAME analyses were performed on a GC-MSQP2010 Plus EI gas chromatograph (Shimadzu, Tokyo, Japan) equipped with a quadruple mass selective detector (Model 5973N; Shimadzu, Tokyo, Japan), a BPX70 fused silica column (120 m × 0.25 mm i.d. × 0.25 μm film thickness; Phenomenex, Torrance, CA, USA) and an injection port. FAME identification was validated using electron impact ionization spectra of FAME and compared to authentic FAME standards and the NIST 2007 reference mass spectra library. All FAME analyses performed on pancreatic samples were based on total ion current chromatograms and/or selected ion-monitoring chromatograms.

Cholesterol and tocopherol determination

Tocopherols and cholesterol were quantified in pancreatic samples using a reversed-phase (RP) liquid chromatographic system according to the methods described by Czauderna et al. (2009b). The liquid chromatographic instrument (Shimadzu, Tokyo, Japan) consisted of an ultra-fast liquid chromatography (UFLC-DAD) system, containing two LC-20ADXP pumps, a SIL-20ACXR autosampler, a CBM-20A communication bus module, a CTO-20A column oven, a DGU-20A5 degasser and an SPD photodiode array detector (DAD) and a Kinetex C18 column (particle size 2.6 μm; Hydro-RP, 150 mm × 2.1 mm i.d.; Phenomenex, Torrance, CA, USA).

Malondialdehyde determination

Chromatographic separations of derivatized MDA from endogenic species in the pancreas samples were conducted using the above-described RP-UFLC-DAD system (Czauderna et al., 2011b), but with a C18 column (Synergi, Hydro-RP, particle size 2.5 μm, 100 Å, 100 mm × 2 mm i.d.; Phenomenex; Torrance, CA, USA).

Calculation of indices

Atherogenic ($_{index}A^{SFA}$) and thrombogenic ($_{index}T^{SFA}$) 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 described by Fernández et al. (2007). The modified atherogenic index ($_{index}A^{SFA+Toc}$) was calculated as follows (Białek et al., 2020):

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

where: $C_{\alpha T}$, $C_{\alpha TAc}$, $C_{\gamma T}$ and $C_{\delta T}$ – concentrations of α-tocopherol, α-tocopheryl acetate, γ-tocopherol and δ-tocopherol, respectively; 1.49, 1.36, 0.15 and 0.05 are the coefficients of biological activity of tocopherols (Zu and Ip, 2003; Rozbicka-Wieczorek et al., 2016a).

Statistical analysis

All statistical analyses of the effects of dietary additives were carried out using the Statistica 12.5 PL software package (StatSoft Inc., Tulsa, OK, USA). Differences were considered significant at $P \leq 0.05$. The results are presented as means and standard errors of means (SEM), except for BW, pancreas weight and FCE (mean ± standard deviation). The Shapiro-Wilk test was used to analyse the normality of the data distribution. The influence of dietary modifications on the examined parameters in all pancreatic samples for variables with normal distribution was tested using one-way ANOVA and Tukey's Honestly Significant Difference (HSD) test. The results for variables without normal distribution were tested using the Kruskal-Wallis test, which is a non-parametric equivalent of one-way ANOVA, with a *post-hoc* multiple comparison test.

Results

The results of the present study demonstrated that FO (rich in n-3LPUFA) and RO (rich in n-6PUFAs), CAc and Se (as SeVI or SeYe), added to the diet, did not cause harmful symptoms (such as vomiting or diarrhoea) as no visual changes were observed in the pancreas or other internal organs, muscles and adipose tissues of the lambs. The results presented in Table 2 indicated that the CAcSeVI diet increased ($P \leq 0.05$) lamb live weight (LW), weight gain (BWG, kg) and feed conversion efficiency (FCE)

compared to the CAc and CAcSeYe diets. On the other hand, all experimental diets, especially the CAc diet, reduced pancreas weight and relative pancreas weight (pancreas weight/final lamb LW) compared to the control diet.

Concentrations of SFA and MUFA in the pancreas

In the current study, a significant increase in the concentrations of C10:0, C14:0, C17:0, C18:0, C20:0, thrombogenic SFA (T-SF), as well as the sum of SFA (ΣSFA) and all FA (ΣFA) was observed in the pancreas ($P \leq 0.05$) of lambs fed the CAc diet compared to the control and CAcSeYe diets (Table 3). Similarly, the CAcSeVI diet led to an increase ($P \leq 0.05$) in the content of C10:0, C14:0, C17:0, C18:0, C20:0, C24:0, T-SFA and ΣFA in the pancreas as compared to the control and CAcSeYe diets. The concentrations of C17:0, T-SFA, ΣSFA and ΣFA in the pancreas of lambs fed the CAcSeYe diet did not differ statistically ($P > 0.05$) relative to the pancreas of lambs fed the control diet. Moreover, all experimental diets did not affect ($P > 0.05$) the concentrations of C12:0, C15:0, C16:0 and atherogenic SFA (A-SFA) in the pancreas when compared to the control diet.

On the other hand, all experimental diets elevated ($P \leq 0.05$) the concentrations of *c7*C16:1, *c9*C16:1, *t11*C18:1 and *c9*C18:1 in the pancreas in comparison to the control diet (Table 4). The experimental diets enriched in CAc, irrespective of the presence of SeVI, increased ($P \leq 0.05$) the total concentration of all assayed MUFA (ΣMUFA) in the pancreas compared to the control and CAcSeYe diets. All experimental diets increased ($P \leq 0.05$) the ΣMUFA to ΣFA (ΣMUFA/ΣFA) concentration ratio and indices of Δ9-desaturation of C16:0 ($^{C16:0}\Delta 9_{index}$), total Δ9-desaturation of C16:0 and C18:0 ($^{\Sigma}\Delta 9_{index}$) and total Δ9-, Δ6-, Δ5 and Δ4-desaturation ($^{\Sigma\Delta 9,6,5,4}FA_{index}$) of FA in the pancreas in comparison to the control diet. The experimental diet enriched in CAc, regardless of the presence of SeYe,

Table 3. Concentrations (μg/g pancreas) of selected individual saturated fatty acids (SFA), all assayed SFA (ΣSFA)[3], all assayed FA (ΣFA), atherogenic[4] ($_{index}A^{SFA}$) and thrombogenic index[5] ($_{index}T^{SFA}$) values and the ratios of ΣSFA concentrations to total concentrations of UFA (ΣSFA/ΣUFA), PUFA (ΣSFA/ΣPUFA), MUFA (ΣSFA/ΣMUFA) and ΣFA (ΣSFA/ΣFA) in the pancreas of lambs

Item	Additive: – Group: Control	CAc CAc	CAc and SeYe CAcSeYe	CAc and SeVI CAcSeVI	SEM	*P*-value
C10:0	3.64^{a}	35.86^{c}	11.73^{b}	32.54^{c}	0.29	0.03
C12:0	37.24	68.36	43.62	44.13	0.42	0.23
C14:0	179.38^{a}	771.07^{d}	280.73^{b}	546.69^{c}	7.89	0.02
C15:0	191.28	228.43	235.74	230.28	11.72	0.09
C16:0	10528.68	12092.91	9990.25	11304.36	91.48	0.23
C17:0	269.80^{a}	396.42^{b}	286.85^{a}	387.36^{b}	7.64	0.04
C18:0	5832.73^{a}	8430.48^{b}	6186.84^{a}	8390.58^{b}	80.75	0.03
C20:0	2.11^{a}	2.98^{b}	2.06^{a}	3.17^{b}	0.05	0.04
C22:0	18.21^{bc}	14.17^{b}	1.14^{a}	23.36^{c}	0.21	0.02
C24:0	16.36^{a}	18.64^{ab}	22.68^{b}	29.23^{c}	0.28	0.02
A-SFA[1]	10746.30	12931.78	10313.75	11894.29	165.42	0.38
A-SFA/ΣFA	0.32^{b}	0.29^{ab}	0.30^{ab}	0.28^{a}	0.01	0.03
T-SFA[2]	16541.68^{a}	21294.38^{b}	16458.06^{a}	202427.16^{b}	176.82	0.02
T-SFA/ΣFA	0.49^{b}	0.48^{ab}	0.47^{a}	0.48^{a}	0.01	0.04
$_{index}A^{SFA}$	0.69^{c}	0.67^{bc}	0.62^{a}	0.64^{ab}	0.01	0.03
$_{index}T^{SFA}$	0.96^{b}	1.00^{c}	0.91^{a}	1.00^{c}	0.01	0.04
ΣSFA	17077.80^{a}	22054.06^{b}	17058.78^{a}	20987.23^{ba}	289.78	0.04
ΣFA	33617.68^{a}	44284.78^{b}	35028.76^{a}	42657.42^{b}	793.58	0.04
ΣSFA/ΣUFA	1.03^{c}	1.01^{bc}	0.94^{a}	1.00^{b}	0.01	0.03
ΣSFA/ΣPUFA	2.37^{a}	3.25^{c}	2.39^{b}	3.15^{c}	0.01	0.04
ΣSFA/ΣMUFA	1.83^{c}	1.46^{a}	1.56^{b}	1.46^{a}	0.01	0.02
ΣSFA/ΣFA	0.51	0.50	0.49	0.49	0.01	0.31

[1] the concentration sum of atherogenic SFA: C12:0, C14:0 and C16:0; [2] the concentration sum of thrombogenic SFA: C14:0, C16:0 and C18:0; [3] the concentration sum of SFA: C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0 and C24:0; [4] the atherogenic index = (C12:0 + 4 × C14:0 + C16:0) / (ΣMUFA + Σn-6PUFA + Σn-3 PUFA) (Morán et al., 2013); [5] the thrombogenic index = (C14:0 + C16:0 + C18:0) / [(0.5 × ΣMUFA + 0.5 × Σn-6PUFA + 3 × Σn-3PUFA) / (Σn-6PUFA)] (Morán et al., 2013); SEM – standard error of the mean; *P* – statistical significances; ^{abc} – different letters within a row indicate significant differences at $P \leq 0.05$

Table 4. Concentrations (μg/g pancreas) of monounsaturated fatty acids (MUFA) and index values of Δ9-desaturation and total FA desaturation in the pancreas

Item	Additive: – Group: Control	CAc CAc	CAc and SeYe CAcSeYe	CAc and SeVI CAcSeVI	SEM	*P*-value
*c7*C16:1	49.76^{a}	183.08^{bc}	155.80^{b}	212.38^{c}	4.93	0.02
*c9*C16:1	266.72^{a}	487.09^{c}	399.38^{b}	438.83^{bc}	7.91	0.02
*t11*C18:1	25.24^{a}	268.82^{b}	338.48^{c}	720.76^{d}	11.83	0.01
*c7*C18:1	216.68^{b}	312.46^{c}	1.24^{a}	1.93^{a}	8.80	0.03
*c9*C18:1	7790.64^{a}	12836.85^{c}	9116.44^{b}	12033.38^{c}	101.63	0.04
*c12*C18:1	1004.70	1026.42	918.38	969.74	26.83	0.32
*c11*C20:1	0.34	0.27	0.31	0.23	0.04	0.37
*c13*C22:1	0.23	0.16	0.27	0.23	0.08	0.31
ΣMUFA[1]	9354.84^{a}	15115.16^{b}	10926.58^{a}	14376.43^{b}	107.63	0.04
ΣMUFA/ΣFA	0.28^{a}	0.32^{b}	0.31^{b}	0.33^{b}	0.01	0.03
$^{C18:0}\Delta9_{index}$ [2]	0.57^{a}	0.60^{b}	0.60^{b}	0.59^{ab}	0.01	0.04
$^{C16:0}\Delta9_{index}$ [3]	0.025^{a}	0.039^{b}	0.038^{b}	0.037^{b}	0.001	0.03
$\Sigma\Delta9_{index}$ [4]	0.30^{a}	0.39^{b}	0.37^{b}	0.39^{b}	0.01	0.04
$^{\Sigma\Delta9,6,5,4}FA_{index}$ [5]	0.50^{a}	0.52^{b}	0.53^{b}	0.52^{b}	0.01	0.03

c – cis; *t – trans*; [1] the concentration sum: *c7*C16:1, *c9*C16:1, *t11*C18:1, *c6*C18:1, *c7*C18:1, *c9*C18:1, *c11*C18:1, *c12*C18:1, *c11*C20:1, *c11*C22:1 and *c13*C22:1; [2] index of Δ9-desaturaturation of C18:0: $^{C18:0}\Delta9_{index}$ = *c9*C18:1 / (*c9*C18:1 + C18:0); [3] index of Δ9-desaturaturation of C16:0: $^{C16:0}\Delta9_{index}$ = *c9*C16:1 / (*c9*C16:1 + C16:0); [4] index of Δ9-desaturaturation of C18:0 and C16:0: index: $^{\Sigma}\Delta9_{index}$ = (*c9*C18:1 + *c9*C16:1) / (*c9*C18:1 + *c9*C16:1 + C18:0 + C16:0); [5] index of the total desaturation of FA; i.e. Δ9-, Δ6-, Δ5- and Δ4-desaturation of FA: $^{\Sigma\Delta9,6,5,4}FA_{index}$ = (ΣMUFA + ΣPUFA) / (C16:0 + C18:0 + C20:0 + C22:0 + C24:0 + ΣMUFA + ΣPUFA); SEM – standard error of the mean; *P* – statistical significances; ^{abc} – different letters within a row indicate significant differences at $P \leq 0.05$

increased ($P \leq 0.05$) Δ9-desaturation of the C18:0 ($^{C18:0}\Delta9_{index}$) index values in the pancreas when compared to the control diet.

Concentrations of PUFA, tocopherols, T-Ch and MDA in the pancreas

In the current study, the concentration of *t7c9*CLA, total CLA isomers (ΣCLA) and the values of the hypocholesterolemic/hypercholesterolemic FA (h/H-Ch) ratio in the pancreas of lambs fed all experimental diets was higher ($P \leq 0.05$) than those in the control diet (Table 5). Similarly, experimental diets containing CAc, irrespective of the presence of SeVI, increased ($P \leq 0.05$) *c9t11*CLA and *c8c11c14*C20:3 concentrations in the pancreas compared to the control and CAcSeYe diets. Moreover, the CAc and CAcSeVI diets reduced ($P \leq 0.05$) the total concentration of n-6PUFA (Σn-6PUFA), as well as the concentration ratios of Σn-6PUFA to Σn-3PUFA (Σn-6PUFA/Σn-3PUFA), ΣLPUFA to ΣFA (ΣLPUFA/ΣFA), Σn-3LPUFA to ΣFA (Σn-3LPUFA/ΣFA) and ΣPUFA to ΣFA (ΣLPUFA/ΣFA) in the pancreas when compared to the control diet. All experimental diets decreased ($P \leq 0.05$) the total concentration ratio of n-6LPUFA to n-3LPUFA (Σn-6LPUFA/Σn-3LPUFA) in the pancreas in comparison to the control diet. All experimental diets, especially those enriched with CAcSeYe or CAcSeVI, reduced Δ9-desaturation of the *t11*C18:1 ($^{t11C18:1}\Delta9_{index}$) index value in the pancreas compared to the control diet. The index values of LA elongation ($^{n\text{-}6ElongC20/C18}$index) and Δ4-desaturation ($\Delta4_{index}$) in the pancreas of lambs fed the CAcSeYe diet were lower ($P \leq 0.05$) than corresponding indices in the pancreas of lambs fed the control, CAc and CAcSeVI diets.

T-Ch accumulation in the pancreas in lambs fed the CAcSeVI diet decreased ($P \leq 0.05$) compared to the control, CAc and CAcSeYe diets (Table 6). Lambs fed the CAc diet had higher ($P \leq 0.05$) pancreas concentrations of γ-tocopherol (γ-T), as well as the sums of α-tocopherol (α-T) and α-tocopheryl acetate (α-TAc) (Σ(α-T+α-TAc)) and all assayed tocopherols (Σall-Ts) than lambs fed the control, CAcSeYe and CAcSeVI diets (Table 6). The CAcSeVI diets led to a decrease ($P \leq 0.05$) in the total concentration of T-Ch, Σ(α-T + α-TAc) and Σall-Ts in the pancreas when compared to the CAcSeYe diet.

The experimental diets containing CAc, regardless of the presence of SeYe, decreased ($P \leq 0.05$) the value of the modified atherogenic index ($_{index}A^{SFA+Toc}$) in the pancreas compared to the control and CAcSeVI diets, whereas the CAcSeVI diet exerted a negligible effect ($P > 0.05$) on the $_{index}A^{SFA+Toc}$ value in the pancreas when compared to the control diet (Table 6).

All experimental diets resulted in a non-significant reduction ($P \leq 0.05$) in pancreatic MDA concentrations in comparison to the control diet, the

Table 5. Concentrations (μg/g pancreas) of polyunsaturated fatty acids (PUFA), concentration ratios of selected PUFA to ΣFA, indices of elongases and desaturases and the hypocholesterolemic/hypercholesterolemic FA (h/H-Ch) ratio in the pancreas

Item	Additive: – Group: Control	CAc CAc	CAs and SeYe CAcSeYe	CAc and SeVI CAcSeVI	SEM	*P*-value
*c9t11*CLA	29.64[a]	67.03[b]	33.68[a]	54.53[b]	0.61	0.03
*t7c9*CLA	0.14[a]	21.59[c]	19.52[c]	0.43[b]	0.27	0.02
ΣCLA[1]	30.16[a]	88.94[b]	53.62[b]	55.08[b]	0.66	0.02
*c9c12*C18:2 (LA)	3025.73	2924.32	2953.80	2779.36	39.41	0.62
*c9c12c15*C18:3 (αLNA)	281.25[b]	325.38[c]	213.81[a]	236.57[a]	5.82	0.04
*c11c14*C20:2	142.58	137.08	132.35	135.14	6.30	0.57
*c8c11c14*C20:3	59.82[a]	71.41[b]	63.69[a]	84.72[c]	1.96	0.03
*c5c8c11c14*C20:4 (AA)	2871.83	2457.42	2858.06	2538.27	31.62	0.27
*c5c8c11c14c17*C20:5 (EPA)	295.46	286.29	328.68	336.83	14.23	0.19
*c7c10c13c16c19*C22:5 (DPA)	341.72	342.18	380.35	331.36	15.48	0.24
*c4c 7c10c13c16c19*C22:5 (DHA)	166.15	158.38	163.31	169.86	8.33	0.38
Σn-3PUFA[2]	1084.35	1111.76	1086.03	1075.32	26.42	0.51
Σn-6PUFA[3]	5960.47[c]	5451.73[a]	5875.36[c]	5399.64[a]	42.72	0.04
ΣPUFA[4]	7216.57	6789.24	7147.03	6666.41	59.36	0.37
Σn-6PUFA/Σn-3PUFA	5.64[b]	4.97[a]	5.51[b]	5.10[a]	0.01	0.03
Σn-6LPUFA	3075.75	2667.23	3054.69	2759.26	67.35	0.33
Σn-3LPUFA	801.68	787.02	871.74	837.36	18.31	0.29
ΣLPUFA[5]	3879.46[bc]	3453.82[a]	3927.42[c]	3597.02[ab]	34.83	0.04
Σn-6LPUFA/Σn-3LPUFA	3.83[c]	3.39[ab]	3.51[b]	3.29[a]	0.01	0.03
Σn-3LPUFA/ΣFA	0.024[b]	0.022[a]	0.025[b]	0.023[a]	0.001	0.04
ΣLPUFA/ΣFA	0.12[b]	0.08[a]	0.11[b]	0.09[a]	0.01	0.03
ΣPUFA/ΣFA	0.21[b]	0.15[a]	0.20[b]	0.16[a]	0.01	0.02
$^{n\text{-}6ElongC20/C18}$index[6]	0.045[bc]	0.045[b]	0.043[a]	0.047[c]	0.001	0.04
$^{n\text{-}3ElongC22/C20}$index[7]	0.54	0.54	0.54	0.50	0.01	0.11
$^{t11C18:1}\Delta 9_{index}$[8]	0.54[c]	0.20[b]	0.09[a]	0.07[a]	0.01	0.02
$\Delta 4_{index}$[9]	0.33[c]	0.32[b]	0.30[a]	0.34[d]	0.01	0.03
$\Delta 5_{index}$[10]	0.98	0.97	0.98	0.97	0.01	0.06
h/H-Ch[11]	1.55[a]	1.70[b]	1.76[c]	1.78[d]	0.01	0.01

CLA – conjugated linoleic acid; *c* – *cis*; *t* – *trans*; [1] the concentration sum of *c9t11*CLA, *t7c9*CLA and t,tCLA isomers (trans-transCLA: 7-7, 8-10, 9-11, 10-12, 11-13 and 12-14); [2] the concentration sum of αLNA, *c6c9c12c15*C18:4 and Σn-3LPUFA (*c11c14c17*C20:3, *c8c11c14c17*C20:3, EPA, DPA and DHA); [3] the concentration sum of LA, *c6c9c12*C18:3 and Σn-6LPUFA (*c11c14*C20:2, *c8c11c14*C20:3, AA and *c7c10c13c16*C22:4); [4] the concentration sum of ΣCLA, Σn-3PUFA and Σn-6PUFA; [5] the concentration sum of Σn-6LPUFA and Σn-3LPUFA; [6] $^{n\text{-}6ElongC20/C18}$index = *c11c14*C20:2 / (*c11c14*C20:2 + LA); [7] $^{n\text{-}3ElongC22/C20}$ index = DPA / (DPA+EPA); [8] index of Δ9-desaturation of *t11*C18:1 = *c9t11*CLA / (*c9t11*CLA + *t11*C18:1); [9] index of Δ4-desaturation of DPA = DHA / (DHA + DPA); [10] index of Δ5-desaturation of C20:3n-6 = AA / (AA + *c8c11c14*C20:3); [11] h/H-Ch ratio = (*c7*C18:1 + *c9*C18:1 + *c12*C18:1 + *c14*C18:1 + *c11*C20:1 + 13C22:1 + LA + αLNA + *c6c9c*12C18:3 + AA + *c11c14*C20:2 + EPA + *c7c10c13c16*C22:4 + DPA) / (C14:0 + C16:0) (Fernández et al., 2007); SEM – standard error of the mean; *P* – statistical significances; [a-d] – different letters within a row indicate significant differences at $P \leq 0.05$

CAc diet most effectively increased ($P \leq 0.05$) the values of the modified PUFA peroxidation indices ($^{Toc/PUFA}MDA_{index}$ and $^{Toc/PUFA\text{-}LPUFA}MDA_{index}$) in the pancreas in comparison to the control, CAcSeYe and CAcSeVI diets. Similarly, lambs fed the CAcSeYe diet showed higher values ($P \leq 0.05$) of these indices in the pancreas than lambs fed the control diet. On the other hand, the CAcSeVI diet led to the most effective reduction ($P \leq 0.05$) in the pancreatic values of $^{Toc/PUFA}MDA_{index}$ and $^{Toc/PUFA\text{-}LPUFA}MDA_{index}$ compared to the control, CAc and CAcSeYe diets.

Discussion

Reactive species (like ROS and RNS) are involved in the pathogenesis of acute and chronic pancreatitis (Newsholme et al., 2019). Clinical investigations have indicated that RNS and ROS production are intimately linked to the development of pancreatic inflammatory disorders. The detrimental impact of highly reactive RNS and ROS is mediated by their direct actions on pancreatic biomolecules (like proteins/enzymes, lipids and nucleic

Table 6. Concentrations of total cholesterol (T-Ch; μg/g pancreas), tocopherols (μg/g pancreas) and MDA (ng/g pancreas)[1], and values of the modified atherogenic index ($_{index}A^{SFA+Toc}$), PUFA peroxidation indices ($^{PUFA}MDA_{index}$ and $^{PUFA\text{-}LHPUFA}MDA_{index}$) in the pancreas

Indices	Diets[5]				SEM	*P*-value
	Control	CAc	CAcSeYe	CAcSeVI		
T-Ch	434.27^{b}	424.38^{ba}	470.64^{b}	350.76^{a}	26.64	0.04
δ-T	0.62	0.81	0.62	0.60	0.09	0.08
γ-T	0.44^{ab}	2.32^{c}	0.39^{a}	0.59^{b}	0.05	0.03
α-T	9.93	10.90	11.29	8.19	0.10	0.09
α-Tac	5.74	7.36	5.45	5.46	0.06	0.23
Σ(α-T + α-TAc)	15.67^{ab}	18.26^{c}	16.73^{bc}	13.65^{a}	0.11	0.04
Σall-Ts[2]	16.72^{ab}	21.39^{c}	17.73^{b}	14.84^{a}	0.11	0.04
$_{index}A^{SFA+Toc}$ [3]	0.034^{b}	0.025^{a}	0.026^{a}	0.032^{b}	0.001	0.03
MDA	2.59	2.57	2.73	2.51	0.10	0.29
$^{Toc/PUFA}MDA_{index}$ [4]	5.99^{b}	8.09^{d}	6.77^{c}	5.59^{a}	0.03	0.04
$^{Toc/PUFA\text{-}LPUFA}MDA_{index}$ [5]	2.29^{b}	3.20^{d}	2.55^{c}	2.13^{a}	0.01	0.03

δ-T – δ-tocopherol; γ-T – γ-tocopherol; α-T – α-tocopherol; α-Tac – α-tocopheryl acetate; MDA – malondialdehyde; [1] MDA concentration (C_{MDA}) was determined immediately after pancreas homogenization; [2] total concentration of all assayed tocopherols: $C_{\Sigma all\text{-}Ts}$ = δ-T + γ-T + α-T + α-Tac; [3] $_{index}A^{SFA+Toc}$ = $_{index}A^{SFA}$ / (1.49 × $C_{\alpha T}$ + 1.36 × $C_{\alpha TAc}$ + 0.15 × $C_{\gamma T}$ + 0.05 × $C_{\delta T}$), where: $C_{\alpha T}$, $C_{\alpha TAc}$, $C_{\gamma T}$ and $C_{\delta T}$ – concentration (μg/g) of α-tocopherol, α-tocopheryl acetate, γ-tocopherol and δ-tocopherol, respectively; [4] $^{Toc/PUFA}MDA_{index}$ = ($C_{\Sigma all\text{-}Ts}$ / C_{PUFA}) × C_{MDA}; [5] $^{Toc/PUFA\text{-}LPUFA}MDA_{index}$ = ($C_{\Sigma all\text{-}Ts}$ / $C_{PUFA+3*LPUFA}$) × C_{MDA}, where: $C_{PUFA+3*LPUFA}$ – total concentration of ΣPUFA and LPUFA multiply by 3 (i.e., PUFA + 3 × ΣLPUFA); SEM – standard error of the mean; P – statistical significances; [a, b,c] – means with different superscripts in the row differ significantly at $P \leq 0.05$

acids) and activation of pro-inflammatory signalling cascades, which subsequently stimulates activation of immune responses (Newsholme et al., 2019). Thus, the main aim of our study was to analyse the possibility that dietary CAc, with or without 0.35 ppm Se (as SeVI or SeYe), could decrease peroxidative damage of PUFA in the pancreas of lambs fed the diets enriched in RO and FO, containing high proportion of pro-healthy n-3LPUFA. Fortunately, our current and previous studies documented that the addition of 0.35 ppm Se (as SeYe or SeVI) to the diet containing RO, FO and CAc did not cause harmful symptoms (such as diarrhoea or vomiting) and visual changes either in the pancreas or in the liver, spleen, kidney, brain and muscles of lambs (Czauderna et al., 2017; Białek and Czauderna, 2019; Białek et al., 2020, 2021). Indeed, only diets supplemented with inorganic Se-compounds (especially selenite or selenides), at concentrations exceeding 5 mg Se/kg diet exerted hepatotoxic and teratogenic effects in lambs or cows, but also in humans (Navarro-Alarcon and Cabrera-Vique, 2008). Fortunately, the results summarized in Table 2 showed that the CAcSeVI diet increased LW and BWG of lambs, as well as FCE compared to the CAc and CAcSeYe diets. In fact, our previous studies demonstrated that an experimental diet containing CAc and SeVI, ensured optimal ruminal fermentation conditions, which was confirmed by the acetic acid/propionic acid ratio in ruminal fluids greater than 2.2:1 (Wolin, 1979; Białek and Czauderna, 2019). Furthermore, the CAcSeVI diet reduced rumen methanogenesis, as it decreased the amount of CH_4 (high-energy compound) and CO_2 in the rumen compared to the control, CAc and CAcSeYe diets (Białek and Czauderna, 2019). In contrast, the CAcSeYe diet increased methanogenesis relative to the control, CAc and CAcSeVI diets (Białek and Czauderna, 2019). Unfortunately, CO_2 and CH_4 (greenhouse gases – waste products) were shown to cause a loss of approx. 8% of the total digestible energy of the diet (Wolin, 1979). Therefore, the CAcSeVI diet in the current study increased LW and BWG of lambs and FCE compared to the CAc and CAcSeY diets (Table 2). Moreover, dietary SeVI (via selenide) can be used to biosynthesize Se-Cys, which is an essential component in Se-enzymes (Suganthi et al., 2019). Se-Cys-containing enzymes increase thyroid hormone biosynthesis and modulate biochemical reactions, especially enzymatic capacity and protein biosynthesis, which results in the stimulation of the metabolic rate in the mammalian organism. Therefore, it is reasonable to suppose that the experimental diet with SeVI most effectively stimulated anabolic processes in sheep. In contrast, Se-Met derived from dietary SeYe had no effect on LW, BWG and FCE in lambs fed the CAcSeYe diet compared to the control and CAc diets, because $tRNA_{Met}$ does not discriminate between Se-Met and methionine (Met) (Navarro-Alarcon and Cabrera-Vique, 2008). Hence, Se-Met derived from SeYe replaces Met in proteins. However, these Se-Met-containing proteins are not considered Se-enzymes (Raymond et al., 2014). In fact, the

impact of Se-Met-containing proteins on thyroid hormone biosynthesis, enzymatic capacity, protein biosynthesis, i.e. the metabolic rate, was shown to be negligible.

Interestingly, all experimental diets, especially the CAc diet, reduced pancreas weight as compared to the control diet. Thus, the current study indicated that the experimental diet supplemented only with CAc was most effective in reducing the mass of pancreatic α-cells (biosynthesizing and secreting the hormone glucagon) in proportion to the reduction of pancreatic weight observed in lambs (Table 2) (Bonnet-Serrano et al., 2018). In contrast, SeYe or SeVI added to the the CAc diet counteracted the reduction of pancreatic α-cell mass by CAc. Moreover, the CAc diet reduced ($P > 0.05$) FCE and BWG of lambs compared to the control diet, whereas it significantly decreased ($P \leq 0.05$) FCE and BWG of lambs in comparison to the experimental diet enriched in CAc and SeVI (Table 2). In fact, CAc-enriched diets stimulated faecal fat excretion, but not with decreased food intake (Ibarra et al., 2011). Consequently, increased faecal fat energy excretion explained the observed reductions in FCE, live weight and BWG of experimental animals fed diets containing CAc. Fortunately, our present study showed that SeVI added to the experimental diet with CAc improved lamb growth parameters compared to the experimental diet containing only CAc.

Effect of experimental diets on fatty acid composition in the pancreas

The current study found that the CAc and CAcSeVI diets stimulated the accumulation of ΣSFA, ΣMUFA, and especially ΣFA in the pancreas compared to the control diet (Tables 3 and 4). Indeed, as previously noted, dietary CAc, irrespective of the presence of SeYe or SeVI, reduced the number of α-cells responsible for biosynthesis and secretion of glucagon, involved in gluconeogenesis, glycogenolysis and fatty acid oxidation. Moreover, dietary CAc reduced pancreatic lipase activity, a key enzyme in the digestion of fat to free fatty acids (f-FA) and glycerol (Ibarra et al., 2011), and decreased f-FA levels resulted in lower rates of mitochondrial oxidation of fatty acids. On the other hand, compared to the CAc and CAcSeVI diets, SeYe addition to the experimental diet with CAc reduced ΣSFA, ΣMUFA and ΣFA contents in the pancreas. Considering the above, we argued that SeYe (containing Se-Met and Se-Cys) supplementation inhibited fatty acid synthetase by reversible binding of HSe-containing metabolite of SeYe to sulfhydryl groups (active sites for acetyl-CoA and/or malonyl-CoA binding) of the enzyme (Combs, 1997).

The results summarised in Table 4 show that the experimental diets containing CAc, regardless of the presence of SeYe, stimulated Δ9-desaturation of C16:0 and C18:0 in the pancreas. This was consistent with previous studies showing that CAc, irrespective of the presence of SeYe, increased Δ9-desaturase capacity by stimulating stearoyl-CoA desaturase mRNA expression in kidneys and adipose tissues (Calvo et al., 2017; Krajewska-Bienias et al., 2017; Białek and Czauderna, 2019; Białek et al., 2020, 2021). In contrast, CAc without or with SeYe addition to the experimental diet reduced C18:0 Δ9-desaturation in the liver and brain compared to the control diet (Rozbicka-Wieczorek et al., 2016a,b). Therefore, we argued that the effect of C without or with Se (especially in the form of SeYe) on the level of Δ9-desaturation depended on tissue type, carbon chain length of desaturated fatty acids (Table 4) and the SFA/UFA concentration ratio in diets, and thus the ratio of C16:0 and C18:0 to Δ9-desaturation products (i.e., *c9*C16:0 and *c9*C18:0) in tissues. In fact, in mammals, a diet rich in PUFA was shown to decrease Δ9-desaturase activity, whereas carbohydrates, cholesterol and low- or free-fat diets were found to increase it (Turpeinen et al., 2002). Interestingly, *t11*C18:1 isomer (TVA) was efficiently Δ9-desaturated to c9t11CLA, whereas Δ9-desatu-ration of isomer *t12*C18:1 to *c9t12*C18:2 could not be detected in mammals (Kuhnt et al., 2005). Moreover, our current and recent studies documented that the presence of double bonds in fatty acid chains (e.g. TVA) decreased Δ9-desaturation in the pancreas of lambs fed experimental diets, especially those containing SeYe or SeVI (Table 5), compared to SFA Δ9-desaturation (e.g. C16:0 or C18:0) in the pancreas and adipose tissue of lambs fed the experimental diets (Table 4) (Krajewska-Bienias et al., 2017; Białek et al., 2020). Dietary CAc contains two phenolic groups, and these groups at position 11 have been found to contribute more significantly to its antioxidant activity (Krajewska-Bienias et al., 2017). Therefore, CAc (like other phenolic diterpenes) and dietary Se (in the form of SeYe and SeVI) decreased oxidative processes in mammalian tissues. Therefore, all experimental diets, particularly with SeYe or SeVI, reduced Δ9-desaturation of TVA containing *trans* double bonds, in contrast to C18:0 and C16:0. Indeed, FA desaturation is an oxidation process that requires two electrons and molecular oxygen; however, oxygen itself is not incorporated into the

FA chain but is completely reduced to water (Bond et al., 2016). Therefore, FA desaturation is quantitatively modified by competitive reactions between acids of the same or different groups, dietary supplements and competition with fatty acids incorporated in tissue lipids.

Effect of experimental diets on T-Ch, tocopherol and MDA contents in the pancreas

Our research showed that SeYe added to the experimental diet elevated T-Ch levels in the pancreas in comparison to those observed in the CAcSeVI group (Table 6). Thus, our current study is consistent with the results of previous investigations in which diets containing higher levels of Se-compounds increased triglyceride contents in the liver (Stranges et al., 2010) and blood serum (Korniluk et al., 2006); this may provide a possible explanation for the lipogenic effects of SeYe-enriched diets. In fact, seleno-proteins and cholesterol are linked through the common use of isopentenyl pyrophosphate for both Sec-tRNA and isoprenoid biosynthesis in the mevalonate pathway (Stranges et al., 2010). Indeed, higher plasma Se (> 1.2 μmol/l) concentrations were associated with increased total and non-HDL cholesterol levels in subjects administered a Se-supplemented diet. These observations raise additional concerns about potential adverse cardiometabolic effects of diets supplemented with higher Se doses. Moreover, our previous study demonstrated that diets enriched in SeVI, and especially SeYe (rich in highly bioavailable Se-Met) were associated with increased T-Ch levels in the fat surrounding the rumen compared to the control diet (Białek and Czauderna, 2019). Similarly, the addition of SeYe to the experimental diet with CAc more effectively increased T-Ch accumulation in the fat surrounding the rumen in comparison to the CAc experimental diet with SeVI (Białek and Czauderna, 2019).

An interesting finding of the present study was that the experimental diet enriched only with CAc increased total pancreatic tocopherol (Σall-Ts) levels to the greatest extent, especially γ-T, compared to control, CAcSeYe and CAcSeVI. Our current and previous studies (Rozbicka-Wieczorek et al., 2016b) demonstrated that CAc increased regeneration of tocopherol radicals (Ts) (i.e. products of tocopherol peroxidation) to the original chemical form of tocopherols. In contrast, SeVI addition to the experimental diet with CAc decreased regeneration efficiency of Ts radicals in the pancreas as compared to the CAc and CAcSeYe diets. Unlike Se-Met (the main Se-compound in dietary SeYe), SeVI is a fairly strong oxidizer, and thus excess SeVI in the diet can be reduced (i.e. catabolised) to selenite or elemental Se. As a consequence, the CAc diet exhibited significant antiatherogenic properties. In fact, the CAc diet decreased the ${}_{\text{index}}\text{A}^{\text{SFA+Toc}}$ value in the pancreas compared to the control and CAcSeVI diets. Thus, our present results are in line with findings of Mathur et al. (2015), who showed that dietary tocopherols, particularly γ-T, played important roles in the prevention of inflammatory symptoms and the pathophysiology of atherosclerosis-related cardiovascular diseases. On the other hand, the CAcSeVI diet reduced Σall-Ts (including γ-T) concentrations in the pancreas compared to the experimental diet containing CAc, irrespective of the presence of SeYe. As a consequence, the experimental diet with CAc and SeVI increased the ${}_{\text{index}}\text{A}^{\text{SFA+Toc}}$ value in the pancreas in comparison to the CAc and CAcSeYe diets. Taking this into account, we concluded that the modified atherogenic index (i.e. ${}_{\text{index}}\text{A}^{\text{SFA+Toc}}$; Table 6) better predicted the process of atherogenesis than the atherogenic index (${}_{\text{index}}\text{A}^{\text{SFA}}$; Table 3); the ${}_{\text{index}}\text{A}^{\text{SFA+Toc}}$ provided more detailed insights into the mechanisms of atherogenesis and therefore possessed better predictive properties. Indeed, the ${}_{\text{index}}\text{A}^{\text{SFA+Toc}}$ value showed that tocopherols (effective antioxidants) decreased the risk of atherogenesis (Saini et al., 2012, Salvayre et al., 2016).

Our current and previous studies also revealed that the experimental diet supplemented with CAc alone increased PUFA peroxidation in the pancreas and liver (Rozbicka-Wieczorek et al., 2016b), as the values of PUFA peroxidation indices (${}^{\text{PUFA}}\text{MDA}_{\text{index}}$ and ${}^{\text{PUFALHPUFA}}\text{MDA}_{\text{index}}$) increased most significantly in the pancreas of lambs fed the CAc diet (Table 6). In fact, previous studies with dietary CAc demonstrated an increase in the content of aldehydes (i.e. volatile species from lipid autoxidation) in meat of lambs fed diets containing higher CAc levels (≥ 1 g/kg diet) (Morán et al., 2013). Moreover, recent research has indicated that dietary CAc stimulates protein oxidation (Afonso et al., 2013; Raes et al., 2015). These findings could be attributed to the high instability of CAc observed during digestion experiments (Raes et al., 2015).

Conclusions

The current study is important to understand the influence of dietary CAc, SeYe and SeVI on the content of FA, tocopherols, T-Ch and the extent of PUFA peroxidation in the pancreas of lambs. Inorganic Se (SeVI) or organic Se (SeYe) added to the experimental diet differently modified LW, BWG,

FCE and the concentration of FA, tocopherols and T-Ch in the pancreas. The experimental diet with CAc, irrespective of the presence of SeYe, reduced atherogenesis in the pancreatic tissue. Thus, the CAc and CAcSeYe diets protected against injuries associated with chronic inflammation, as well as stimulated repair of blood vessel walls in the pancreas. Moreover, the CAcSeVI diet, and especially the CAc diet, stimulated fatty acid biosynthesis in the pancreas compared to the control and CAcSeYe diets.

The present study provides useful knowledge for nutritionists carrying out further research aimed at improving the health and welfare of farm animals.

Conflict of interest

The Authors declare that there is no conflict of interest.

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