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Dietary selenite and conjugated linoleic acid isomers influence fatty acid concentrations in the liver and femoral muscles of rats*

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ABSTRACT

The aim of the study was to determine the influence of diets containing 1.5% conjugated linoleic acid isomers (CLAmix) and/or a high or low level of selenite (0.2 ppm Se ($_{L}$Se) or 0.5 ppm Se ($_{H}$Se)) on the body weight gain (BWG), feed conversion efficiency (FCE), and concentrations of CLA isomers and other fatty acids (FA) in the liver and femoral muscle of female rats fed *ad libitum* these experimental diets for 6 weeks. It was found that the diet containing the CLAmix and 0.5 ppm Se ($_{H}$Se) resulted in the highest increase in BWG and FCE compared with values for BWG and FCE of all other groups. This diet most efficiently increased the concentration of Se in the liver and muscles. The content of CLA isomers in muscles was ~20-fold higher than in the liver. There were usually no differences in the concentrations of *c9t11*CLA, *t10c12*CLA and the sum of CLA isomers in the liver and muscles of rats fed the diet with CLAmix with or without $_{H}$Se, whereas these values in the liver and muscles were usually lower for rats fed the diet enriched in CLAmix and 0.2 ppm Se ($_{L}$Se) compared with those fed the diet with CLAmix. The diets enriched in CLAmix and/or Se (as $_{L}$Se or $_{H}$Se) caused a decrease in the Δ9-desaturase index only in the liver. The current study shows that the highest values of the Δ4-, Δ5- and Δ6-desaturase indexes were in muscles of rats fed the diet enriched in CLAmix. This diet most efficiently increased the value of the Δ4-desaturase index in the liver, the desaturase responsible for the most efficient increase in the content of *c4c7c10c13c16c19*C22:6n-3 in the liver. Our study indicates that dietary CLAmix stimulated β-oxidation of FA in skeletal muscles, while promoting the accumulation of FA, particularly n-3 polyunsaturated fatty acids (PUFA), in the liver. The diet enriched in CLAmix and $_{H}$Se assured satisfactory ratios of PUFA to saturated FA and PUFA to the sum of all assayed FA in the liver and

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muscles. These findings are valuable information for nutritionists carrying out research to improve the nutritive value of food for human health. Further studies are necessary to thoroughly investigate the effects of dietary CLA isomers as well as interactions of CLA isomers and Se compounds on the human health.

KEY WORDS: rats, selenite, CLA isomers, liver, femoral muscles, fatty acids, desaturases, elements, atherogenic index, thrombogenic index

INTRODUCTION

Food is the main source of selenium (Se) and intake of this essential element depends on its content in diets and the amount of food consumed (Navarro-Alarcon and López-Martínez, 2000; Czauderna et al., 2009). Recent studies (Surai, 2004; Boosalis, 2008; Navarro-Alarcon and Cabrera-Vique, 2008) have indicated that an adequate adult human diet should have at least 40 μg/day of Se to support the maximum expression of Se-enzymes and that supranutritional intake of Se (300 μg/day) appears to decrease cancer risk. Deprivation of Se is associated with reduced antioxidant protection, energy production and redox regulation as a consequence of suboptimal expression of many of the Se-cysteine-containing enzymes (Surai, 2004, 2006; Thomson, 2004). Previous studies have also found that dietary Se protects animals against toxicity associated with high exposure to heavy metals like mercury, lead, cadmium or silver (Czauderna et al., 1995; Cabrera et al., 1996; Cabañero et al., 2007; Mousa et al., 2007).

Many Se-compounds exist in animal, plant tissues, and diets (Rayman, 2004; Gammelgaard et al., 2008). Selenate and selenite incorporate directly into the Se pool when used in the synthesis of specific Se-proteins (like Se-cysteine- or Se-methionine-proteins) and Se-containing proteins, independent of their origin (animal or vegetable) (Thomson, 2004; Navarro-Alarcon and López-Martínez, 2000; Navarro-Alarcon and Cabrera-Vique, 2008). In general, the mammals metabolize various dietary Se-compounds into selenide (as HSe^-), which seems to be the common point for regulating Se metabolism, and especially of Se-cysteine (Se-Cys) containing-proteins (Se-Cys-proteins) (Navarro-Alarcon and López-Martínez, 2000). Recent studies have found that Se as selenite (SeIV) added to diets is more efficiently accumulated in mammalian organisms than dietary Se as selenate (Navarro-Alarcon and Cabrera-Vique, 2008). Moreover, dietary Se compounds can be metabolized to Se-Cys, which is an essential part of Se-Cys-proteins. Se-Cys-proteins play a key role in mammalian oxidative defense by virtue of its incorporation into antioxidant enzymes such as Se-Cys, which, is the redox active site of Se-enzymes (Tapiero et al., 2003; Boldizarova et al., 2005). Currently, about thirty Se-proteins have been indentified in mammals,

but the physiological function of only less than half of them is well known. Among them, the best understood Se-Cys-protein appears to be cytosolic glutathione peroxidase, which functions as an antioxidant by removing reactive oxygen substances (Boldižárová et al., 2005). In agreement with the above, our recent studies revealed that dietary organic-Se (Czauderna et al., 2007) or inorganic-Se (Czauderna et al., 2004a) stimulated the accumulation of unsaturated fatty acids (UFA), conjugated linoleic acid (CLA) isomers in particular. Several studies have also documented that CLA isomers also act as effective antioxidants, while only a few investigations revealed CLA isomers' ability to increase fatty acid (FA) oxidation. Recent studies using rats also found that the concentration of polyunsaturated fatty acids (PUFA), especially in serum cholesterol esters and phospholipids, was also positively correlated with the Se concentration in the diet (Crespo et al., 1995).

Considering the above, we hypothesized that dietary SeIV and CLA isomers stimulated the yield of the accumulation of Se in the liver and femoral muscles, as well as in the concentration of CLA isomers and other UFA in the femoral muscle and liver of rats. Positive health effects attributed to Se-compounds (Surai, 2006; Boosalis, 2008) and CLA isomers, particularly *cis9trans11*CLA (*c9t11*CLA) in living organisms (Park and Pariza, 2007), essentially related to protection against oxidative stress, made it desirable to study the extent to which dietary Se compounds and CLA isomers may contribute to FA concentrations, especially CLA isomers and PUFAn-3 in the liver and femoral muscles of rats. Thus, the major objective of the current study was to investigate the influence of dietary Se as sodium selenite on the FA level in selected rat tissues. In our pilot study of interactions between CLA isomers and SeIV, rats were used as model animals for monogastric animals like pigs.

MATERIAL AND METHODS

Animals, housing, diets and sampling

The experiment was carried out on 48 female rats (Wistar, Hsd Brl Han: WIST), 8 weeks of age and with an initial body weight of about 195.4±0.8 g. The animals were housed and handled in accordance with protocols approved by the Local Animal Care and Use Committee (The Agricultural University of Warsaw, Poland). The animals were housed individually in plastic cages at a temperature of 22±1°C with a 12 h light-dark cycle and relative humidity of 50-60%. Each group comprised eight rats. The rats were fed the standard Labofeed H diet produced by

the Feeds and Concentrates Production Plant in Kcynia (Poland), according to the recommendations of Pastuszewska et al. (2000). For the current study, the standard Labofeed H diet was enriched in 0.2 ppm Se as selenite (Table 1). During the 7-day preliminary period the rats were fed a standard Labofeed H diet offered at sub-maintenance level to reduce the rats' body fat (Table 2). Next, the rats were fed *ad libitum* for 6 weeks the experimental diets supplemented with 1.5% CLA isomer mixture (CLAmix), 0.2 ppm ($_{L}$Se) or 0.5 ppm ($_{H}$Se) Se as Na_2SeO_3 (Table 2). Feed intake and body weight of rats were measured weekly. The rats were killed at the end of the six week experiment. The liver and femoral muscles were removed, weighed, and frozen. Fatty acids (FA), selenium (Se), zinc (Zn), iron (Fe), calcium (Ca) and magnesium (Mg) were analysed in the liver and muscles. All tissues samples were analysed individually. The concentrations of all FA and elements were calculated based on freeze-dried liver and muscle samples.

Table 1. Chemical composition and energy content of the basal diet (Pastuszewska et al., 2000)

Item	Labofeed H[1] g/100 g diet
Dry matter[2]	88.2 ± 0.9
In dry matter	
crude protein	21.8 ± 1.3
lysine	1.31
methionine and cysteine	0.76
tryptophan	0.28
threonine	0.87
crude fibre	3.85
crude fat	3.0 ± 0.8
ash	5.9 ± 0.6
N total	3.77
N protein	3.17
P total	0.75
nono-carbohydrates[3]	5.75
starch	30.3
Energy value[4], MJ, ME/kg	13.9

[1] means of 9 samples; ingredients: maize, wheat, oat flakes, green meal, soyabean oilmeal, fish meal, soya oil, vitamins (per kg diet; IU: vit. A 10 096, vit. D_3 2000; mg: vit. E 86.1, vit. K_1 3, vit. B_1 15.7, vit. B_2 16.0, vit. B_6 5.24, vit. B_{12} 81, biotin 0.2, folic acid 3.03, nicotinic acid 79.3, pantothenic acid 25.5; g: choline 2.02; minerals (contained per g diet; mg: Na - 3.60, K - 8.30, Ca - 10.68; P - 7.60) and trace elements (contained per g diet; mg: Se as Na_2SeO_3 - 0.2, Cu - 13.9, Zn - 98, Mn - 112, Fe - 698, Mg - 1653); [2] the concentrations of main fatty acids, (μg per g DM diet: C8:0 - 37; C10:0 - 6; C12:0 - 11; *cis9cis12cis15* C18:3 (αLNA) - 8; *cis6cis9cis12*C18:3 (γLNA) - 715; C14:0 - 11; *cis9cis12*C18:2 (LA) - 429; C16:0 - 250; *cis9*C18:1 - 187; *cis6*C18:1 - 112; C18:0 - 89; C20 - 11; C22 - 2.4; Σsaturated fatty acids (SFA) - 417; Σpoly-unsaturated fatty acids (PUFA) - 1499; Σassayed fatty acids (ΣFA) - 1915; [3] compounds with chemical formula: $C_nH_{2n}O_n$, where n=5 or 6; [4] the mean from 3 samples

Reagents and analytical methods

All chemicals were analytical grade and organic solvents were of HPLC grade. Dichloromethane (DCM), KOH, NaOH, Na_2SO_4 and conc. HCl were purchased from POCH (Gliwice, Poland). Acetonitrile, methanol, and n-heptane (99%, GC) were supplied by Lab-Scan (Ireland), while the CLA isomer mixture (2.1% *tt*CLA, 7.1% *c11t13*CLA, 40.8% *c9t11*CLA, 41.3% *t10c12*CLA, 6.7% *c8t10*CLA and 2.0% *cc*CLA) by Industrial Chemistry Research Institute (Warsaw, Poland). The concentration ratio ($R_{c9t11\text{CLA}/t10c12\text{CLA}}$) of *c9t11*CLA to *t10c12*CLA in the dietary CLA isomer mixture was 0.9879. Fatty acid methyl ester (FAME) standards and 25% BF_3 in methanol were purchased from Supelco and Sigma (USA).

Water used for the preparation of mobile phases and chemical reagents was prepared using an Elix™ water purification system (Millipore). The mobile phases were filtered through a 0.45 μm membrane filter (Millipore).

Saponification and fatty acid extraction

The liver and muscles were frozen, lyophilized and the obtained residue was stored at -20°C until assayed. Finely powdered biological samples (~50 mg) were placed in vials and treated with a mixture of 2 ml of 2 M KOH in water and 2 ml of 1 M KOH in methanol. Next, 50 μl of the internal standard (IS) solution (17 mg ml^{-1} nonadecanoic acid in chloroform) were added to the obtained mixture. The resulting mixture was flushed with argon (Ar) for ~4 min. The vial was then sealed and the mixture vortexed and heated under Ar at 95°C for 10 min, cooled for 10 min at room temperature, and sonicated for 10 min. The resulting mixture was protected from the light and stored in the sealed vial under Ar at ~22°C overnight. Next, 3 ml of water were added to the hydrolysate and the solution was again vortexed. The obtained solution was acidified with 4 M HCl to ~pH 2 and free fatty acids were extracted four times with 3 ml of DCM. Extraction was repeated 4 times using 3 ml of n-hexane. The upper n-hexane layer was combined with the DCM layer, and next the resulting organic phase was dried with ~0.1 g of Na_2SO_4. The organic solvents were removed under a stream of Ar at room temperature. The obtained residue was stored at -20°C until base- and acid-catalyzed methylation.

Preparation of fatty acid methyl esters (FAME) and element analysis

Two ml of 2 M NaOH in methanol were added to the residue while mixing, then flushed with Ar, and reacted for 1 h at 40°C. After cooling the reaction mixture to ~4°C, 2 ml of 25% BF in methanol were added, flushed with Ar, and heated for 1 h at 40°C. To the cooled reaction mixture 5 ml of water were added and then

FAME were extracted with 5 ml of n-hexane. The supernatant was transferred to a GC vial.

The Se concentration in the liver and muscles was analysed by the fluorimetric method of Rodriguez et al. (1994), while the levels of Zn, Fe, Mg and Ca in lyophilized liver and muscle samples were determined by flame (air-acetylene) atomic absorption spectrometry (PU9100X Atomic Absorption Spectrometer, UNICAM, Philips) (Czauderna et al., 2007).

Analytical equipment

The analyses of all FAME were performed on a SHIMADZU GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica capillary column (120 m × 0.25 mm i.d. × 0.25 μ m film thickness; SHIM-POL, quadrupole mass selective (MS) detector (Model 5973N) and injection port. Helium as the carrier gas operated at a constant pressure (223.4 kPa) and flow rate of 1 ml/min. Injector and MS detector temperatures were maintained at 200 and 240ºC, respectively. The total FAME profile in a one μl sample at a split ratio of 10:1 was determined using the column temperature gradient programme. The column was operated at 70°C for 4 min, then the temperature programmed at 12°C/min to 150°C, held for 6 min, programmed at 8°C/min to 168°C, held for 27 min, programmed at 0.75°C/min to 190°C, held for 10 min, programmed at 1.8°C/min to 210°C, held for 15 min, programmed at 6°C/min to 234°C, held for 4 min, programmed at 6°C/min to 236°C, held for 20 min.

FAME identification was validated based on electron impact ionization spectra of FAME and compared with authentic FAME standards and NIST 2007 reference mass spectra library.

Statistical analyses

Results are presented as means of 8 individually analysed samples of liver and muscles. Mean values in columns having the same superscripts are significantly different at $^{a,b}P<0.05$ and $^{A,B}P<0.01$, while differences at $^{\alpha,\beta}P=0.1$ are indicated as tendencies. These one-factorial statistical analyses of the effects of SeIV or the CLA isomer mixture in the diets were conducted using the non-parametric Mann-Whitney U test for comparing independent experimental groups. Statistical analyses of the interactions between CLAmix and $_{L}$Se or $_{H}$Se (i.e. CLA x $_{L}$Se and CLA x $_{H}$Se) were performed using two factorial ANOVA analysis; interactions of CLAmix x $_{L}$Se and CLAmix x $_{H}$Se were significant at the $^{x,y}P<0.05$ and $^{X,Y}P<0.01$ levels, respectively. Statistical analyses were performed using the Statistica v. 6 software package (2002; www.statsoft.pl).

RESULTS AND DISCUSSION

The effects of experimental diets on rat weight and concentration of Se, Zn, Cu, Fe, Ca and Mg in liver and muscles. During the one-week preliminary period of submaintenance feeding on the control unsupplemented diet (9 g/day), all rats lost 16.0±1.2 g body weight. As expected, no macroscopic lesions or pathological changes were found in the liver, femoral muscles or in any other organs of rats fed the diets enriched in 1.5% CLAmix and/or 0.2 or 0.5 ppm SeIV. Indeed, our previous studies documented that diets enriched in 2 ppm of Se as selenate (Czauderna et al., 2004a) or 1.2 ppm Se as selenized yeast (Czauderna et al., 2007, 2009) would not be toxic for rats because only chronic feeding of inorganic Se compounds at a rate of more than 5 ppm can be hepatotoxic and teratogenic in animals and humans (Tapiero et al., 2003). In the current study, it has been shown (Table 2) that the body mass gain of rats fed the diet enriched in $_{L}$Se or $_{H}$Se resulted in an increase ($P<0.05$) in body weight gain (BWG) in comparison with the control group ($P=0.023$ and $P=0.028$, respectively). On the other hand, no significant differences ($P>0.05$) in BWG were found between the control and experimental rats fed CLAmix with ($P>0.05$) or without ($P>0.05$) $_{L}$Se and $_{H}$Se, although values for BWG were greater for animals fed the diet enriched in CLAmix regardless of the presence of $_{L}$Se. The diet containing CLAmix and $_{H}$Se resulted in the highest BWG increase compared with values for BWG of all other groups, however, no significant differences ($P>0.05$) in these values were found. This is consistent with the findings that addition of $_{H}$Se to the diet with CLAmix significantly increased ($P<0.05$) the BWG of rats compared with rats fed the diet containing only CLAmix.

Feeding the diet containing CLAmix and SeIV increased FCE (g/g) in rats (the interaction CLA x $_{L}$Se and CLA x $_{H}$Se significance: $P<0.05$) in comparison with the control rats and rats fed the diet containing CLAmix, $_{L}$Se or $_{H}$Se (Table 2). Similar results were observed when selenate (2 ppm Se) or selenized yeast (1.2 ppm Se) were present in the rats' diets enriched in 1% *t10c12*CLA or 2% CLA isomer mixture (Czauderna et al., 2003; Korniluk et al., 2007). Although there were no statistical differences ($P>0.05$) between FCE in rats fed the diets enriched in SeIV, these values were greater for rats fed the diet with $_{L}$Se or $_{H}$Se. Similarly, there were no statistical differences between liver fresh mass in rats fed the diets containing CLAmix and/or SeIV (as $_{L}$Se or $_{H}$Se), however, this value was lowest for animals fed the diet enriched in $_{L}$Se, while highest for the $_{H}$Se+CLA rat group. Interestingly, these changes in liver masses positively correlated with the total concentration of all assayed fatty acids (ΣFA) in the rat livers (Table 3).

Table 2. Dietary effects of 1.5% CLA isomer mixture (CLAmix) and sodium selenite ($_{L}$Se and $_{H}$Se) on the feed conversion efficiency (FCE; g body mass gain/g feed intake)[1], body weight gain (BWG)[1] and liver fresh mass (g) of rats and the concentrations[2] (ppm) of Se, Zn, Cu, Fe, Mg, Ca in the liver and femoral muscles of rats after 6 weeks feeding with experimental diets

Group	Additives	Body weight, g		BWG	FCE	Liver mass	Liver						Femoral muscles[4]				
		initial	adapted[3]	g	g/g	g	Se	Zn	Cu	Fe	Ca	Mg	Se	Zn	Fe	Ca	Mg
Control[5]	-	196.0	180.4	50.0^{ab}	0.068	8.718	5.1^{α}	120	6.3^{AB}	1036^{α}	$206^{ab\alpha}$	580^{A}	0.28^{Aa}	49	68	246^{a}	869
$_{L}$Se	0.2 ppm Se	194.7	176.9	53.5^{a}	0.071^{a}	8.251	5.2^{a}	124	7.2	991^{β}	160^{α}	560^{B}	0.29^{B}	50	61^{A}	190	873^{a}
$_{H}$Se	0.5 ppm Se	195.7	180.1	57.1^{b}	0.074^{c}	8.723	5.4^{b}	122^{α}	9.1^{AC}	1113^{α}	120^{a}	616^{C}	0.41^{ab}	49	68^{a}	154^{a}	853
CLA	CLAmix	196.5	181.7	51.9^{c}	0.069^{b}	8.486	$5.5^{\alpha c}$	128	$10.2^{BD\alpha}$	1061	144^{b}	682^{A}	0.56^{A}	46	78	219^{b}	836
$_{L}$Se+CLA[6]	0.2 ppm Se CLAmix	195.3	180.3	54.8	0.075^{abx}	8.495	5.8^{ax}	130	8.2^{α}	1078^{β}	142	704^{B}	0.58^{B}	52	92^{A}	202	819^{a}
$_{H}$Se+CLA[6]	0.5 ppm Se CLAmix	194.7	177.0	59.9^{c}	0.081^{cy}	8.800	6.1^{bcy}	129^{α}	12.6^{CD}	1097	190	693^{C}	0.61^{b}	52	79^{a}	164^{b}	848

[1] - BWG and FCE after feeding for 6 weeks days with the experimental diets enriched in CLAmix and/or SeIV

[2] - the concentrations of assayed elements in dry mass (DM); the liver and femoral muscle samples were freeze-dried

[3] - the body weight (g) of individually adapted rats after 7 days of submaintenance feeding (9 g/the Labofeed H diet/a day/a rat)

[4] - the concentration of Cu in the muscles was below the quantification limit

[5] - means in columns sharing the same letter are significantly different: ^{a,b} $P<0.05$ and ^{A,B} $P<0.01$

[6] - interactions of CLAmix x $_{L}$Se and CLAmix x $_{H}$Se, significant at ^{x,y} $P<0.05$ and ^{X,Y} $P<0.01$, respectively

Table 3. The effect of the diets enriched in CLAmix and 0.2 ppm and 0.5 ppm Se as sodium selenite ($_{L}$Se and $_{H}$Se) on the concentration of CLA isomers[1], *cis9*C18:1, C18:0, arachidonic acid (AA), PUFAn-3, PUFAn-6, MUFA, SFA, ΣFA and on values of Δ9-desaturase index, atherogenic and thrombogenic indexes and the concentration ratio of PUFAn-6/PUFAn-3 in the liver and femoral muscles of rats

Group	*c9t11*[1] mg/g	*t10c12*[1] mg/g	ΣCLA[8] mg/g	Δ9-index[2]	*cis9* C18:1 mg/g	index-A-SFA[3]	index-T-SFA[4]	*cis11* C18:1 mg/g	AA µg/g	PUFA n-3	PUFA n-6	n-6 n-3[5]	MUFA mg/g	SFA[6] mg/g	ΣFA[6] mg/g
Liver															
control	-[7]	-	-	0.16^{α}	2.19^{α}	0.29	0.69	0.67	4.79^{a}	3.76	12.08	3.21^{Aa}	3.07	13.82^{a}	32.74^{α}
$_{L}$Se	-	-	-	0.13	1.67^{α}	0.29^{α}	0.68	0.50	4.45^{α}	3.61	10.69	2.96^{a}	2.31	12.34^{b}	28.95^{a}
$_{H}$Se	-	-	-	0.14	1.93	0.28	0.65	0.56^{a}	4.74	4.19^{a}	11.35^{a}	2.70^{A}	2.69	13.37^{A}	31.61^{b}
CLA	0.18	0.087^{α}	0.27^{α}	0.11^{α}	1.89	0.34	0.63	0.53	$1.96^{\beta a}$	6.01	9.19	1.53^{α}	2.49	16.43^{a}	34.30^{α}
$_{L}$Se+CLA	0.14	0.077^{α}	0.22^{α}	0.12	1.84	0.32^{α}	0.70	0.40	$4.38^{\alpha\beta}$	4.43	10.61	2.39	2.29	14.58^{b}	32.06^{a}
$_{H}$Se+CLA	0.18	0.083	0.26	0.11	1.69	0.28	0.63	0.40^{a}	5.21	4.94^{a}	12.77^{a}	2.59^{α}	2.11	14.74^{A}	34.75^{b}
Femoral muscles															
control	-	-	0.30	0.47^{ab}	17.33	0.33^{α}	0.32^{A}	2.79	1.44^{A}	12.97	23.64	1.82^{A}	20.52	20.56	83.36^{α}
$_{L}$Se	-	-	0.30	0.46^{ac}	14.12	0.34^{A}	0.32^{B}	2.35^{α}	1.36^{B}	11.31^{a}	20.23	1.79^{B}	16.77	17.80	70.60
$_{H}$Se	-	-	0.28	0.45^{b}	13.00	0.34^{a}	0.33^{C}	2.16	1.24^{C}	10.30^{b}	18.60	1.80^{C}	15.41	16.55	64.93^{α}
CLA	3.17	1.95^{a}	5.79	0.46^{d}	15.63	$0.36^{b\alpha}$	0.39^{A}	2.35^{a}	0.67^{ADa}	9.46	21.26	2.25^{A}	18.30	19.40	76.62
$_{L}$Se+CLA	2.68^{α}	1.70	5.07^{α}	0.44^{cd}	13.10	0.37^{Ab}	0.40^{B}	$1.71^{\alpha a}$	0.86^{Ba}	8.39^{a}	18.45	2.20^{B}	15.22	17.39	66.64
$_{H}$Se+CLA	3.19^{α}	1.62^{a}	5.99^{α}	0.46	14.46	0.36^{a}	0.38^{C}	2.07	0.93^{CD}	9.20^{b}	20.30	2.21^{C}	17.22	18.38	73.24

[1] - *c9t11*: *c9t11*CLA; *t10c12*: *t10c12*CLA; ΣCLA: the concentration sum of *c9t11*CLA, *t10c12*CLA, *c11c13*CLA, *c11t13*CLA, *t7c9*CLA, *c9c11*CLA and *tt*CLA; [2] - Δ9-desaturase index: (*c9*C14:1 + *c9*C16:1 + *c9*C18:1)/(*c9*C14:1 + *c9*C16:1 + *c9*C18:1 + C14:0 + C16:0 + C18:0); [3] - atherogenic index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFAn-6 + PUFAn-3) (Ulbricht and Southgate, 1991); [4] - thrombogenic index: (C14:0+C16:0+C18:0)/0.5*MUFA + 0.5*PUFAn-6 + 3*PUFAn-3 + PUFAn-3/PUFAn-6) (Ulbricht and Southgate, 1991); [5] - the concentration ratio of PUFAn-6/PUFAn-3; [6] - SFA: the concentration sum of saturated fatty acids (from C8:0 to C24:0); ΣFA - the concentration sum of all assayed fatty acids; [7] - below the quantification limit; [8] - the concentration ratios ($R_{c9t11CLA/t10c12CLA}$) in the liver: CLA group - 2.0689; $_{L}$Se+CLA group - 1.8182; $_{H}$Se+CLA group - 2.1687; the concentration ratios ($R_{c9t11CLA/t10c12CLA}$) in muscles: CLA group - 1.6256; $_{L}$Se+CLA group - 1.5765; $_{H}$Se+CLA group - 1.9691

The experimental diets changed significantly ($P<0.05$) or only numerically ($P>0.1$) the concentrations of Se, Zn, Fe, Cu, Mg and Ca in the liver and muscles of rats (Table 2). We found that the diets enriched in LSe or HSe increased the concentrations of Se in the liver and muscles, although only the diet with HSe significantly elevated ($P<0.05$) the concentration of Se in muscles. Surprisingly, the diet containing CLAmix increased the concentration of Se in the liver (a tendency: $P=0.067$) and muscles ($P<0.05$). Data from this study documented that dietary 1.5% CLAmix revealed antioxidative properties in the liver and muscles, reflecting better incorporation of Se into liver and muscle tissue proteins. Indeed, selenite added to diets is taken up through the small intestine and then either methylated (i.e. excess of Se), followed by excretion, or assimilated *via* selenide into Se-Cys and subsequently incorporated into Se- Cys-proteins *via* the UGA codon (Surai, 2004, 2006). As can be seen from the current study, dietary CLAmix stimulated selenite-metabolism into proteins containing Se-Met or Se-Cys, reflecting more efficient incorporation Se in the liver and muscles compared with the control rats and rats fed the diet enriched in $_{L}$Se or $_{H}$Se. Consequently, the addition of CLAmix to the diets containing $_{L}$Se or $_{H}$Se increased ($P<0.05$) the concentration of Se in both tissues compared with rats fed the diet enriched in only $_{L}$Se or $_{H}$Se. Thus, in contrast to selenate (Czauderna et al., 2003, 2004a) and Se as selenized yeast (Czauderna et al., 2007, 2009), selenite added to the diet with CLAmix is a source of Se more efficiently assimilated into the liver and muscles of rats.

Dietary $_{H}$Se highly significantly increased ($P<0.01$) the concentration of Cu in the liver compared with the control rats, although the value of the concentration of Cu was also greater in rats fed the diet with $_{L}$Se. The addition of CLAmix to the diet with $_{H}$Se elevated the concentration of Cu in the liver ($P<0.01$) compared with the control rats and rats fed the diet containing CLAmix. Thus, the presented results document that dietary Se and CLAmix stimulated the incorporation of Cu into the liver in dose-dependent manner.

Dietary SeIV, especially $_{H}$Se, decreased the concentration of Ca in the liver and muscles compared with the control rats. The addition of CLAmix to the diet enriched in SeIV usually decreased the effect of SeIV on the concentration of Ca in both tissues. On the other hand, supplementing CLAmix to the diet enriched in $_{L}$Se or $_{H}$Se resulted in an increase in the concentration of Mg in the liver in comparison with $_{L}$Se and $_{H}$Se experimental ($P<0.01$) and control rats. There were no appreciable differences in the concentration of Zn in the liver and muscles between rats fed the diets enriched in $_{L}$Se, $_{H}$Se or CLAmix, but the Zn concentration increased in the liver of rats fed the diet containing CLAmix and SeIV (i.e. $_{L}$Se, $_{H}$Se) compared with the control rats and rats fed the diets with $_{L}$Se or $_{H}$Se.

Although there were no significant differences in the concentration of Fe in the liver and muscles of rats fed the diet containing $_{L}$Se, $_{H}$Se or CLAmix, the Fe

concentrations in the liver and muscles were usually greater in rats fed the diet enriched in CLAmix and SeIV (i.e. $_{L}$Se, $_{H}$Se) compared with other groups.

Fatty acid concentrations in the liver and muscles of rats fed experimental diets. As expected, the experimental diets containing CLAmix, regardless of the presence of SeIV ($_{L}$Se or $_{H}$Se), resulted in a significant increase in the concentration of *c9t11*CLA, *t10c12*CLA and concomitant increase the concentration sum of CLA isomers (ΣCLA) in the liver and muscles (Table 3). The concentration of CLA isomers in muscles was ~20-fold higher than in the liver (Table 3). Although there were no considerable differences in the concentrations of both isomers and ΣCLA in the liver and muscles of rats fed the diet containing CLAmix and $_{H}$Se, the values of the concentration in the liver and muscles were usually lower for rats fed the diet enriched in CLAmix and $_{L}$Se compared with rats fed the diet enriched in CLAmix. Surprisingly, the diet enriched in CLAmix and $_{H}$Se resulted in a increase in the concentration of *c9t11*CLA and ΣCLA in muscles in comparison with rats fed the diet with CLAmix $_{L}$Se. In the current study, the concentration ratios of *c9t11*CLA to *t10c12*CLA in the liver (from 2.169 to 1.818) and muscles (from 1.969 to 1.576) of rats fed the diets enriched with CLAmix were, regardless of the presence of SeIV, higher (Table 3) compared with the concentration ratio of these isomers (i.e. $R_{c9t11\text{CLA}/t10c12\text{CLA}} = 0.9879$) in the CLA isomer mixture added to the rats' diets. Thus, our current results are in agreement with those of Alasnier et al. (2002) and our previous studies (Czauderna et al., 2003, 2004, 2007, 2009; Korniluk et al., 2007) in which the *t10c12* and *t10t12* isomers were also more efficiently driven through β-oxidation in the cells of muscles, kidneys, adipose tissue or liver than their *9,11* homologues. Moreover, the addition of the lower amount of SeIV to the diet with CLAmix slightly decreased the $R_{c9t11\text{CLA}/t10c12\text{CLA}}$ value in the liver (1.818) and muscles (1.576) (Table 3). Interestingly, our experiment demonstrated that the addition of the higher amount of SeIV (a strong oxidant) to the diet with CLAmix ($_{H}$Se+CLAmix) stimulated driving *t10c12*CLA through the β-oxidation pathway in comparison with the CLA group or rats fed the diet enriched in CLAmix and the lower amount of SeIV. On the other hand, the diet enriched in CLAmix and $_{H}$Se showed a tendency ($P<0.01$) to increase the concentration of *c9t11*CLA and ΣCLA in muscles and increased these values in the liver compared with the $_{L}$Se+CLA group.

The experimental diet enriched in $_{L}$Se or $_{H}$Se decreased the Δ9-desaturase index (Δ9-index) in the liver (Table 3), therefore, it also decreased the concentration of *c9*C18:1 in the liver and muscles. Considering these results and our previous studies (Korniluk et al., 2007), we could argue that dietary inorganic Se (as selenite (SeIV) and selenate) and organic Se (i.e. selenized yeast) revealed a similar influence on the capacity of Δ9-desaturation. Similarly, diets containing CLAmix, regardless of the addition of SeIV (selenite), resulted in a decrease in the Δ9-desaturase index

(Δ9-index) in the liver, consequently, decreased the concentration of *c9*C18:1 in the liver and muscles. Moreover, these values were smaller in comparison with the Δ9-index in the liver of rats fed the diets containing $_{L}$Se or $_{H}$Se. Our current investigations are thus consistent with our previous studies (Czauderna et al., 2003, 2004a), which also documented that dietary 2 ppm inorganic Se as selenate or 1.2 ppm Se as Se-Y and especially 1-2% CLA isomer(s) decreased the capacity of Δ9-desaturase in the liver and other organs of rats. The decrease in the capacity of Δ9-desaturase in rat liver and other organs is probably due to inhibited stearoyl-CoA desaturase mRNA expression (Alasnier et al., 2002; Korniluk et al., 2007). The results presented herein are in agreement with other studies documenting that mRNA expression for Δ9-desaturase and enzyme activity is known to be strongly modulated by several nutrients (like PUFA or fructose), drugs (e.g., sterculic acid), or hormones such as leptin and insulin (Waters et al., 2009). Moreover, recent studies showed that the half-life of Δ9-desaturase mRNA was decreased in a dose-dependent manner by dietary PUFAn-6 (e.g., LA or AA) (Waters et al., 2009).

The experimental diets, especially enriched in only SeIV, resulted in minute changes in values of the atherogenic index (A-SFA index) in the liver and muscles of rats, although the diet enriched in CLAmix increased the A-SFA index in the liver (+17%) and muscles (+10%) compared with the control rats (Table 3). Interestingly, the addition of CLAmix to the diet containing $_{L}$Se showed a tendency to increase (P=0.09) the A-SFA index in the liver compared with the $_{L}$Se group and numerically in muscles compared with the $_{L}$Se group (+9%) and the control rats (+12%). Similarly, the diets containing CLAmix, regardless of the addition of $_{L}$Se or $_{H}$Se, resulted in an increased T-SFA index values in the muscles (19-25%), while no noticeable influence was found in the liver of rats in comparison with the control rats.

So, the above results confirm our recent studies concerning the A-SFA and T-SFA indexes in femoral muscles of rats fed diets with CLA isomers and organic Se as Se-Y (Czauderna et al., 2009). In summary, our current studies suggest that dietary CLA isomers and inorganic Se as selenite or organic Se as Se-Y increased values of the A-SFA and T-SFA indexes in muscles of monogastric animals.

The diets enriched in CLAmix, $_{L}$Se or $_{H}$Se decreased the concentration of *c11*C18:1 in the liver and muscles in comparison with the control group (Table 3). Furthermore, the addition of CLAmix to the diet containing $_{L}$Se or $_{H}$Se amplified the decrease in the *c11*C18:1 concentration in both tissues. This effect could be related to the increased yield of fatty acid β-oxidation by dietary SeIV, and especially CLA isomers. Indeed, the current results and our previous studies (Czauderna et al., 2004a, 2007) documented that dietary CLA isomer mixture (1 or 2%) in rat diets tended to reduce MUFA and SFA in muscle, especially saturated fatty acids (SFA) containing from 10 to 18 carbon atoms, most probably due to an increase

in the rate of lipolysis and enhanced fatty acid β-oxidation in the skeletal muscles of the experimental animals fed the mixture of the CLA isomers (Alasnier et al., 2002; Park and Pariza, 2007). Based on the concentration sums of all assayed fatty acids (ΣFA) in muscles (Table 3) and other studies (Alasnier et al., 2002) we argue that the anti-obesity effect of a CLA isomer mixture, *t10c12*CLA in particular, was documented in rats fed diets enriched in the CLAmix with or without SeIV (as $_{L}$Se or $_{H}$Se).

The diet enriched in CLAmix resulted in a decrease in the concentration of arachidonic acid (AA) in the liver ($P<0.05$) and muscles ($P<0.01$) in comparison with the control rats (Table 3). Moreover, the addition of $_{L}$Se or $_{H}$Se to the diet containing CLAmix also led to a decrease in the AA concentrations in muscles compared with the control rats (41 and 36%, respectively). Our recent studies (Czauderna et al., 2003, 2004a,b, 2007) on the fatty acid metabolism also reinforce the finding that CLA isomers could be metabolized *in vivo* into long-chain conjugated PUFA (elongation and desaturation products of CLA) using the same enzymes (i.e. elongase and desaturases) as linoleic acid (LA) (Alasnier et al., 2002; Park and Pariza, 2007). Thus, competition of CLA isomers with LA for the same enzymes resulted in a lower yield of AA formation in both tissues. Moreover, CLA isomers, especially *c9t11*CLA and *t10c12*CLA, are incorporated into the sn-2 position of phospholipids. Since the sn-2 position of phospholipids is the primary location for AA as well (Park and Pariza, 2007), it was not surprising to observe the negative correlation between CLA isomers and AA in tissues of the studied animals. Thus, the above results indicate that dietary SeIV and, especially, CLA isomers can inhibit cyclooxygenase activity, which is the rate-limiting enzyme for prostaglandin synthesis (Park and Pariza, 2007).

The above results are consistent with our results regarding the concentration of PUFAn-3, PUFAn-6 and the concentration ratio of PUFAn-3 to PUFAn-6 in the liver of rats fed the diets enriched in CLAmix. One explanation for this influence of dietary CLA isomers is that CLAmix competed in particular with LA and its metabolites (i.e. PUFAn-6) for the same enzymes (i.e. elongase and desaturates), which can result in decreasing in the concentration long-chain PUFAn-6, like AA in the liver (Park and Pariza, 2007). Dietary CLA isomers stimulated PUFAn-3 (especially α-LNA anabolites) accumulation in the liver as well as the liver concentration of SFA (Table 3) due to inhibiting stearoyl-CoA desaturase activity (Park and Pariza, 2007). Concomitantly, values of the concentration of MUFA in the liver of rats fed the diet containing CLAmix, regardless of the addition of $_{L}$Se or $_{H}$Se were lower in comparison with the control group, as the diets containing CLAmix resulted in a decrease in the value of the Δ9-desaturase index in the liver. Taken together, dietary CLAmix increased the concentration sum of FA (ΣFA) in the liver (Table 3), while the diet containing 1.5% CLAmix or SeIV, especially

$_{H}$Se, decreased the concentration of ΣFA in muscles. Moreover, there is also evidence in our previous studies of a negative relationship between dietary 1 and 2% CLA isomer mixture as well as 1.2 ppm Se, as selenized yeast (Czauderna et al., 2007) and 2 ppm Se as selenate (Czauderna et al., 2004a) on the concentration of ΣFA in the femoral muscles of rats. As can be seen from the results in Table 3, the diets with CLAmix, $_{L}$Se or $_{H}$Se, as well as diets containing CLAmix and SeIV, resulted in decreasing the concentration of PUFAn-3, PUFAn-6, MUFA and SFA in muscles, as in skeletal muscles, dietary CLAmix stimulated fatty acid oxidation. Indeed, *in vivo* studies (Alasnier et al., 2002; Park and Pariza, 2007) showed that fatty acid oxidation in muscles is directly linked to body fat use as energy. The current study also established a negative relationship between the concentration of *c9c12c15*C18:3 (αLNA), *c9c12*C18:2 (LA), C18:0, atherogenic (A-SFA) and thrombogenic (T-SFA) saturated fatty acids in muscles and the presence of extra CLAmix in the diet. Moreover, the addition of $_{L}$Se or $_{H}$Se to the diet enriched in CLAmix amplified a decrease in the concentration of these fatty acids in muscles in comparison with rats fed the diet enriched in CLAmix (Table 4). On the other hand, the diet containing CLAmix resulted in an increase in the concentration of αLNA and *c4c7c10c13c16c19*C22:6 (C22:6n-3) in the liver ($P<0.01$) compared with the control group. Similarly, the addition of CLAmix to the diet enriched in $_{L}$Se or $_{H}$Se stimulated the accumulation of αLNA ($P=0.003$ and $P=0.002$, respectively), *c7c10c13c16c19* C22:5 ($P=0.093$ and $P=0.012$, respectively) and C22:6n-3 ($P=0.074$ and $P=0.001$, respectively) in the liver in comparison with rats fed the diet containing only $_{L}$Se or $_{H}$Se.

In the current study there was negative relationship between the concentrations of LA, C18:0, A-SFA, T-SFA, MUFA, PUFA, SFA as well as ΣFA in the liver and muscles of rats fed the diet containing $_{H}$Se or, in particular, $_{L}$Se. Thus, the present results concur with our previous findings, which also documented that a diet enriched in Se as selenate or selenized yeast decreased the concentration of ΣFA in femoral muscles and liver of rats (Czauderna et al., 2004a,b; 2007). Data presented in the current and previous investigation regarding dietary Se have important implications for the development of dietary strategies to decrease the concentration of ΣFA in the liver, and especially in muscles.

Dietary CLAmix increased the Δ4-desaturase index (Δ4-index) in the liver ($P<0.05$) and muscles ($P<0.01$) compared with the control group (Table 4). In agreement with this finding, it has been demonstrated that the concentrations of *c7c10c13c16c19*C20:5 (C20:5n-3; the substrate for the elongase) and *c7c10c13c16c19*C22:5 (C22:5n-3; the substrate for the Δ4-desaturase) decreased in the liver and muscles of rats fed the diet containing CLAmix compared with the control group. Since dietary CLA isomers stimulated FA, especially long-chain PUFAn-3 (i.e. α-LNA anabolites), synthesis in the liver, the highest concentration

Table 4. The effect of the diets enriched in CLAmix and 0.2 ppm and 0.5 ppm Se as sodium selenite ($_{L}$Se and $_{H}$Se) on the concentration of α-linolenic acids (αLNA), linoleic acid (LA), atherogenic (A-SFA) and thrombogenic (T-SFA) saturated fatty acids[1], long-chain polyunsaturated fatty acids and values of the concentration ratio of selected groups of fatty acids, Δ4-, Δ5- and Δ6-desaturase indexes in the liver and femoral muscles of rats

Group	αLNA mg/g	LA mg/g	C18:0 mg/g	A-SFA mg/g	T-SFA mg/g	Δ4-index[2]	Δ5-index[3]	Δ6-index[4]	PUFA/SFA	PUFA/ΣFA	UFA[5]/ΣFA	C20:3 n-6[6] μg/g	C20:5 n-3[6] mg/g	C22:5 n-3[6] mg/g	C22:6 n-3[6] mg/g
Liver															
control	0.37^{AB}	7.16	7.68^{Aa}	5.28	$13.0^{a\alpha}$	0.703^{a}	0.973	0.982^{a}	1.146	0.484	0.578	133^{α}	$1.05^{\alpha\beta}$	0.73	1.65^{A}
$_{L}$Se	0.42^{C}	6.11	7.02^{Ba}	4.70	$11.7^{A\alpha}$	0.700^{α}	0.972^{α}	0.980	1.159	0.494	0.574	128	$0.95^{\alpha\gamma}$	0.67^{α}	1.57^{α}
$_{H}$Se	0.51^{AD}	6.44^{A}	7.69^{C}	5.01^{a}	12.7^{B}	0.683^{A}	0.965	0.974^{a}	1.163	0.492^{α}	0.577	173^{α}	1.21^{β}	0.79^{a}	1.69^{B}
CLA	1.37^{B}	7.05	$9.33^{A\alpha}$	5.81	15.1^{a}	0.870^{a}	0.964^{β}	0.990^{α}	0.963	0.448^{β}	0.521	73	0.94	0.48	3.19^{Ab}
$_{L}$Se+CLA	0.63^{C}	6.01	$8.41^{B\alpha}$	5.40	13.8^{A}	0.715^{α}	$0.971^{\alpha\beta}$	0.979	1.042	0.474	0.545	129	0.81^{γ}	0.84^{α}	$2.11^{\alpha bx}$
$_{H}$Se+CLA	0.71^{D}	7.31^{A}	8.55^{C}	5.43^{a}	14.0^{B}	0.732^{A}	0.972	0.980^{α}	1.213	$0.515^{\alpha\beta}$	0.576	152	0.88^{x}	0.89^{a}	2.42^{B}
Femoral muscles															
control	10.44	22.1	4.18	15.7	19.5	0.652^{A}	0.963^{A}	0.998	2.057	0.507	0.753	$54^{\alpha A}$	0.150^{A}	0.82^{a}	1.53^{a}
$_{L}$Se	8.90	18.8	3.79	13.5	17.0	0.657^{α}	0.964	0.997^{B}	2.026	0.510	0.748	51	0.149^{B}	0.78^{A}	1.49^{A}
$_{H}$Se	8.01	17.3	3.55	12.5	15.9	0.662^{B}	0.965^{B}	0.997	1.991	0.508	0.745^{A}	45^{α}	0.144^{C}	0.73^{B}	1.43^{B}
CLA	7.98	20.3	3.79	15.1	18.7	0.720^{A}	1.000^{ACD}	1.000^{CD}	2.006	0.508	0.747^{a}	-[7]	0.022^{ADE}	0.36^{a}	$0.92^{a\alpha}$
$_{L}$Se+CLA	6.83	17.3	3.50	13.4	16.7	0.711^{α}	0.966^{C}	0.998^{BC}	1.957	0.511	0.739^{a}	30^{A}	0.065^{BD}	0.37^{A}	0.91^{A}
$_{H}$Se+CLA	7.39	18.6	3.57	14.1	17.5	0.723^{B}	0.954^{BD}	0.998^{D}	2.047	0.514	0.749^{A}	46^{X}	0.073^{CE}	0.39^{B}	$1.02^{B\alpha}$

[1] - A-SFA - the concentration sum of C12:0, C14:0 and C16:0; T-SFA: the concentration sum of C14:0, C16:0 and C18:0

[2] - Δ4-desaturase index = *c4c7c10c13c16c19*C22:6/(*c4c7c10c13c16c19*C22:6+*c7c10c13c16c19*C22:5)

[3] - Δ5-desaturase index = *c5c8c11c14*C20:4/(*c8c11c14*C20:3+*c5c8c11c14*C20:4)

[4] - Δ6-desaturase index = *c9c12*C18:2/(*c9c12*C18:2+*c11c14c17*C20:3n-3)

[5] - the concentration ratio of unsaturated fatty acids (MUFA+PUFA) and the sum of all assayed fatty acids

[6] - C20:3n-6 - *c8c11c14*C20:3; C20:5n-3 - *c5c8c11c14c17*C20:5; C22:5n-3 - *c7c10c13c16c19*C22:5; C22:6n-3 - *c4c7c10c13c16c19*C22:6

[7] - below the quantification limit

of C22:6n-3 was also found in the liver of rats fed the diets containing CLAmix. On the other hand, the addition of $_{L}$Se or $_{H}$Se to the diet enriched in CLAmix slightly lowered the concentration of C22:6n-3 in the liver compared with the CLA group, while was higher than in the liver of rats fed the diet containing only $_{L}$Se ($P<0.07$) or $_{H}$Se ($P<0.001$). Lastly, the diet enriched in CLAmix, regardless of the addition of $_{L}$Se or $_{H}$Se, resulted in a decrease of C22:6n-3 in muscles compared with the control rats ($P<0.05$) or rats fed the diet with Se ($P<0.001$) or $_{H}$Se ($P<0.002$). The present trial has demonstrated that dietary CLA isomers stimulated β-oxidation of fatty acids in skeletal muscles, and promoted the accumulation of fatty acids, particularly PUFAn-3 in the liver. On the other hand, dietary CLAmix considerably decreased the concentration of *c8c11c14*C20:3 (C20:3n-6) in the liver and, especially, in femoral muscles. These results indicated that dietary CLA isomers were able to act as a competitive substrate for Δ6-desaturase, the enzyme responsible for the conversion of LA to *c6c9c12*C18:3 (γLNA) (the rate-limiting step in the conversion of LA to C20:3n-6 and then to arachidonic acid). Therefore, dietary CLA isomers, as a competitive substrate, considerably decreased the concentrations of AA (Table 3) and C20:3n-6 (Table 4) in the liver and muscles. The current investigation showed a positive influence of dietary CLAmix on the Δ5-desaturase index (Δ5-index) only in muscles ($P=0.009$), while the addition of $_{L}$Se or $_{H}$Se to the diet, regardless of the presence of CLAmix, had a negligible effect on the Δ5-index in the liver and muscles (Table 4). The present study demonstrated that the highest values of the Δ4-, Δ5- and Δ6-desaturase indexes were found in muscles of rats fed the diet enriched in CLAmix. We attributed this elevation of the index values and preferential β-oxidation to the lower concentration of products of Δ4-, Δ5- and Δ6-desaturations especially in muscles (i.e. C20:3n-6, AA, C20:5n-3, C22:5n-3 and C22:6n-3; Tables 3 and 4).

In the current study, although there were no statistically significant changes in the concentration ratio of PUFA to SFA (PUFA/SFA), ΣFA (PUFA/ΣFA) and unsaturated fatty acids (UFA) to ΣFA (UFA/ΣFA) in the liver and muscles of rats fed the experimental diets, the diet enriched in CLAmix decreased (-16%) the PUFA/SFA ratio in the liver in comparison with control rats (Table 4). We attributed this effect in the liver to the lower Δ9-index in the liver of rats fed the diet enriched in CLAmix compared with the control rats ($P=0.07$).

CONCLUSIONS

The presented study demonstrated that the rat diet enriched in the CLA isomer mixture and higher-dose selenite efficiently stimulated body mass gain and feed conversion efficiency, as well as the concentration of Se, Zn and CLA isomers in

the liver and muscles. Moreover, this diet assured satisfactory values of the PUFA/SFA and PUFA/ΣFA ratios in the liver and muscles. These findings are valuable information for nutritionists carrying out research to improve the nutritive value of food for human health. However, numerous further studies are necessary to thoroughly investigate the effects of dietary CLA isomers as well as the interaction of CLA isomers and Se compounds on the animal and human health. However, numerous further studies are necessary to thoroughly investigate the effects of dietary CLA isomers as well as the interaction of CLA isomers and Se compounds on the animal and human health.

REFERENCES

Alasnier C., Berdeaux O., Chardigny J.M., Sébédio J.L., 2002. Fatty acid composition and conjugated linoleic acid content of different tissues in rats fed individual conjugated linoleic acid isomers given as triacylglycerols. J. Nutr. Biochem. 13, 337-345

Boldižárová K., Grešáková Ľ., Faix Š., Mellen M., Leng Ľ., 2005. Antioxidant status of lambs fed on diets supplemented with selenite or Se-yeast. J. Anim. Feed Sci. 14, 245-253

Boosalis M.G., 2008. The role of selenium in chronic disease. Nutr. Clin. Pract. 23, 152-160

Cabañero A.I., Madrid Y., Camara C., 2007. Mercury–selenium species ratio in representative fish samples and their bioaccessibility by an in vitro digestion method. Biol. Tr. Elem. Res. 119, 195-211

Cabrera C., Lorenzo M.L., DeMena C., López M.C., 1996. Chromium, copper, iron, zinc, manganese and selenium levels in dairy products: in vitro study of absorbable fractions. Int. J. Food Sci. Nutr. 47, 331-339

Crespo A.M., Reis M.A., Lanca M.J., 1995. Effect of selenium supplementation on polyunsaturated fatty acids in rats. Biol. Tr. Elem. Res. 47, 335-341

Czauderna M., Kowalczyk J., Korniluk K., 2007. Effect of dietary conjugated linoleic acid mixture and selenized yeast on concentrations of selected fatty acids and mineral elements in rats. Arch. Anim. Nutr. 61, 135-150

Czauderna M., Kowalczyk J., Niedźwiedzka K.M., Leng Ľ., Cobanova K., 2009. Effect of dietary selenized yeast and CLA isomer mixture on the fatty and amino acids concentration in the femoral muscles and liver of rats. J. Anim. Feed Sci. 18, 348-361

Czauderna M., Kowalczyk J., Niedźwiedzka K.M., Wąsowska I., Pastuszewska B., 2004a. Conjugated linoleic acid (CLA) content and fatty acids composition of muscle in rats fed isomers of CLA and selenium. J. Anim. Feed Sci. 13, 183-196

Czauderna M., Kowalczyk J., Niedźwiedzka K.M., Wąsowska I., Pastuszewska B., Bulska E., Ruszczyńska A., 2004b. Liver and body mass gain, content of CLA isomers, other fatty acids in the liver of rats fed CLA isomers and selenium. J. Anim. Feed Sci. 13, 353-369

Czauderna M., Kowalczyk J., Wąsowska I., Niedźwiedzka K.M., Pastuszewska B., 2003. The effects of selenium and conjugated linoleic acid (CLA) isomers on fatty acid composition, CLA isomer content in tissues, and growth of rats. J. Anim. Feed Sci. 12, 865-881

Czauderna M., Sierakowska S., Sitowska B., 1995. Use of INAA to study Se, Sb, Zn and Co levels of yeast cells. Appl. Radiat. Isot. 46, 1295-1298

Gammelgaard B., Gabel-Jensen C., Stürup S., Hansen H.R., 2008. Complementary use of molecular and element-specific mass spectrometry for identification of selenium compounds related to human selenium metabolism. Anal. Bioanal. Chem. 390, 1691-1706

Korniluk K., Czauderna M., Kowalczyk J., 2007. The influence of dietary conjugated linoleic acid isomers and high-selenized yeast on the fatty acid profile of spleen, pancreas and kidneys of rats. J. Anim. Feed Sci. 16, 121-139

Mousa S.A., O'Connor L., Rossman T.G., Block E., 2007. Pro-angiogenesis action of arsenic and its reversal by selenium-derived compounds. Carcinogenesis 28, 962-967

Navarro-Alarcon M., Cabrera-Vique C., 2008. Selenium in food and the human body: A review. Sci. Total Environ. 400, 115-141

Navarro-Alarcon M., López-Martínez M.C., 2000. Essentiality of selenium in the human body: relationship with different diseases. Sci. Total Environ. 249, 347–71

Navas-Acien A., Bleys J., Guallar E., 2008. Selenium intake and cardiovascular risk: what is new? Curr. Opin. Lipidol. 19, 43-49

Park Y., Pariza M.W., 2007. Mechanisms of body fat modulation by conjugated linoleic acid (CLA). Food Res. Int. 40, 311-323

Pastuszewska B., Ochtabińska A., Morawski A., 2000. A note on the nutritional adequacy of stock diets for laboratory rats and mice. J. Anim. Feed Sci. 9, 533-542

Rayman M.P., 2004. The use of high-selenium yeast to raise selenium status: How does it measure up? Brit. J. Nutr. 92, 557-573

Rodriguez E.M., Sanz M.T., Romero C.D., 1994. Critical study of fluorometric determination of selenium in urine. Talanta 4, 2025-2031

Surai P.E., 2004. New source in selenium supplementation. In Expanding horizons. In: Proceedings from Alltech's 18th European, Middle East and African Lecturer Tour, 2004. Nottingham University Press, Nottingham (UK)

Surai P.F., 2006. Selenium in Nutrition and Health. Nottingham University Press, Nottingham (UK)

Tapiero H., Townsend D.M., Tew K.D., 2003. The antioxidant role of selenium and seleno-compounds. Biomed. Pharmacotherapy 57, 134-144

Thomson C.D., 2004. Assessment of requirements for selenium and adequacy of selenium status: a review. Eur. J. Clin. Nutr. 58, 391-402

Ulbricht T.L.V., Southgate D.A.T., 1991. Coronary heart disease: seven dietary factors. Lancet 338, 985-992

Waters S.M., Kelly J.P., Boyle P.O., Moloney A.P., Kenny D.A., 2009. Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of Δ9-desaturase in muscles of beef cattle. J. Anim. Sci. 87, 244-252