Influence of dietary selenium level on the concentration of conjugated linoleic acid isomers, other fatty acids and amino acids in the liver and femoral muscles of rats

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ABSTRACT: The purpose of the present study was to determine the influence of diets containing conjugated linoleic acid isomers (CLAmix) with or without low ($_{L}$Se) or high ($_{H}$Se) concentration of selenised yeast (SeY) on 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 rats. The investigation was performed on 48 female rats (Wistar, Hsd Brl Han: WIST), aged 8 weeks with an initial body weight of 195.7 ± 0.8 g. After one week of submaintenance feeding, for 6 weeks the rats were fed *ad libitum* the Labofeed H diet supplemented with 1.5% CLAmix with or without low (0.2 μg Se/g diet) or high (0.5 μg Se/g diet) concentration of Se as SeY. The diet enriched with $_{L}$Se or $_{H}$Se elevated the BWG of rats compared with the control group, while the $_{L}$Se diet resulted in the most efficient increase in BWG. The diet containing CLAmix, irrespective of the presence of SeY, stimulated the accumulation of Se in the liver and muscles in comparison with the diet enriched with SeY. The addition of SeY to the diet enriched with CLAmix stimulated the accumulation of *cis*9*trans*11 (*c*9*t*11), *t*10*c*12 and *cc* isomers of CLA in the liver. The diet containing CLAmix and $_{L}$Se most efficiently increased the concentration of these CLA isomers in muscles. The diets enriched with SeY increased the concentration of essential, endogenous and total amino acids (AA) in the liver, whereas the diets enriched with SeY or CLAmix reduced the concentration of these AA in muscles. The diets enriched with CLAmix and/or SeY decreased the Δ9-desaturase index in the liver and muscles compared with the control group. These diets increased Δ4-, Δ5- and Δ6-desaturase indexes in muscles, but significantly reduced the elongase index compared with the control group. Our study shows that dietary CLAmix increased the concentration of C14:0, C18:0 and the sum of saturated FA (SFA) in the liver, whereas the diets enriched with SeY, irrespective of the presence of CLAmix, slightly reduced the concentration of C14:0, C18:0, and SFA in muscles compared with the control group. The diets containing SeY and/or CLAmix increased the accumulation of arachidonic acid (ArA), linolenic acid (αLNA) and *c*4*c*7*c*10*c*13*c*16*c*19C22:6 in the liver compared with the control rats. Negative effects of dietary SeY and/or CLAmix on the accumulation of αLNA, *c*5*c*8*c*11*c*14*c*17C20:5, *c*7*c*10*c*13*c*16*c*19C22:5, *c*4*c*7*c*10*c*13*c*16*c*19C22:6, linoleic acid, ArA and *c*8*c*11*c*14C20:3 in muscles were found out. The CLAmix diet increased the Δ4-, Δ5-desaturase and elongase indexes in the liver. It also increased the Δ4-, Δ5- and Δ6-desaturase indexes in muscles, but significantly reduced the elongase index compared with the control group. The finding that the diet with CLAmix and $_{H}$Se fed to rats decreased total FA and most efficiently increased the content of Se and essential AA in muscles is valuable information for nutritionists carrying out research on farm animals to improve the nutritive value of food from the aspect of human health.

Keywords: selenised yeast; conjugated linoleic acid isomers; rats; liver; femoral muscles; fatty acids; amino acids; minerals

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Selenium (Se) in tissues of mammals reflects the chemical form of Se and its level in consumed diets. Therefore, it is important to know its abundance or deficiency in food and diets and to determine the correct balance of Se and its effect on the level of other substances in farm animals and in humans consuming livestock products. Se supplementation of livestock diets increases the nutritional quality of animal products. Se is usually dosed in two forms: inorganic compounds (mostly selenite or selenate) or organic forms such as selenised yeast (SeY), in which selenomethionine (Se-Met) is the prevalent form of Se (Rayman, 2004). Endogenous Se is present in fluids and tissues of mammals as selenocysteine (Se-Cys), which is the functional core of ~25 Se-proteins (like isozymes of the glutathione-peroxidase (GPx) family or thioredoxin reductases) or Se-Met, which can be bound non-specifically to proteins (Se-Met-proteins) (Suzuki, 2005; Juniper et al., 2008). Se incorporated into Se-Met-proteins can be utilised in mammals during periods of suboptimal Se dosages. Despite the different metabolic pathways for Se-containing compounds, all Se compounds seem to ultimately enter the Sec-loaded tRNA pool to be used for Se-Cys-protein synthesis (Schomburga et al., 2004). These Se-proteins (i.e. Se-enzymes) are involved in diverse metabolic processes and display unique qualities. Numerous studies have shown that nearly half of these Se-Cys-proteins (like the GPx family or selenoprotein P) protect against oxidative stress. Indeed, the GPx enzyme recycles glutathione, reducing lipid peroxidation by catalysing the reduction of peroxides, including hydrogen peroxide. All Se-enzymes in their reduced state catalyse the breakdown of lipid hydroperoxides and hydrogen peroxides in mammalian cells (Navarro-Alarcon and Cabrera-Vique, 2008). Furthermore, the antioxidant properties of Se in these enzymes can help to alleviate the damage caused by ultraviolet-β radiation in humans and animals.

It was found out in numerous studies on laboratory animals that the concentration of polyunsaturated fatty acids (PUFA) was positively correlated with the level of Se in diets (Crespo et al., 1995). Considering the above and the potential importance of conjugated linoleic acid (CLA) isomers and Se in living organisms, essentially related to protection against oxidative stress, it was desirable to study the extent to which various dietary levels of SeY may contribute to the concentration of CLA isomers and other fatty acids (FA), especially PUFAn-3, in the liver and femoral muscles of rats. Indeed, CLA isomers are believed to play an important role in many physiological functions and may act to convey body composition or to modulate immune function (Hur et al., 2007). CLA isomers are also believed to inhibit tumorigenesis by interfering in the metabolism of some carcinogens. Moreover, some CLA isomers are antioxidative, antiatherosclerotic and hypolipidemic.

Therefore, the main purpose of the present study was to establish the effect of different amounts of Se (as SeY) added to a diet enriched with CLA isomers (CLAmix) on the concentrations of selected amino acids and fatty acids in the liver and femoral muscles of rats. The second objective of the present study was to investigate the influence of these dietary additives on the growth of rats and concentrations of Se, Zn, Fe, Cu, Ca, and Mg in the liver and muscles.

MATERIAL AND METHODS

Animals, housing, diets, experimental design and sampling

The experiment was conducted with 48 female rats (Wistar, Hsd Brl Han: WIST) aged 8 weeks, of an initial body weight about 195.7 ± 0.8 g. The animals were housed and handled in accordance with protocols approved by the Local Animal Care and Use Committee (Agricultural University of Warsaw, Poland). The animals were housed individually in metabolic 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 a commercial diet for laboratory rats and mice (the trade name Labofeed H diet). This diet was purchased from the Feeds and Concentrates Production Plant in Kcynia, MORAWSKI Co., Poland (www.wp-morawski.com.pl/Labofeed/labofeed). Since 1998 the Labofeed H diet has been produced according to ISO 9001 Standards (Pastuszewska et al., 2000). For our studies, this commercial diet containing 0.13 μg Se/(g diet) (the natural content of Se) was enriched with only 0.2 μg Se/(g diet) as selenite (Table 1). Therefore, the total concentration of Se in this commercial rat diet was 0.33 μg Se/(g diet).

Table 1. Chemical composition and energy content of the basal diet[#] (the Labofeed H diet manufactured by MORAWSKI Co., Poland) and selenised yeast (SeY)

Item	Content	Item	Content
Composition of the basal diet*		**Composition of selenized yeast (SeY)**	
Dry matter[d] (g/100 g diet)	88.2 ± 0.9	Se (mg/g DM)	1.8
Crude protein (g/100 g diet)	21.8 ± 1.3	Sum of identified Se species (%)	88.3
Lysine (g/100 g diet)	1.31	Seleno-methionine (%)	83.0
Methionine and cysteine (g/100 g diet)	0.76	Seleno-cysteine (%)	5.0
Tryptophan (g/100 g diet)	0.28	Selenite (%)	0.3
Threonine (g/100 g diet)	0.87	Fatty acids[$] (mg/g DM)	
Crude fibre (g/100 g diet)	21.8 ± 1.3	C16:0	9.0
Crude fat (g/100 g diet)	3.0 ± 0.8	*cis*9C16:1	4.1
Ash (g/100 g diet)	5.9 ± 0.6	C18:0	13.6
N total	3.77	*cis*9C18:1	11.3
N protein	3.17	*cis*11C18:1	0.8
P total	0.75	*cis*9*cis*12C18:2 (LA)	14.7
Mono-carbohydrates	5.75	*cis*9*cis*12*cis*15C18:3 (αLNA)	0.16
Starch	30.3		
Metabolize energy[t] (MJ/kg)	13.9		

[#]Means of 9 samples

*Ingredients: maize, wheat, oat flakes, green meal, soya-bean oil-meal, fish meal, soya oil, vitamins (per kg diet: vit. A 10 096 U, vit. D_3 2000 IU, vit. E 86.1 mg, vit. K 1 3 mg, vit. B_1 15.7 mg, vit. B_2 16.0 mg, vit. B_6 5.24 mg, vit. B_{12} 81 mg, biotin 0.2 mg, folic acid 3.03 mg, nicotinic acid 79.3 mg, pantothenic acid 25.5 mg; choline 2.02 g); minerals (contained per g DM diet: Na – 3.60 mg; K – 8.30 mg; Ca – 10.68 mg; P – 7.60 mg) and trace elements (contained per kg DM diet: Se as Na_2SeO_3 – 0.2 mg; Cu – 13.9 mg; Zn – 98 mg; Mn – 112 mg; Fe – 698 mg; Mg – 1653 mg)

[d]The concentrations of main fatty acids (mg per kg DM diet): C8:0 – 37; C10:0 – 6; C12:0 – 11; *cis*9*cis*12*cis*15 C18:3 (αLNA) – 8; *cis*6*cis*9*cis*12C18:3 (γLNA) – 715; C14:0 – 11; *cis*9*cis*12C18:2 (LA) – 429; C16:0 – 250; *cis*9C18:1 – 187; *cis*6C18:1 – 112; C18:0 – 89; C20 – 11; C22 – 2.4; Σ saturated fatty acids (SFA) – 417; Σ polyunsaturated fatty acids (PUFA) – 1499; Σ assayed fatty acids (ΣFA) – 1915

[t]The mean from 3 samples

[$]Main fatty acid peaks (i.e. 95% of peak areas of all fatty acids in a GC-MS chromatogram)

During a one-week preliminary period the rats were fed the Labofeed H diet offered at a sub-maintenance level to reduce the rats' body fat (Table 2). Next, for 6 weeks the rats were fed *ad libitum* the experimental diets supplemented with 1.5% CLA isomer mixture (CLAmix), 0.2 μg Se/(g diet) ($_L$Se) or 0.5 μg Se/(g diet) ($_H$Se), Se as selenised yeast (SeY) (Tables 1 and 2). Feed intake and body weight of rats were measured weekly. At the end of the six-week experiment the rats were euthanized with CO_2. The liver and femoral muscles were removed, weighed, and frozen until analysed. The concentrations of fatty acids (FA), selenium (Se), zinc (Zn), iron (Fe), copper (Cu), calcium (Ca) and magnesium (Mg) were determined in the liver and muscles. All liver and muscle samples were analysed individually. The concentrations of FA, Zn, Fe, Cu, Ca, and Mg were calculated based on freeze-dried liver and muscle samples.

Reagents

All chemicals were of 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% *c*11*t*13CLA, 40.8% *c*9*t*11CLA, 41.3% *t*10*c*12CLA, 6.7% *c*8*t*10CLA, and 2.0% *cc*CLA) by the Industrial Chemistry Research Institute (Warsaw, Poland). The concentration ratio ($R_{c9t11CLA/t10c12CLA}$) of *c*9*t*11CLA to *t*10*c*12CLA in the dietary CLA isomer mixture was 0.9879. Fatty acid methyl ester (FAME) standards and 50% BF_3 in methanol were purchased from Supelco and Sigma (Bellefonte, Pennsylvania, USA).

The selenised yeast (Se-Saccharomyces cerevisiae) was donated by Sel-Plex (a non-commercial yeast sample; Alltech Inc., Nicholasville, USA). About 83% of the total Se concentration of selenised yeast (SeY) is represented by Se in the form of Se-Met incorporated into proteins of Saccharomyces cerevisiae (Table 1) (Rayman, 2004; Weiss and Hogan, 2005).

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

Analytical methods

The Se concentration in the liver and muscles was analysed by the fluorimetric method of Rodriguez et al. (1994) using fresh samples of these tissues. The concentrations of Se were calculated per dry mass (DM) using the relation between fresh mass and mass of freeze-dried liver and femoral muscle samples. The concentrations of Zn, Fe, Cu, Mg, and Ca in freeze-dried liver and muscle samples were determined by flame (air-acetylene) atomic absorption spectrometry (Korniluk et al., 2006).

The methods of alkaline hydrolysis and base- and acid-catalyzed methylation of fatty acids (i.e. the derivatization) were used as previously described (Czauderna et al., 2007a). The methylated CLA isomers (CLA-ME) were determined using silver ion liquid chromatography with photodiode array detection (Ag^+-HPLC-DAD) according to Czauderna et al. (2005).

The concentrations of methylated FA (i.e. FAME and CLA-ME) and Zn, Fe, Cu, Mg, and Ca were calculated based on freeze-dried liver and muscle samples.

Analytical equipment

The analyses of methylated fatty acids (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 µm film thickness); SHIM-POL, a quadrupole mass selective (MS) detector (Model 5973N) and an injection port. Helium as the carrier gas operated at a constant pressure (223.4 kPa) and flow rate of 1 ml per min. Injector and MS detector temperatures were maintained at 200 and 240°C, respectively. The total FAME profile in a 1-µl sample at the split ratio of 10:1 was determined using the column temperature gradient programme, which was described previously (Czauderna et al., 2005).

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

Methylated CLA isomers (CLA-ME) were determined using a Waters 625 LC system that included a controller for gradient elution and two pumps (Waters Model 515). The apparatus consisted of a Waters 712 WISP autosampler, two ion-exchange columns loaded with silver ions (250 × 4.6 mm Chrompack ChromSper 5 µm Lipids, the Netherlands) and a Waters 996 photodiode array detector (Czauderna et al., 2005).

The concentrations of Zn, Fe, Cu, Mg, and Ca in lyophilised liver and muscle samples were determined using a PU9100X Atomic Absorption Spectrometer (UNICAM, Philips) (Korniluk et al., 2006).

Statistical analyses

Results are presented as means ± SD of 8 individually analysed samples of the liver and muscles. The one-factorial statistical analyses of the effects of SeY or the CLAmix in the experimental diets were conducted using the non-parametric Mann-Whitney U test for comparing independent experimental groups. Statistical analyses of the interactions between CLAmix and $_LSe$ ($_LSe_{CLA}$) and CLAmix and $_HSe$ ($_HSe_{CLA}$) were performed using two-factorial ANOVA analyses. Differences were considered significant at the $P < 0.05$ (*) and $P < 0.01$ (**) levels, while at $P < 0.1$ ($^{\alpha}$) differences were indicated as tendencies. Statistical analyses were performed using the Statistica v. 6 software package (Statistica, 2002).

Table 2. Dietary effects of 1.5% CLA isomer mixture (CLAmix) and selenised yeast (SeY) on feed conversion efficiency (FCE; g body weight gain/g feed intake)[#], body weight gain (BWG)[#] and liver fresh mass (g) of rats and the concentrations* (μg/g) of Se, Zn, Cu, Fe, Mg, Ca in the liver and femoral muscles of rats after 6 weeks of feeding the experimental diets

Group	Additives per g diet	Body weight (g) Initial	Body weight (g) adapted[&]	BWG (g)	FCE (g/g)	Liver mass (g)	Liver						Femoral muscles[$]				
							Se[t]	Zn	Cu	Fe	Ca	Mg	9Se[t]	Zn	Fe	Ca	Mg
Control[Ł]	–	196.0	180.4	50.0^{ab}	0.068	8.718	4.3^{α}	120	6.3^{AB}	1036^{α}	$206^{ab\alpha}$	580^{A}	0.25^{Aa}	49	68	246^{a}	869
$_{L}Se^{Ł}$	0.2 μg Se	196.7	179.2	58.1^{a}	0.076^{a}	8.408	4.6^{a}	129	10.0	1047^{β}	149^{α}	607^{B}	0.44^{B}	47	74^{A}	256	892^{a}
$_{H}Se^{Ł}$	0.5 μg Se	195.0	177.3	55.6^{b}	0.075^{c}	8.315	5.1^{b}	125^{α}	8.9^{AC}	1067^{α}	134^{a}	651^{C}	0.72^{ab}	44	79^{a}	230^{a}	844
CLA[Ł]	CLAmix	196.5	181.7	51.9^{cd}	0.069^{b}	8.486	$4.6^{\alpha c}$	128	$10.2^{BD\alpha}$	1061	144^{b}	682^{A}	0.49^{A}	46	78	219^{b}	836
$_{L}Se_{CLA}^{\S}$	0.2 μg Se CLAmix	195.3	178.3	56.1^{d}	0.073^{abx}	8.482	4.9^{ax}	129	12.1^{α}	984^{β}	217	668^{B}	0.64^{B}	47	81^{A}	325	829^{a}
$_{H}Se_{CLA}^{\S}$	0.5 μg Se CLAmix	194.7	177.4	54.6^{c}	0.076^{cy}	8.361	5.3^{bcy}	135^{α}	13.0^{CD}	1045	183	665^{C}	0.82^{b}	52	93^{a}	247^{b}	859

[#]FCE and BWG after feeding for 6 weeks with the experimental diets enriched with CLAmix and/or Se-yeast

*The concentrations of assayed elements in dry mass (DM); the liver and femoral muscles were freeze-dried

[&]The body weight (g) of individually adapted rats after 7 days of submaintenance feeding (9 g the Labofeed H diet per day per rat)

[$]The concentration of Cu in the muscles was below the quantification limit

[Ł]Means in columns sharing the same letter are significantly different

$^{a,b}P < 0.05$ and $^{A,B}P < 0.01$; [§]Interactions of CLAmix × $_{L}$Se and CLAmix × $_{H}$Se are significant at $^{x,y}P < 0.05$ and $^{X,Y}P < 0.01$, respectively

[t]Fresh liver and muscle samples were used to determine Se by the fluorimetric method

Table 3. The effect of diets enriched with CLAmix and 0.2 or 0.5 μg Se/(g diet) as SeY ($_{L}$Se and $_{H}$Se) on the concentration of CLA isomers (μg/g)[&], essential (EAA; mg/g DM) and endogenous (NEAA; mg/g DM) amino acids, the sum of amino acids (ΣAA; mg/g DM), methionine (Met; mg/g DM), *c*9C18:1 (mg/g), SFA (mg/g), MUFA (mg per g), PUFAn-3 (mg/g), PUFAn-6 (mg/g), the sum of FA (ΣFA; mg/g), values of Δ9-desaturase ($\Delta9_{index}$)[$], atherogenic ($A_{index}$)[*], thrombogenic ($T_{index}$) [π]indexes[đ] in the liver and muscles of rats

Group	Control	$_{L}$Se	$_{H}$Se	CLA	$_{L}Se_{CLA}$	$_{H}Se_{CLA}$	Significance of effects analysis				
							one factorial			interactions	
							$_{L}$Se	$_{H}$Se	CLA	$_{L}Se_{CLA}$	Se_{CLA}
Liver											
*tt*CLA	–[h]	–	–	2.1[Aa]	6.5[A]	3.6[a]	–	–	–	–	–
*cc*CLA	–	–	–	0.9[ab]	1.3[a]	1.4[b]	–	–	–	–	–
*c*9t11	–	–	–	181[a]	231	255[a]	–	–	–	–	–
*t*10c12	–	–	–	87	118	122	–	–	–	–	–
ΣCLA	–	–	–	271[ab]	367[a]	402[b]	–	–	–	–	–
EAA[Ł]	262	278	279	272	266	268	NS	NS	NS	NS	NS
NEAA[Ł]	280	310	312	282	256	306	NS	NS	NS	α	NS
ΣAA[Ł]	542	589	591	554	522	574	NS	NS	NS	α	NS
Met[Ł]	14.5	15.4	20.2	13.0	8.6	10.5	NS	α	NS	*	*
$\Delta9_{index}$	0.156	0.132	0.123	0.115	0.119	0.129	NS	NS	α	NS	NS
A_{index}	0.287	0.256	0.263	0.340	0.257	0.264	α	NS	NS	NS	NS
T_{index}	0.688	0.604	0.632	0.634	0.566	0.577	**	NS	NS	α	α
*c*9C18:1	2.19	1.88	1.84	1.89	1.93	2.01	NS	NS	NS	NS	NS
SFA	13.8	14.0	14.8	16.4	15.4	15.4	NS	NS	*	NS	NS
MUFA	3.07	2.63	2.50	2.49	2.50	2.67	NS	NS	NS	NS	α
$PUFA_{n-3}$	3.76	4.79	4.84	6.01	5.72	5.40	**	*	**	NS	α
$PUFA_{n-6}$	12.1	12.5	12.8	9.2	14.4	13.7	NS	NS	NS	α	α
ΣFA	32.7	34.0	34.9	34.3	38.2	37.4	NS	NS	NS	*	*

RESULTS

The effect of dietary SeY and CLAmix on body weight gain, feed conversion efficiency and levels of elements in liver and muscles

The influence of diets enriched with CLAmix and/or SeY on body weight gain (BWG), feed conversion efficiency (FCE), liver weight and concentrations of Se, Zn, Fe, Cu, Ca, and Mg in both tissues is summarized in Table 2. As can be seen from the obtained results, the diet enriched with $_{L}$Se or $_{H}$Se elevated the BWG of rats ($P < 0.05$) compared with the control group, while the lower concentration of extra Se in the diet resulted in the most efficient increase in the BWG of rats. On the other hand, the diet containing only CLAmix exerted a negligible influence on BWG in comparison with control rats, whereas the diets with CLAmix and SeY, regardless of the concentration of extra SeY, increased the value of BWG compared with rats fed the control diet or the diet enriched with CLAmix, although no statistically significant interactions (i.e. $_{L}$Se x CLAmix, $_{H}$Se × CLAmix) were determined ($P > 0.05$).

The diets containing SeY (as $_{L}$Se or $_{H}$Se), regardless of the presence of CLAmix, resulted in a decrease in the liver weight in comparison with the control group, however, these differences

Table 3 to be continued

Group	Control	$_{L}$Se	$_{H}$Se	CLA	$_{L}$Se$_{CLA}$	$_{H}$Se$_{CLA}$	Significance of effects analysis				
							one factorial			interactions	
							$_{L}$Se	$_{H}$Se	CLA	$_{L}$Se$_{CLA}$	Se$_{CLA}$
Femoral muscles											
ttCLA	–	–	–	26^{ab}	41^{a}	56^{b}	–	–	–	–	–
ccCLA	–	–	–	16	18	17	–	–	–	–	–
c9t11	–	–	–	3172^{a}	3586	2596^{a}	–	–	–	–	–
t10c12	–	–	–	1954	2260	1570	–	–	–	–	–
ΣCLA	–	–	–	5888^{ab}	6932^{a}	4857^{b}	–	–	–	–	–
EAA^{Ł}	320	263	188	211	290	325	NS	*	NS	α	NS
NEAA^{Ł}	376	345	252	275	354	369	NS	*	NS	NS	NS
ΣAA^{Ł}	696	608	440	485	644	694	NS	*	NS	α	NS
Met^{Ł}	19.1	19.5	12.3	15.4	20.1	21.3	α	*	NS	NS	α
$\Delta 9_{index}$	0.474	0.454	0.457	0.458	0.458	0.459	*	α	α	NS	NS
A_{index}	0.332	0.337	0.335	0.365	0.359	0.367	NS		α	NS	α
T_{index}	0.320	0.329	0.336	0.389	0.380	0.408	NS	α	**	NS	α
c9C18:1	17.3	13.0	13.3	15.6	15.5	13.7	*	*	NS	NS	*
SFA	20.6	16.5	16.6	19.4	19.6	17.0	*	NS	NS	NS	NS
MUFA	20.5	15.2	15.6	18.3	18.4	15.8	*	*	NS	NS	*
$PUFA_{n-3}$	13.0	10.6	10.0	9.5	9.8	7.7	α	α	*	NS	α
$PUFA_{n-6}$	23.6	18.9	19.1	21.3	21.6	18.7	*	*	NS	NS	NS
ΣFA	83.4	64.5	64.8	76.6	78.6	66.1	*	*	NS	NS	NS

[&]Means in rows sharing the same letter are significantly different

[a,b]$P < 0.05$ and [A,B]$P < 0.01$; ΣCLA – a sum of CLA isomers

[$]Δ9-desaturase index = (*c*9C14:1 + *c*9C16:1 + *c*9C18:1)/(*c*9C14:1 + *c*9C18:1 + *c*9C18:1 + C14:0 + C16:0 + C18:0)

*The atherogenic index = (C12:0 + 4*C14:0 + C16:0)/(MUFA +PUFAn-6 + PUFAn-3)

[π]The thrombogenic index = (C14:0 +C16:0+ C18:0)/0.5*MUFA + 0.5*PUFAn-6 + 3*PUFAn-3 + PUFAn-3/PUFAn-6)

[d] Ulbricht and Southgate (1991)

[Ł]The content of amino acid(s) in the dry mass of the liver and muscles (i.e. mg/g DM)

[h]Below the quantification limit (L_Q)

were not significant ($P > 0.05$) (Table 2). On the other hand, the Se concentration increased in the liver and muscles of rats fed the diets enriched with SeY and with or without CLAmix compared with control rats, although this increase was relatively lower in the liver than in muscles. The diet containing CLAmix, irrespective of the presence of SeY, stimulated the accumulation of Se in both tissues in comparison with the diet enriched with SeY (as $_{L}$Se or $_{H}$Se). All experimental diets also caused a numerical or statistically significant increase in the concentrations of Zn, Cu, and Mg in the liver, whereas they resulted only in an increase in the Fe concentration in muscles in comparison with control rats (Table 2).

The effect of experimental diets on the concentration of CLA isomers, amino acids and PUFA in liver and muscles

As can be seen from the data summarized in Table 3, the addition of SeY to the diet enriched with CLAmix elevated the concentration of *c*9*t*11,

Table 4. The effect of diets enriched with CLAmix and 0.2 or 0.5 μg Se/(g diet) as SeY ($_{L}$Se and $_{H}$Se) on the concentrations (mg/g) of C14:0, linolenic acid (αLNA), linoleic acid (LA), C18:0, *c*8c11*c*14C20:3 (C20:3$_{n-6}$), *c*5c8*c*11*c*14C20:4 (ArA), *c*5c8*c*11*c*14*c*17C20:5 (C20:5$_{n-3}$), *c*7*c*10c13*c*16*c*19C22:5 (C22:5$_{n-3}$), *c*4*c*7*c*10*c*13*c*16*c*19C22:6 (C22:6$_{n-3}$), values of the PUFA-to-SFA, PUFA-to-ΣFA, UFA-to-ΣFA, PUFAn-6-to-PUFAn-3 (n-6/n-3) ratios, values of the Δ4-(Δ4$_{index}$)$^{\&}$, Δ5-(Δ5$_{index}$)$^{\$}$ and Δ6-(Δ6$_{index}$)$^{\#}$ desaturase, elongase (Elong$_{index}$)* indexes in the liver and muscles of rats

Group	Control	$_{L}$Se	$_{H}$Se	CLA	$_{L}$Se$_{CLA}$	$_{H}$Se$_{CLA}$	Significance of effects analysis				
							one factorial			interactions	
							$_{L}$Se	$_{H}$Se	CLA	$_{L}$Se$_{CLA}$	$_{H}$Se$_{CLA}$
Liver											
C14:0	0.053	0.051	0.052	0.066	0.051	0.052	NS	NS	NS	NS	NS
αLNA	0.366	0.562	1.088	1.365	0.699	0.490	**	**	**	α	*
LA	7.16	6.79	7.23	7.05	8.00	7.72	NS	NS	NS	NS	NS
C18:0	7.69	8.30	8.86	9.33	8.83	8.47	*	**	**	NS	NS
C20:3n-6	0.134	0.209	0.139	0.0 73	0.175	0.223	**	NS	NS	*	*
ArA	4.79	5.54	5.40	5.21	6.05	5.61	NS	NS	NS	α	NS
C20:5n-3	1.05	1.36	1.16	0.939	0.974	1.12	**	α	NS	NS	*
C22:5n-3	0.701	0.906	0.793	0.477	0.952	0.973	*	NS	NS	NS	*
C22:6n-3	1.65	1.96	1.79	3.19	3.05	2.77	*	NS	**	*	α
PUFA SFA	1.146	1.234	1.188	1.134	1.322	1.261	*	NS	NS	*	NS
PUFA ΣFA	0.484	0.510	0.504	0.496	0.532	0.518	**	NS	NS	α	NS
UFA ΣFA	0.578	0.587	0.576	0.562	0.598	0.589	NS	NS	NS	NS	NS
n-6/n-3	3.21	2.61	2.64	2.06	2.51	2.54	**	**	**	α	NS
Δ4$_{index}$	0.701	0.684	0.693	0.870	0.762	0.740	NS	NS	*	α	α
Δ5$_{index}$	0.973	0.964	0.975	0.986	0.972	0.962	*	NS	α	NS	NS
Δ6$_{index}$	0.982	0.970	0.981	0.990	0.979	0.972	**	NS	NS	NS	NS
Elong $_{index}$	0.599	0.601	0.594	0.663	0.506	0.535	NS	NS	*	*	α

*t*10*c*12 and *cc* isomers of CLA in the liver in comparison with the concentrations of these isomers in the liver of rats fed the diet with CLAmix only; the increase in the concentration of these isomers in the liver was observed with the increase in the SeY concentration in the diet with CLAmix. On the other hand, the lower concentration of extra SeY in the diet containing CLAmix most efficiently increased the concentration of these CLA isomers in muscles. The higher concentration of SeY in the diet with CLAmix resulted in a decrease in the concentrations of *c*9*t*11CLA and *t*10*c*12CLA in muscles in comparison with the control group.

The diets enriched with SeY (as $_{L}$Se and $_{H}$Se) increased the concentration of methionine (Met), essential (EAA), endogenous amino acids (NEAA) and the sum of all amino acids (ΣAA) in the liver, although no significant differences ($P > 0.05$) in these values were found out (Table 3). On the other hand, these diets usually resulted in a decrease in the concentration of these amino acids in muscles, however, significant differences ($P < 0.05$) in these values were determined in muscles of rats fed the diet enriched with the higher concentration of SeY (i.e. $_{H}$Se). The results show that the addition of CLAmix to the diet containing $_{L}$Se or $_{H}$Se reduced the effect of dietary $_{L}$Se or $_{H}$Se on the concentrations of EAA, NEAA, ΣAA and Met in the liver and muscles compared with these values in the liver and muscles of rats fed the diets with $_{L}$Se or $_{H}$Se.

The diets enriched with CLAmix and/or SeY (as $_{L}$Se or $_{H}$Se) reduced the values of Δ9-desaturase index (Δ9$_{index}$) in the liver and muscles of rats

Table 4 to be continued

Group	Control	$_{L}$Se	$_{H}$Se	CLA	$_{L}$Se$_{CLA}$	$_{H}$Se$_{CLA}$	Significance of effects analysis				
							one factorial			interactions	
							$_{L}$Se	$_{H}$Se	CLA	$_{L}$Se$_{CLA}$	$_{H}$Se$_{CLA}$
Femoral muscles											
C14:0	1.090	0.822	0.766	0.908	0.933	0.702	NS	α	NS	NS	α
αLNA	10.44	8.09	8.06	7.98	8.10	6.15	αα	α	α	NS	*
LA	22.1	17.7	17.9	20.3	20.2	17.6	*	α	NS	NS	NS
C18:0	4.18	3.51	3.52	3.80	3.84	3.20	NS	NS	NS	NS	NS
C20:3n-6	0.054	0.040	0.034	– [π]	0.038	0.019	NS	α	**	NS	NS
ArA	1.44	1.14	1.09	0.67	0.91	0.80	**	**	**	α	α
C20:5n-3	0.150	0.118	0.098	0.023	0.065	0.043	NS	α	**	*	*
C22:5n-3	0.818	0.710	0.643	0.360	0.354	0.339	NS	*	**	NS	NS
C22:6n-3	1.53	1.34	1.23	0.92	0.97	0.95	*	**	**	NS	NS
PUFA SFA	2.05	1.99	1.96	2.01	2.07	1.96	NS	NS	NS	NS	NS
PUFA ΣFA	0.507	0.508	0.503	0.508	0.517	0.504	NS	NS	NS	NS	NS
UFA ΣFA	0.753	0.744	0.743	0.747	0.751	0.743	NS	NS	NS	NS	NS
n-6/n-3	1.823	1.845	1.900	2.247	2.209	2.439	NS	NS	**	NS	α
$\Delta4_{index}$	0.652	0.654	0.657	0.720	0.732	0.737	NS	NS	**	NS	α
$\Delta5_{index}$	0.963	0.966	0.970	1.000	0.960	0.977	NS	NS	**	NS	NS
$\Delta6_{index}$	0.997	0.998	0.998	1.000	0.998	0.999	NS	*	**	NS	NS
Elong $_{index}$	0.155	0.143	0.132	0.977	0.156	0.113	NS	NS	**	NS	α

[&]The Δ4-desaturase index = *c*4*c*7*c*10*c*13*c*16*c*19C22:6/(*c*4*c*7*c*10*c*13*c*16*c*19C22:6 + *c*7*c*10*c*13*c*16*c*19C22:5)

[$]The Δ5-desaturase index = *c*5*c*8*c*11*c*14C20:4/(*c*8*c*11*c*14C20:3 + *c*5*c*8*c*11*c*14C20:4)

[#] The Δ6-desaturase index = *c*9*c*12C18:2/(*c*9*c*12C18:2 + *c*11*c*14*c*17C20:3)

*The elongase index = *c*5*c*8*c*11*c*14*c*17C20:5/(*c*5*c*8*c*11*c*14*c*17C20:5 + *c7c10c13c16c19C22*:5)

compared with control animals (Table 3), however, in the present trials differences in $\Delta9_{index}$ values were usually non-significant ($P > 0.05$). Moreover, changes in concentrations of *c*9C18:1 and MUFA in the liver and muscles were also consistent with changes in $\Delta9_{index}$ values in both tissues of the examined rats.

The present study documented the positive influence of SeY (as $_{L}$Se or $_{H}$Se) in the diets with or without CLAmix on values of atherogenic (A_{index}) and thrombogenic (T_{index}) indexes in the liver (Table 3), although differences in A_{index} and T_{index} values were usually non-significant ($P > 0.05$). On the other hand, there was a negligible effect of dietary SeY on A_{index} and T_{index} values in muscles, whereas the simultaneous addition of CLAmix and SeY to the diet resulted in an increase in A_{index} and T_{index} values in muscles. The diet containing CLAmix and the higher concentration of SeY (i.e. $_{H}$Se) however showed a tendency ($P < 0.1$) to increase A_{index} and T_{index} values in muscles. Rats fed the diet containing CLAmix tended to increase the A_{index} ($P = 0.07$) and significantly ($P < 0.01$) increased the T_{index} in muscles, whereas the dietary CLAmix elevated only the A_{index} in the liver, although no significant differences ($P > 0.05$) between these values were found out (Table 3).

The results show (Tables 3 and 4) that dietary CLAmix increased the concentration of C14:0, C18:0 as well as the sum of saturated fatty acids (SFA) in the liver compared with the control group ($P = 0.12$, $P < 0.01$ and $P < 0.05$, respectively) and non-significantly the concentration of C14:0, C18:0 and SFA in the liver in comparison with rats fed the

diet containing only SeY (as $_{L}$Se or $_{H}$Se). The addition of $_{L}$Se or $_{H}$Se to the diet with CLAmix slightly reduced the concentration of C14:0, C18:0, and SFA in the liver compared with this value in the liver of rats fed the diet containing only CLAmix. On the other hand, the diets enriched with SeY (as $_{L}$Se or $_{H}$Se), regardless of the presence of CLAmix, slightly reduced the concentration of C14:0, C18:0, and SFA in muscles compared with the control group, although no significant differences ($P > 0.05$) in these values were usually observed.

In the present study there was usually a significant positive influence of dietary SeY (as $_{L}$Se or $_{H}$Se) and/or CLAmix on the accumulation of arachidonic acid (ArA), linolenic acid (αLNA), *c*4*c*7*c*10*c*13*c*16*c*19C22:6 (C22:6n-3) and PUFAn-3 in the liver compared with control rats (Tables 3 and 4). On the other hand, negative effects of dietary SeY and/or CLAmix on the accumulation of PUFAn-3 (αLNA, *c*5*c*8*c*11*c*14*c*17C20:5, *c*7*c*10*c*13*c*16*c*19 C22:5 and C22:6n-3) and PUFAn-6 (linoleic acid, ArA and *c*8*c*11*c*14C20:3) in muscles were found out. Increased values of the PUFA/SFA and PUFA/ΣFA ratios were observed in the liver of rats fed the diets containing SeY (as $_{L}$Se or $_{H}$Se) or both CLAmix and SeY (as $_{L}$Se or $_{H}$Se) compared with control rats, although statistically significant effects or a tendency were noticed only in the livers of rats fed the diet containing $_{L}$Se or $_{L}$Se and CLAmix ($P < 0.05$, $P < 0.1$, for the interactions, respectively). On the other hand, no statistically significant differences in the values of the PUFA/SFA and PUFA/ΣFA ratios in muscles were observed among all rat groups. Similarly, dietary treatments did not affect the values of the UFA/ΣFA ratios in the liver and muscles.

The simultaneous addition of CLAmix and SeY (as $_{L}$Se or $_{H}$Se) to the diet significantly increased the concentration of the sum of all assayed fatty acids (ΣFA) in the liver (the interaction: $P < 0.05$), while the diet containing only $_{L}$Se, $_{H}$Se or CLAmix non-significantly elevated the concentration of ΣFA in this organ. Dietary $_{L}$Se or $_{H}$Se treatments reduced ($P < 0.05$) the concentration of ΣFA in muscles in comparison with the control group, whereas the addition of CLAmix to the diet, regardless of the presence of SeY, non-significantly decreased the concentration of ΣFA in muscles.

The values of the PUFAn-6/PUFAn-3 ratio in the liver were significantly decreased ($P < 0.01$) by dietary SeY (as $_{L}$Se or $_{H}$Se) or CLAmix, whereas no statistically significant interactions of CLAmix × SeY (as $_{L}$Se or $_{H}$Se) were observed ($P < 0.1$ and $P > 0.1$, respectively). The diet enriched with CLAmix resulted in a statistically significant increase ($P < 0.01$) in the PUFAn-6/PUFAn-3 ratio in muscles. Similarly, interactions of CLAmix × SeY (as $_{L}$Se or $_{H}$Se) caused an increase in the ratio of PUFAn-6/PUFAn-3 in muscles, although no significant differences were observed ($P < 0.1$ and $P > 0.1$, respectively).

The CLAmix diet resulted in higher values of Δ4-, Δ5-desaturase (i.e. $\Delta4_{index}$, $\Delta5_{index}$) and elongase ($Elong_{index}$) indexes in the liver ($P < 0.05$, $P < 0.1$, $P < 0.05$, respectively) compared with the control group (Table 4). Similarly, this diet caused an increase in the $\Delta4_{index}$, $\Delta5_{index}$ and $\Delta6_{index}$ in muscles ($P < 0.01$) and significantly reduced ($P < 0.01$) the elongase index compared with the control group. The addition of SeY (as $_{L}$Se or $_{H}$Se) to the diet, regardless of the presence of CLAmix, exerted a small influence on the values of desaturase indexes in the liver and muscles, with the exception of $\Delta4_{index}$ values in the liver and muscles of rats fed the diet with CLAmix and SeY (as $_{L}$Se or $_{H}$Se). The diet enriched with $_{L}$Se or $_{H}$Se resulted in small changes in the values of the $Elong_{index}$ in the liver and muscles. Surprisingly, the diet containing only CLAmix considerably decreased ($P < 0.01$) the value of the $Elong_{index}$ in muscles.

DISCUSSION

The present studies demonstrate the ability of supplemented Se-yeast, regardless of the presence of CLAmix in the diet, to increase the body weight gain of the examined animals. Fortunately, neither macroscopic lesions nor toxic symptoms of SeY and CLA isomers were observed in rats fed experimental diets. Indeed, diets containing up to 2 mg Se/kg would not be toxic to rodents (like rats or mice) (McDowell et al., 2005; Korniluk et al., 2006).

The results of this study indicate that dietary 0.5 μg Se/g diet as SeY exerts an inhibitory effect on the BWG in comparison with the diet enriched with 0.2 ppm Se. Thus, we argue that the diet containing 0.5 μg Se/g diet catalyses hydrosulphide oxidation, which inhibits protein synthesis in rats (Navarro-Alarcon and Cabrera-Vique, 2008). Indeed, the diet containing the higher content of Se (i.e. 0.5 μg Se/g diet) most efficiently decreased the concentrations of Met, EAA and NAA in muscles.

Considering our previous studies (Czauderna et al., 2004a,b; Korniluk et al., 2006) and the present

study, we argue that only the lower concentration of Se (i.e. 0.2–0.5 μg Se/g diet) in the diet, regardless of the presence of CLA isomers, elevated body weight gain and improved feed conversion efficiency. Our previous and present studies support the concept that a Se pro-oxidative effect of diets containing 1.2–2 μg Se/g diet (Czauderna et al., 2004a,b; Korniluk et al., 2006) is due to the catalysis of hydrosulphide oxidation that results in an inhibitory effect on the yield of protein synthesis (Navarro-Alarcon and Cabrera-Vique, 2008). Moreover, the supranutritional levels of Se in diets may act as a pro-oxidant, which can result in peroxidative damage of unsaturated fatty acids (particularly PUFA) as well as in stimulation of oxidative stress in living organisms (Czauderna et al., 2004a,b; Juniper et al., 2008). Consequently, a diet containing 2 μg Se/(g diet) as selenate reduced body weight gain and feed conversion efficiency in rats (Czauderna et al., 2003, 2004a,b), while the diet enriched with 0.2 μg Se/(g diet) as SeY most effectively increased body weight gain and feed conversion efficiency (Table 2) as well as considerably increased protein synthesis in the liver, as the concentration of amino acids (AA) in this organ was higher than in control rats and practically similar to the concentration of AA in the liver of rats fed the diet with 0.5 μg Se/g diet as SeY.

The present study demonstrated that the addition of CLAmix to a diet enriched with $_{L}$Se or $_{H}$Se positively correlated with the accumulation of Se in liver and muscles. Our present studies are thus in agreement with our previous investigations (Czauderna et al., 2007b) in which the addition of an individual isomer (1%) or an isomer mixture of CLA (2%) to a diet enriched with SeY (i.e. 1.2 μg Se/g diet) also stimulated the accumulation of Se in the liver and femoral muscles of rats. The possible explanation is that dietary CLAmix leads to increased oxygen consumption associated with significantly increased oxidation of fat (Belury, 2002). This higher oxygen consumption and formation of fat-oxidation products stimulated the biosynthesis of the Se-proteins that mainly protect against oxidative stress (Schomburga et al., 2004; Suzuki, 2005). So, we suggest that the stimulation of Se-protein biosynthesis by dietary CLAmix resulted in increasing the concentration of Se in the liver and muscles of rats fed the diet enriched with both CLAmix and SeY (as $_{L}$Se or $_{H}$Se).

The present study also investigated relationships between the experimental diets and the concentrations of CLA isomers in the liver and muscles. The addition of 0.2 and 0.5 μg Se/g diet to the diet enriched with CLAmix stimulated the incorporation of CLA isomers, with the exception of *t*,*t* isomers of CLA, in the liver; the concentrations of isomers increased as the Se content in the diet increased. Therefore, we suggest that the low level of Se in the Labofeed H diet (i.e. 0.13 μg Se/g as the natural content of Se and extra 0.2 μg Se/g as selenite; Table 1) is the main reason why the 7-day preliminary period followed by dietary extra Se stimulated the assimilation of Se into the rats' organisms. Considering the above, we supposed that the metabolites of Se in the liver positively interacted with CLA isomers. On the other hand, the addition of the lower amount of SeY ($_{L}$Se) to the diet containing CLAmix resulted in the increase in concentrations of *cc*, *c*9t11, *t*10*c*12 and in the sum of CLA isomers (ΣCLA) in muscles. The chemical form of Se added to the diet is relevant to this finding; Se-Met, the main component of dietary SeY, is incorporated into muscle protein in place of methionine (Weiss and Hogan, 2005). So, it seems reasonable to suggest that formed Se-Met-proteins in muscles stimulated the incorporation of these CLA isomers into muscles. On the other hand, the addition of the higher amount of Se (0.5 μg Se/g diet] to the diet containing CLAmix reduced the concentrations of *cc*, *c*9*t*11, *t*10*c*12 and ΣCLA isomers and elevated the level of Se in muscles. Considering these facts, we argue that the higher concentration of Se in muscles exerts the antagonistic and pro-oxidative effects on *cc*, *c*9*t*11, *t*10*c*12 and ΣCLA isomers in muscles.

Our present results (Table 3) are in agreement with the observations of Alasnier et al. (2002) and our previous studies (Czauderna et al., 2003, 2004a,b, 2007b; Korniluk et al., 2006) in which *t*10*c*12CLA and *t*10*t*12CLA are more efficiently driven through β-oxidation in cells of liver, muscles, kidneys or adipose tissue in rodents than their 9,11 homologues. Therefore, in the present studies, the values of the ratio (R = *c*9*t*11CLA/*t*10*c*12CLA) of these isomers in the liver (1.958–2.090) and muscles (1.587–1.654) of rats fed the diet enriched with CLAmix, regardless of the presence of SeY, were higher compared with the same ratio in the CLA isomer mixture (CLAmix) added to the Labofeed H diet (R = 0.988). Moreover, the present studies clearly indicate that the β-oxidation rate of *t*10*c*12CLA relative to the β-oxidation rate of *c*9*t*11CLA is higher in the liver than in muscles.

Our previous experiments (Czauderna et al., 2004b) demonstrated that dietary *t*,*t* isomers of CLA are preferentially metabolised in comparison with the metabolism of dietary *c*,*c*, *c*9*t*11 and *t*10*c*12CLA isomers. Considering our current results and previous studies (Czauderna et al., 2004b) it can be concluded that the addition of Se (as SeY or selenate) to the diet containing CLA isomers reduced the metabolism of *t*,*t* isomers of CLA in comparison with the diet containing only CLA isomers. Thus, dietary inorganic Se (as selenate) and organic Se (as SeY) exhibited a similar influence on the metabolic capacity of tt isomers of CLA in rats.

As expected, the diets containing CLAmix, irrespective of the presence of SeY, decreased the Δ9-desaturation capacity of C18:0. This is consistent with our previous results (Czauderna et al., 2003, 2004a,b, 2007b; Korniluk et al., 2006) showing that CLA isomers and/or selenate are responsible for a change in the levels of monounsaturated (MUFA) and saturated fatty acids (SFA) in tissues of animals fed CLA isomer-enriched diets due to a decrease in the Δ9-desaturation of FA such as C16:0 or C18:0 (Alasnier et al., 2002). Recent studies (Alasnier et al., 2002; Belury, 2002; Kang et al., 2004; Korniluk et al., 2006; Czauderna et al., 2007b) have demonstrated that CLA isomers, particularly *t*10*c*12CLA, caused a reduction in Δ9-desaturase capacity, inhibited stearoyl-CoA desaturase mRNA expression and fatty acid synthesis (e.g. compare ΣFA concentrations in muscles, Table 3). Similarly, in the present study, the value of the Δ9-desaturase index in the liver and muscles of rats fed a diet enriched with CLAmix and/or SeY decreased compared with that in the liver and muscles of control rats (Table 3). The experimental diet enriched only with SeY also decreased the Δ9-desaturase capacity, and thus the concentration of *c*9C18:1 in both tissues (Table 3), while the concentration of C18:0 in the liver and muscles increased (Table 4). Similarly, the addition of inorganic Se as selenate to diets decreased also the concentration of *c*9C18:1 in the liver, femoral muscles and spleen of rats (Czauderna et al., 2004a,b; Niedźwiedzka et al., 2006).

The diet enriched with CLAmix resulted in increased values of the atherogenic index (A_{index}) in the liver and muscles of rats, likewise, the addition of CLAmix to the diet with SeY also caused an increase in the values of the A_{index} and thrombogenic index (T_{index}) in muscles. Considering the above results, it seems reasonable to suggest that dietary CLAmix can be responsible for accelerating atherogenesis and increasing platelet aggregation (Ulbricht and Southgate, 1991). Fortunately, the diets enriched with 1.5% CLAmix, irrespective of the presence of SeY, increased the concentrations of αLNA, C22:6n-3, the sum of polyunsaturated fatty acids n-3 (PUFAn-3) and the ratio PUFAn-6 to PUFAn-3 (PUFAn-6/PUFAn-3) in the liver. On the other hand, the diets enriched with CLAmix with or without SeY decreased the accumulation concentrations of αLNA, C22:6n-3 and the value of PUFAn-6/PUFAn-3 in muscles. So, the present results are in agreement with our previous studies (Korniluk et al., 2006) showing that a diet containing less than 2% of CLA mixture increased the concentration of αLNA, C22:6n-3 and PUFAn-3 in the liver, whereas diets containing 1 or 2% of CLA mixture decreased the concentration of these fatty acids in femoral muscles of rats (Czauderna et al., 2007b). The possible mechanism through which the liver accumulation of long-chain PUFAn-3 (especially C22:6n-3) was increased by CLA isomers, particularly *t*10*c*12, is by changes in the capacity of Δ6-, Δ4-desaturations and elongation of these long-chain PUFAn-3 to very-long chain PUFAn-3 (Alasnier et al., 2002; Heird and Lapillonne, 2005). Moreover, these dietary CLA isomers inhibited enzymes involved in the synthesis of eicosanoids from long-chain PUFAn-3 (Belury, 2002; Heird and Lapillonne, 2005). Another reason may be that elongated and desaturated metabolites of CLA isomer(s) compete for the same enzymes as long-chain PUFAn-3. The above explanations are consistent with our results regarding the sum of concentrations of all assayed fatty acids (ΣFA) in the liver of rats fed the diet containing CLAmix with or without SeY. Indeed, dietary CLAmix and/or SeY resulted in an increase in the content of ΣFA in the liver (i.e. stimulated symptoms of fatty liver).

The diet containing CLAmix with or without SeY reduced the concentrations of assayed fatty acids in muscles; indeed, dietary CLA isomers reduced the adiposity of animals (Belury, 2002). The ability of CLA isomers to reduce adipose tissue was linked with the stimulation of adipocyte apoptosis and/or differentiation. Moreover, dietary CLA isomers elevated oxygen consumption concomitantly with increased oxidation of fat in animals (Belury, 2002). Considering the above results, we suggest that dietary CLAmix, irrespective of the presence of SeY, decreased

the nutritive value of meat of monogastric animals, as the value of the PUFAn-6-to-PUFAn-3 ratio increased in femoral muscles of rats fed the diet containing CLAmix with or without SeY. Fortunately, no noticeable harmful influence on the values of the PUFA/SFA, PUFA/ΣFA and UFA/ΣFA ratios in muscles of rats fed the experimental diets was found out.

The present study documented that the influence of diets containing CLAmix with or without SeY on the capacity of Δ4-, Δ5-, Δ6-desaturases and elongase was tissue-specific. In truth, the experimental diets usually elevated the capacity of these desaturases and decreased the yield of elongase only in muscles. On the other hand, the diet enriched with CLAmix elevated the elongase index ($Elong_{index}$) in the liver with the result that the concentration of C22:6n-3 in this organ increased (Table 4).

CONCLUSIONS

Feeding CLA isomers, irrespective of the addition of dietary SeY, increased the concentration of CLA isomers in the liver and muscles of rats. Our results documented that the addition of CLAmix to the diet with SeY stimulated the accumulation of Se in the liver and muscles. The finding that CLAmix and 0.5 μg Se/g diet as SeY fed to animals considerably decreased the content of the sum of all assayed fatty acids and most efficiently increased the concentration of Se in the muscles is valuable information for nutritionists carrying out research on farm animals to improve the nutritive value of food from the aspect of human health.

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