The effect of conjugated linoleic acid isomers, selenite and selenized yeast supplemented to the diet on the fatty acid profile in the pancreas of rats[*]

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ABSTRACT

The influence of dietary conjugated linoleic acid (CLA) isomers (CLAmix) and/or Se as Na_2SeO_3 (SeIV) or selenized yeast (SeY) on the relative body weight gain (RBWG) of rats, pancreas weight, concentrations of CLA isomers and of other fatty acids (FA) in the pancreas were studied. For 6 weeks, female rats Wistar (aged 8 weeks) were fed *ad libitum* the Labofeed H diet enriched in 1.5% CLAmix, 0.2 ppm Se ($_L$Se), 0.5 ppm Se ($_H$Se) as SeIV (i.e. $_L$SeIV or $_H$SeIV, respectively), or SeY (i.e. $_L$SeY or $_H$SeY, respectively). Each group contained eight rats. The results documented that dietary $_L$SeIV and CLAmix most effectively increased the RBWG of rats and feed conversion efficiency, while the diet with $_H$SeIV most efficiently increased pancreas weight compared with the control rats. The diets enriched in CLAmix and Se as SeIV or SeY increased the sum of saturated fatty acids (SFA), the atherogenic SFA, A-SFA index, thrombogenic SFA (T-SFA), and the T-SFA index in the pancreas of rats compared with the control group. The diets containing CLAmix and Se as SeIV or SeY stimulated the accumulation of *cis9trans11*CLA, *trans10cis12*CLA, as well as the sum of all CLA isomers in the pancreas compared with rats fed the diet enriched in CLAmix. These diets increased the concentration of n-6 polyunsaturated FA (n-6 PUFA) and decreased the level of long-chain n-6 PUFA (Ln-6 PUFA) in the pancreas compared with the control rats and rats fed the diets with SeIV or SeY. The diets containing CLAmix and Se (as SeIV or SeY) increased the concentration of n-3 PUFA and Ln-3 PUFA in the pancreas compared with the control rats. Our study findings documenting that dietary Se and/or CLAmix and Se (as SeIV or SeY) considerably increased the concentration of PUFA, especially n-3 PUFA, improved the n-6 PUFA/n-3 PUFA ratio in the pancreas are valuable information for scientists carrying out research to improve animal and human health.

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INTRODUCTION

Selenium (Se) is an essential trace element for humans and animals. Se is a component of a number of important Se-enzymes required for such functions as anticancer defence, reduction of inflammation, DNA synthesis, fertility, reproduction and thyroid hormone production (e.g., thyroid hormone deiodinase families or thioredoxin reductase) (Rayman, 2004; Suzuki, 2005; Boosalis, 2008; Juniper et al., 2008; Navarro-Alarcon and Cabrera-Vique, 2008). Moreover, previous investigations have shown that feeding diets enriched in selenium (Se) as selenite (SeIV), selenate (SeVI), or selenized yeast (SeY) to animals results in effective induction of antioxidant enzymes, indicating that the animal converts the dietary form of Se to Se-cysteine (Se-Cys), which is an essential amino acid for the synthesis of these Se-proteins (e.g., glutathione peroxidise family) (Whanger, 2004; Navarro-Alarcon and Cabrera-Vique, 2008; Yu et al., 2008). Therefore, these protective effects of dietary Se on unsaturated fatty acids (UFA), especially conjugated linoleic acid (CLA) isomers, in tissues and organs of mammals may also be plausible. Indeed, in our previous studies we found that dietary Se (as SeIV, SeVI or SeY) and/or CLA isomers influence the concentration of fatty- and amino acids in the liver, femoral muscles, heart, kidneys or spleen of rats (Czauderna et al., 2004, 2007a, 2009a,b; Korniluk et al., 2007). Considering the above, we hypothesized that a diet enriched in CLA isomers with or without selenite (SeIV) or selenized yeast (SeY) affected the concentration of fatty acids in the pancreas of rats. The pancreas is an important dual-function gland, having features of both endocrine and exocrine glands. The part of the pancreas with endocrine function is made up of cell clusters called islets of Langerhans. In contrast to the endocrine pancreas, which secretes hormones into the blood, the exocrine pancreas synthesizes digestive enzymes and pancreatic juice, and secretes them into the small intestine through a system of exocrine ducts in response to the small-intestine hormones, secretin and cholecystokinin. Digestive enzymes include trypsin,chymotrypsin, pancreatic lipase, and pancreatic amylase, and are produced and secreted by acinar cells of the exocrine pancreas.

In our recent studies we found that the addition of SeVI or SeY to a diet enriched in CLA isomers affected the deposition of CLA isomers in tissues and selected organs of rats (Czauderna et al., 2004, 2007a, 2009a,b; Korniluk et al., 2007). Therefore, the objective of our study was to investigate the influence of different chemical forms of Se added to a diet on the deposition of CLA isomers and other fatty acids (FA) in the rat pancreas, which has features of endocrine and exocrine glands.

MATRERIAL AND METHODS

Animals, housing, diets, experimental design and sampling

The experiment was conducted with 80 female rats (Wistar, Hsd Brl Han: WIST), aged 8 weeks with an initial body weight of 195.7±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 rats 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 the 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.wpmorawski.com.pl/Labofeed/labofeed). The Labofeed H diet has been produced according to ISO 9001 Standards since 1998. For our studies, this commercial diet containing 0.13 ppm Se (the natural content of Se) was enriched in only 0.2 ppm Se as selenite (Table 1). Therefore, the total concentration of Se in this commercial rat diet was 0.33 ppm Se.

During the one-week preliminary period the rats were fed the standard Labofeed H diet offered at a sub-maintenance level to reduce body fat (Table 1). Next, for 6 weeks the rats were fed *ad libitum* the experimental diets supplemented with 1.5% CLA isomer mixture (CLAmix), 0.2 ppm Se or 0.5 ppm Se as Na_2SeO_3 (i.e. $_LSeIV$ or $_HSeIV$, respectively) and SeY (i.e. $_LSeY$ or $_HSeY$, respectively) (Table 1). Feed intake and body weight of rats were measured weekly. At the end of the six-week experiment the rats were euthanized by CO_2. The pancreases were removed, weighed, and frozen until analysed. All tissue samples from each rat were analysed individually. The concentrations of all fatty acids (FA) were calculated based on freeze-dried pancreas samples.

Reagents

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 the Industrial Chemistry Research Institute (Warsaw, Poland). The concentration ratio ($R_{c9t11CLA/t10c12CLA}$) of *c9t11*CLA to *t10c12*CLA in the dietary CLA isomer mixture was 0.988. Fatty

Table 1. Dietary effects of 1.5% CLA isomer mixture (CLAmix) and selene (SeIV) or selenized yeast (SeY) on the relative body weight gain (RBWG), total feed intake (per rat), feed conversion efficiency (FCE; g body weight gain/g feed intake), and pancreas fresh masses (g) of rats after 6 weeks feeding with experimental diets[1]

Group	Additives	Body weight, g		Relative body weight gain[2], weeks			Total feed intake[4] g	FCE[5] g/g	Pancreas mass[5] g
		Initial $BW_{initial}$	Adapted[3] $BW_{adapted}$	2	4	6			
Control[6]	-	196.0	180.4	0.2192^a	$0.2597^{q\beta}$	0.277^{aa}	737.2^{ab}	0.068	0.967^a
$_L$SeIV	0.2 ppm SeIV	194.7	176.9	0.2135^a	$0.2807^{q\lambda}$	0.302	750.2	0.079	1.026^β
$_H$SeIV	0.5 ppm SeIV	195.7	180.1	0.2026^b	0.2726^A	0.317^a	764.4^a	0.075	1.109^{ab}
$_L$SeY	0.2 ppm SeY	196.7	179.2	0.2300^β	0.2785^β	0.324^a	761.4^b	0.076^a	1.061
$_H$SeY	0.5 ppm SeY	195.0	177.3	0.2146	0.2755	0.313	738.0	0.075^c	0.965
CLA	CLAmix	196.5	181.7	0.2013^a	0.2544^γ	$0.286^{b\beta}$	712.3^c	0.069^b	1.050^a
$_L$SeIV$_{CLA}$	0.2 ppm SeIV CLAmix	195.3	180.3	0.1899^a	0.2609^λ	0.304	723.5	0.075	$0.928^{q\beta}$
$_H$SeIV$_{CLA}$	0.5 ppm SeIV CLAmix	194.7	177.0	0.2286^{bx}	$0.3025^{\lambda\gamma x}$	0.339^{bx}	735.5	0.081	0.982^b
$_L$SeY$_{CLA}$	0.2 ppm SeY CLAmix	194.9	178.3	0.2074^β	0.2811	0.314^β	766.1^{cx}	0.073^{abx}	0.979
$_H$SeY$_{CLA}$	0.5 ppm SeY CLAmix	194.7	177.4	0.2104	0.2763	0.306	716.7	0.076^{cy}	0.938

[1] means in columns sharing the same letter are significantly different: ^{a,b}P <0.05 and ^{A,B}P <0.01; $^{a,\beta}$P<0.1 differences were taken as tendencies; interactions of CLAmix x $_L$Se and CLAmix x $_H$Se (Se as SeIV or SeY), significant at ^{x,y}P<0.05 and ^{X,Y}P<0.01, respectively; [2] the Relative body weight gain (RBWG) after feeding for 2, 4 and 6 weeks with the experimental diets enriched in CLAmix with or without Se (as $_L$SeIV, $_H$SeIV, $_L$SeY or $_H$SeY); RBWG = (BW$_{2, 4 or 6 weeks}$ - BW$_{adapted}$)/BW$_{adapted}$ (BW - the body weight); the ratio of $c9t11$CLA to $t10c12$CLA in the dietary CLA isomer mixture: 0.988

[3] 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 body weight of all adapted rats: 179.0 ± 6.1 g

[4] during the whole experimental period (42 days);

[5] average pancreas fresh masses

[6] the standard Labofeed H diet contained 0.2 ppm Se as selenite and 0.13 ppm Se as the natural content of Se

acid methyl ester (FAME) standards, sodium selenite (Na_2SeO_3) and 50% BF_3 in methanol were purchased from Supelco and Sigma (USA).

The selenized yeast (Se-*Saccharomyces cerevisiae*) was donated by Sel-Plex (a non-commercial yeast sample; Alltech In., USA). About 83% of the total Se concentration of selenized yeast (SeY) represents Se in the form of Se-methionine (Se-Met) incorporated into proteins of *Saccharomyces cerevisiae* (Rayman, 2004; Weiss and Hogan, 2005).

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).

Analytical methods

The methods of alkaline hydrolysis and base- and acid-catalysed methylation of fatty acids (i.e. the derivatization) were as previously described (Czauderna et al., 2009a). 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. (2007b).

The concentrations of methylated FA (i.e. FAME and CLA-ME) in pancreases were calculated based on freeze-dried pancreas 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 μ m 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/min. Injector and MS detector temperatures were maintained at 200 and 240ºC, respectively. The total FAME profile in a one μl sample at the split ratio of 10:1 was determined using the column temperature gradient programme, which was as previously described (Czauderna et al., 2009a).

FAME identification was validated based on electron impact ionization spectra of FAME and compared with authentic FAMS standards and the NIST **(wyjaśnić skrót!)** 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 x 4.6 mm Chrompack ChromSper 5 μm Lipids, the Netherlands) and a Waters 996 photodiode array detector (Czauderna et al., 2007b).

Statistical analyses

Results are presented as means±SD of 8 individually analysed samples of rat pancreases. 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 $_L$Se ($_L$Se$_{CLA}$) and CLAmix and $_H$Se ($_H$Se$_{CLA}$) 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 labeled tendencies. Statistical analyses were performed using the Statistica v. 6 software package (Statistica, 2002; www.statsoft.pl).

RESULTS AND DISUSSION

The influence of dietary CLA isomers and Se on rat body weight gain and pancreas weight. Our results regarding the concentration of additives in the diets as well as the influence of the examined diets on the growth performance of rats are summarized in Table 1. As expected, no macroscopic lesions or pathological changes were found in the pancreas or other internal organs and tissues of rats fed the diets enriched in CLA isomers and/or 0.2 ppm or 0.5 ppm Se as SeIV or SeY. Thus, the current results are consistent with our recent studies (Korniluk et al., 2007; Czauderna et al., 2009a,b) corroborating that only chronic feeding of Se compounds at a rate of more than 5 ppm Se can be teratogenic and hepatotoxic in animals and humans (Tapiero et al., 2003).

It was found that supplementation of the diet with CLAmix tended to decrease (P=0.07) the relative body weight gain (RBWG) of rats compared with the control rats during the two weeks of the experiment (Table 1). On the other hand, the effects of dietary 1.5% CLAmix on body weight gain were rather small after 6 weeks of the experimental period. It seems reasonable to assume that dietary CLAmix is responsible, especially after 2 weeks of the experiment, for the reduction of fat, whereas these isomers in the diet after 4 weeks of the experimental period stimulated the accumulation of water (Javadi et al., 2007). Indeed, a negative correlation between body fat and body water content is observed in rodents fed the diet enriched in CLA isomers (Javadi et al., 2007).

Interestingly, the addition of 0.2 ppm Se as SeIV to the diet containing CLAmix exhibited a stronger influence on diminishing the RBWG of rats compared with the control rats, and especially, on the body gain of rats fed the diet with $_L$SeIV (P<0.05) during the two weeks of the experiment. Our results also documented that the diet with the higher concentration of SeY (organic Se), regardless of

the presence of CLAmix, had an inhibitory effect on body protein synthesis (Navarro-Alarcon and Cabrera-Vique, 2008) compared with the diets containing the lower level of SeY. On the other hand, the diet with the higher content of SeIV (inorganic Se), especially containing CLAmix, was not associated with the detrimental effect of Se accumulated in rat bodies. The explanation for these effects is that the accumulation of Se is lower in bodies of rats fed the diet with inorganic Se in comparison with dietary organic Se (Czauderna et al., 2007a, 2009a,b; Niedźwiedzka et al., 2007). Our results documented that dietary $_L$SeIV and CLAmix is the most beneficial for rats. This conclusion is supported by the higher values of RBWG of rats and feed conversion efficiency (FCE) (Table 1). Similarly, the diet containing $_H$SeIV, $_L$SeY or $_H$SeY, irrespective of the presence of CLAmix, numerically increased FCE values compared with the control rats. Moreover, in our studies, the diet enriched to a higher concentration of SeIV ($_H$SeIV) or the lower concentration of SeY ($_L$SeY), effectively increased the RBWG of rats in comparison with the control rats, especially after 6 weeks of the experiment. Furthermore, the diet containing the lower concentration of SeY was more efficient than the diet with the higher concentration of SeIV in increasing RBWG. These results might be associated with the more efficient retention of organic Se in tissues of animals compared with dietary inorganic Se (Han et al., 2009). On the other hand, the higher concentration of SeY ($_H$SeY) in the diet more efficiently reduced rat growth (due to upregulation of anabolic processes in rats) compared with dietary $_L$SeY. Indeed, our recent papers documented that dietary Se as SeY was accompanied by higher retention of Se in rat tissues in comparison with selenite or selenate (Czauderna et al., 2004, 2007a, 2009a,b; Niedźwiedzka et al., 2007). We suggest that the higher retention of Se in the rats fed the diet with $_H$SeY upregulated anabolic processes in rats.

On the other hand, the addition of 1.5% CLAmix to the diet decreased total feed intake and FCE. Our results are supported by several other studies, which have indicated that CLA isomer *t10c12* in particular, due to its geometric and positional structure, is the most potent CLA isomer in terms of antiobesity activity, thus, the one which most efficiently reduces feed intake, body weight gain, as well as increases energy expenditure (Alasnier et al., 2002; Belury, 2002; Czauderna et al., 2007a; 2009a,b; Niedźwiedzka et al., 2007).

As can be seen from the data in Table 1, the diet containing $_L$SeIV, $_H$SeIV, $_L$SeY or CLAmix numerically or statistically increased pancreas weight compared with the control group. Thus, it can be concluded that less available inorganic Se (i.e. dietary $_L$SeIV or $_H$SeIV) and lower amounts of dietary SeY stimulated pancreas growth. On the other hand, the higher amount of dietary organic Se ($_H$SeY) had a detrimental effect on pancreas growth in comparison with rats fed the diets enriched in $_L$SeIV, $_H$SeIV or $_L$SeY. Indeed, the higher amount of dietary organic

Se as $_{H}$SeY resulted in greater deposition of Se in the pancreas and other organs compared with dietary $_{H}$SeIV or $_{L}$SeY. Thus, we suggest that the high pancreatic concentration of Se (mainly as Se-methionine) catalyses hydrosulphide oxidation (Javadi et al., 2007) which exerts an inhibitory effect on pancreas growth compared with rats fed the diets enriched in $_{L}$SeIV, $_{H}$SeIV, $_{L}$SeY or CLAmix. On the other hand, the CLAmix x Se interactions (regardless of the level and chemical form of Se) did not influence pancreas weight (Table 1).

Effect of the diets with CLAmix and Se on pancreatic contents of saturated and monounsaturated FA, and CLA isomers. One of the most prominent effects of dietary supplementation with CLAmix and Se as SeIV or SeY (regardless of the concentration of extra Se), is an increase in the sum of saturated fatty acids (SFA), atherogenic SFA (A-SFA), A-SFA index, and thrombogenic SFA (T-SFA), T-SFA index, as well as individual saturated fatty acids such as C14:0, C16:0, in the pancreas of rats compared with the control group (Tables 2 and 3). This effect on the concentration of these fatty acids might be due to the lower capacity of Δ9-, Δ6- and Δ5-desaturase in the pancreas of rats fed the diet with CLAmix and Se as SeIV or SeY (Table 3). Moreover, this decrease in the capacity of these desaturases is concomitant with the decrease in the pancreatic concentration of *c9*C18:1, the sum of monounsaturated fatty acids (MUFA), and with the increase in the A-SFA and T-SFA indexes.

Interestingly, the diet enriched in $_{L}$SeY decreased the concentration of SFA, T-SFA, as well as individual saturated fatty acids such as C14:0, C16:0 in the pancreas (Table 2). Unfortunately, this diet also resulted in a decrease in the capacity of Δ9-desaturase in this organ (Table 3). Consequently, the addition of the lower amount of SeY to the diet decreased the concentration of *c9*C18:1, as well as MUFA in the pancreas of rats.

The diets containing CLAmix and Se as SeIV or SeY (regardless of the level of extra Se) stimulated the accumulation of *c9t11*CLA, *t10c12*CLA, as well as the sum of all assayed CLA isomers in the pancreas in comparison with rats fed the diet enriched in only CLAmix. Our studies documented that the concentration ratio of *c9t11*CLA to *t10c12*CLA in the pancreas of rats fed the diets enriched with the CLAmix was, regardless of the presence of extra Se, higher compared with the ratio ($R_{c9t11CLA/t10c12CLA}$) of these isomers in the CLA isomer mixture added to the diets (i.e. 1.82-1.52 vs 0.988; see Tables 1 and 2). Thus, our present results are in agreement with those of Alasnier et al. (2002) and our previous study (Czauderna et al., 2007a, 2009a,b, 2010), in which *t10c12*CLA and *t10t12*CLA were also more efficiently driven through β-oxidation in the tissues of the examined animals than their *9,11* CLA-homologues. Interestingly, our studies documented that the $R_{c9t11CLA/t10c12CLA}$ values were lower in the pancreas of rats fed the diet containing CLAmix and Se (as SeIV or SeY) compared with

Table 2. The effect of the diets enriched in 1.5% CLAmix, 0.2 ppm ($_L$) or 0.5 ppm ($_H$) Se as selenite (SeIV) or selenized yeast (SeY) on the concentration of C14:0 (mg/g), C16:0 (mg/g), c9C18:1 (mg/g), LA (mg/g), α-LNA (mg/g), c6c9c12C18:3 (γ-LNA; μg/g), individual CLA isomers and the sum of CLA isomers (ΣCLA; μg/g), c8c11c14C20:3 (C20:3n-6; μg/g), c11c14c17C20:3 (C20:3n-3; mg/g), c5c8c11c14C20:4 (C20:4n-6; μg/g), c5c8c11c14c17C20:5 (C20:5n-3; μg/g), c7c10c13c16c19C22:5 (C22:5n-3; μg/g), c4c7c10c13c16c19C22:6 (C22:6n-3; μg/g), the sum of saturated FA (SFA; mg/g), atherogenic SFA (A-SFA; mg/g) and thrombogenic SFA (T-SFA; mg/g) in the pancreas of rats

Item	Control	$_L$SeIV	$_H$SeIV	$_L$SeY	$_H$SeY	CLA	$_L$SeIV	$_H$SeIV	$_L$SeY	$_H$SeY	$_L$SeIV	$_H$SeIV	$_L$SeY	$_H$SeY	CLA	$_L$SeIV	$_H$SeIV	$_L$SeY	$_H$SeY
	Control					CLA					One factorial analysis					Interactions, CLA x			
C14:0	0.96	0.86	0.97	0.67	0.87	0.89	1.14	1.07	1.18	1.07	N	N	N	N	N	α	N	N	α
C16:0	20.8	19.9	21.1	17.3	21.1	22.0	29.2	28.1	27.4	26.0	N	N	N	N	N	**	*	*	N
c9C18:1	19.7	18.1	18.9	14.4	18.5	16.2	20.9	23.5	19.2	19.6	N	N	N	N	N	α	**	*	N
LA	22.4	23.6	23.7	20.6	24.2	23.8	28.6	27.1	27.3	26.0	*	**	α	α	α	*	**	α	N
γ-LNA	77	124	155	89	125	42	28	18	15	1.0	N	**	N	N	α	*	**	α	**
α-LNA	6.4	7.2	7.7	6.0	7.2	6.5	7.4	6.6	6.8	6.3	N	N	N	N	N	N	N	N	N
c9t11CLA	0.62	0.79	0.76	0.26	3.7	2653	3071	3027	3147	2805	N	N	N	N	**	N	N	N	N
t7c9CLA	1.02	-[2]	-	-	-	129	163	40	164	146	N	N	N	N	**	N	N	N	N
c1t13CLA	0.43	-	-	-	-	557	348	347	383	340	N	N	N	N	**	N	N	N	N
t10c12CLA	-	-	-	-	0.83	1461	2018	1923	2013	1763	N	N	N	N	**	N	N	N	N
c9c11CLA	-	-	0.14	-	-	48	49	45	45	48	N	N	N	N	**	N	N	N	N
ΣCLA[1]	2.09	0.82	0.93	0.31	4.65	4899	5697	5413	5791	5143	N	*	N	N	**	-	-	-	-
Ratio$_{isomers}$[3]	-	-	-	-	-	1.82[a]	1.52[b]	1.57[b]	1.56[b]	1.59[b]	-	-	-	-	-	-	-	-	-
C20:3n-6	67	105	91	80	91	40	44	30	50	47	*	N	N	N	*	*	N	N	N
C20:3n-3	1.87	2.20	2.21	2.43	2.34	1.85	2.54	2.57	2.82	2.52	N	N	N	*	N	*	α	α	N
C20:4n-6	24	40	43	36	38	5	4	1	6	8	*	*	α	N	**	*	N	N	N
C20:5n-3	565	743	742	849	807	558	728	654	706	722	α	**	**	*	N	*	N	**	N
C22:5n-3	206	372	407	398	389	250	315	291	341	325	**	**	**	**	N	*	N	**	N
C22:6n-3	286	413	443	473	453	287	393	424	473	432	*	*	*	N	N	N	N	α	N
SFA	28.5	27.1	28.5	23.9	28.7	29.6	39.5	37.6	37.3	35.3	N	N	N	N	N	**	N	*	α
A-SFA[4]	21.8	20.8	22.2	21.3	22.1	23.0	30.4	29.2	28.7	27.2	**	**	*	*	N	**	**	**	**
T-SFA[5]	27.7	26.4	27.7	23.3	28.0	29.0	38.7	36.9	36.5	34.5	N	N	N	N	N	**	α	**	α

[1] ΣCLA - the sum of assayed tt, ct/tc and cc isomers of CLA; [2] below the quantification limit, L_Q (i.e. L_Q = 3 x the detection limit)

[3] the concentration ratio of c9t11CLA to t10c12CLA; means in rows sharing the different letter are significantly different: [a,b]P <0.05

[4] A-SFA: the sum of: C12:0, C14:0 and C16:0; [5] T-SFA: the sum of C14:0, C16:0 and C18:0

Table 3. The effect of diets enriched in 1.5% CLAmix and 0.2 ppm (L) or 0.5 ppm (H) Se as selenite (SeIV) or selenized yeast (SeY) on the concentration of MUFA (mg/g), PUFA (mg/g), PUFAn-3 (mg/g), long-chain PUFAn-3[1] (LPUFAn-3; mg/g), PUFAn-6 (mg/g), long chain PUFAn-6[2] (LPUFAn-6; μg/g), values of the ratio of PUFAn-6 to PUFAn-3 (n-6/n-3), the sum of assayed fatty acids (ΣFA; mg/g), the ratio of PUFA to SFA (PUFA/SFA), MUFA to SFA (MUFA/SFA), UFA to a sum of FA (UFA/SFA), PUFA to all assayed FA (PUFA/ΣFA), [3]Δ9-, [4]Δ6-, [5]Δ5-, [6]Δ4-desaturase and [7]elongase (Elongas$_{index}$) indexes, atherogenic (A-SFA$_{index}$) indexes, thrombogenic (T-SFA$_{index}$) indexes[8] in the pancreas of rats

Item	Control		CLA					One factorial analysis						Interactions, CLAx			
	Control	SeIV$_L$ / SeY$_L$ / SeIV$_H$ / SeY$_H$	CLA	SeIV$_L$ / SeY$_L$ / SeIV$_H$ / SeY$_H$				SeIV$_L$	SeY$_L$	SeIV$_H$	SeY$_H$	SeY	CLA	SeIV$_L$	SeY$_L$	SeIV$_H$	SeY$_H$
MUFA	29.8	24.9 / 23.8 / 26.4 / 25.4	20.5	26.0 / 28.0 / 24.4 / 25.0				N	N	N	N	N	α	α	α	*	N
PUFA	32.3	35.2 / 31.3 / 36.1 / 38.9	—	46.5 / 43.6 / 45.0 / 42.1				N	N	N	N	N	α	N	N	N	N
PUFAn-3	9.7	11.4 / 10.5 / 12.0 / 11.6	10.1	12.1 / 11.1 / 11.8 / 11.0				N	N	N	N	N	N	N	N	N	N
LPUFAn-3	2.95	3.75 / 3.91 / 3.83 / 4.01	2.96	3.98 / 3.94 / 4.36 / 4.02				α	**	α	*	*	*	N	α	α	*
PUFAn-6	22.6	23.8 / 21.5 / 24.1 / 24.5	25.4	30.7 / 29.1 / 29.4 / 27.8				N	N	N	N	N	N	N	N	N	N
LPUFAn-6	119	149 / 146 / — / 164	186 / 134	—				N	N	N	α	N	α	N	N	N	*
n-6/n-3	2.45	2.10 / 2.00 / 2.01 / 2.11	2.53	2.54 / 2.62 / 2.48 / 2.54				N	N	N	α	N	N	N	N	N	N
ΣFA	90.6	87.2 / 82.9 / 91.0 / 90.3	89.0	111.9 / 109.1 / 106.6 / 102.4				N	N	*	N	N	*	*	α	*	N
PUFA/SFA	1.137	1.266 / 1.302 / 1.310 / 1.259	1.313	1.176 / 1.162 / 1.207 / 1.192				N	N	N	N	N	**	**	*	*	α
MUFA/SFA	1.012	0.919 / 0.928 / 0.823 / 0.885	0.694	0.658 / 0.745 / 0.654 / 0.708				N	α	N	N	N	**	**	N	N	N
UFA/FA	0.429	0.416 / 0.422 / 0.396 / 0.402	0.410	0.344 / 0.340 / 0.358 / 0.351				N	N	α	N	N	**	**	N	α	N
PUFA/ΣFA	0.357	0.404 / 0.396 / 0.418 / 0.400	0.437	0.415 / 0.400 / 0.422 / 0.411				α	*	*	α	α	**	**	*	*	*
Δ9$_{desat}$	0.457	0.408 / 0.452 / 0.421 / 0.439	0.378	0.351 / 0.403 / 0.364 / 0.383				α	α	α	α	*	**	**	**	α	α
Δ6$_{desat}$ ·10^{-5}	358	528 / 685 / 388 / 502	173	94 / 69 / 54 / 15				*	*	N	*	N	α	α	**	α	*
Δ5$_{desat}$	0.270	0.321 / 0.277 / 0.312 / 0.294	0.114	0.080 / 0.014 / 0.102 / 0.139				N	N	N	N	N	**	**	*	N	N
Δ4$_{desat}$	0.579	0.521 / 0.526 / 0.543 / 0.538	0.535	0.555 / 0.593 / 0.581 / 0.571				N	N	N	N	N	N	N	N	α	N
Elongas$_{index}$	0.226	0.224 / 0.234 / 0.290 / 0.247	0.223	0.255 / 0.282 / 0.292 / 0.286				N	N	N	N	N	N	N	N	N	N
A-SFA$_{index}$	0.354	0.355 / 0.346 / 0.354 / 0.359	0.410	0.442 / 0.429 / 0.438 / 0.427				α	N	N	α	α	**	**	*	*	*
T-SFA$_{index}$	0.508	0.454 / 0.451 / 0.452 / 0.468	0.545	0.598 / 0.597 / 0.585 / 0.583				N	N	N	N	N	N	*	N	α	N

[1] LPUFAn-3 - the sum of c11c14c17C20:3, c8c11c14c17C20:4, c5c8c11c14c17C20:5, c7c10c13c16c19C22:5 and c4c7c10c13c16c19C22:5;
[2] LPUFAn-6 - the sum of c11c14C20:2, c8c11c14C20:3 and c5c8c11c14C20:4(AA); [3] Δ9$_{desat}$ - Δ9-desaturase index = (c9C14:1+c9C16:1+c9C18:1)/(c9C14:1+c9C18:1+c9C18:1+C14:0+C16:0+C18:0); [4] Δ6$_{desat}$ - Δ6-desaturase index = c6c9c12C18:3/(c6c9c12C18:3+c9c12C18:2); [5] Δ5$_{desat}$ - Δ5-desaturase index = c5c8c11c14C20:4/(c5c8c11c14C20:4+c8c11c14C20:3n-6); [6] Δ4$_{desat}$ - Δ4-desaturase index = c4c7c10c13c16c19C22:6/(c4c7c10c13c16c19C22:6+c7c10c13c16c19C22:5)/(c7c10c13c16c19C22:5+c5c8c11c14c17C20:5); [7] Elongas$_{index}$ - the elongase index = c7c10c13c16c19C22:5/(c7c10c13c16c19C22:5+c5c8c11c14c17C20:5); [8] The atherogenic index (A-SFA$_{index}$) = (C12:0+4*C14:0+C16:0)/(MUFA+PUFAn-6+PUFAn-3); the thrombogenic index (T-SFA$_{index}$) = (C14:0+C16:0+C18:0)/0.5*MUFA+; 0.5*PUFAn-6+3*PUFAn-3+PUFAn-3/PUFAn-6) (Ulbricht and Southgate, 1991)

the $R_{c9t11CLA/t10c12CLA}$ value in the pancreas of rats fed the containing diet only CLAmix (Table 2). Therefore, it can be hypothesized that dietary SeIV (selenite) or SeY (i.e. organic Se) diminished the efficiency of β-oxidation of *t10c12*CLA and *t10t12*CLA in the pancreas of rats. Selenate added to rat diets exhibited a similar effect on the β-oxidation of *t10c12*CLA and *t10t12*CLA (Niedźwiedzka et al., 2007).

Effect of the diets with CLAmix and Se on pancreatic contents of selected polyunsaturated FA. As expected, the experimental diets enriched in CLAmix and Se (as $_L$SeIV, $_H$SeIV, $_L$SeY or $_H$SeY) numerically increased the concentration of linoleic acid (LA) in the pancreas in comparison with the control group as well as with rats fed the diets with a single additive (SeIV, SeY, or CLAmix) (Table 2). On the other hand, the diets containing both additives significantly decreased the concentration of γ-linolenic acids (γ-LNA) in the pancreas (the negative interactions) compared with the control group or rats fed the diet containing only extra Se or CLAmix. The increase in the concentration of LA (the substrate) and decrease in the concentration of γ-LNA (the product) in the pancreas might be related to the decrease in the value of the Δ6-desaturase index in the pancreas of rats fed the diets containing CLAmix and extra Se (as SeIV or SeY) (Table 3). Indeed, the diets enriched in both additives significantly reduced the capacity of pancreas desaturase in comparison with the control group. Also the diet with CLAmix decreased this desaturase index, while the diets with extra Se as SeIV or SeY increased the value of the Δ6-desaturase index compared with the control group. Consequently, the concentration of γ-LNA in the pancreas changed in good accordance with changes of these values of the Δ6-desaturase index in the pancreas of rats fed the diet enriched in $_L$SeIV, $_H$SeIV, $_L$SeY, $_H$SeY or CLAmix, respectively. A similar effect of the experimental diets was found on the Δ5-desaturase index in the rat pancreas (Table 3).

The concentration of *c8c11c14*C20:3 (C20:3n-6) and *c5c8c11c14*C20:4 (C20:4n-6) decreased in the pancreas of rats fed the diets enriched in CLAmix and Se (as SeIV or SeY) compared with the control rats or rats fed the diets containing only a single additive (Table 2). These differences in the concentrations of C20:3n-6 and C20:4n-6 are related to differences in the pancreatic concentration of the precursor of these long-chain fatty acids (i.e. γ-LNA) . Indeed, C20:3n-6 is the elongation product of γ-LNA, while C20:4n-6 is the product of elongation and Δ5-desaturation of γ-LNA (Belury, 2002; Czauderna et al., 2007a). Our results documented that the concentration of γ-LNA (the substrate) in the pancreas determined the biosynthesis yield of long-chain polyunsaturated n-6 fatty acids (i.e. C20:3n-6 and C20:4n-6).

The concentrations of *c11c14c17*C20:3 (C20:3n-3), *c5c8c11c14c17*C20:5 (C20:5n-3), *c7c10c13c16c19*C22:5 (C22:5n-3) and *c4c7c10c13c16c19*C22:6

(C22:6n-3) increased in the pancreas of rats fed the diets enriched in CLAmix and SeIV or SeY, regardless of the concentration of extra Se (Table 2). Based on findings from a number of laboratories (e.g., Belury et al., 2002), current results and our recent studies (Korniluk et al., 2007), we argue that dietary CLA isomers, especially in the presence of Se as selenite or SeY, reduced desaturase and elongate anabolites of LA (e.g., eicosanoid synthesis from n-6 PUFA precursors), while stimulating the eicosanoid biosynthesis from n-3 PUFA, i.e. the pathway for eicosanoid synthesis from n-3 PUFA precursors. Also the diet enriched in SeIV or SeY, irrespective of the concentration of extra Se, increased the concentration of these long-chain n-3 PUFA, i.e. PUFA n-3-derived eicosanoids. The above findings are in good accordance with changes in the concentration of n-3 LPUFA and n-6LPUFA in the pancreas of rats fed the experimental diets (Table 3). Indeed, the diets enriched in CLAmix and Se (as SeIV or SeY) increased the concentration of n-3 LPUFA, the sum of fatty acids (ΣFA), and decreased the content of n-6 LPUFA in the pancreas in comparison with the control rats. Similarly, the diets with only SeIV or SeY, regardless of the level of extra Se, increased the concentration of Ln-3 PUFA, the PUFA/SFA and PUFA/ΣFA ratio, whereas decreased the n-6 PUFA/n-3 PUFA ratio (n-6/n-3) in the pancreas compared with the control group. In addition, other experimental diets increased the PUFA/ΣFA ratio in the pancreas compared with the control rats, although the diet enriched in CLAmix with or without $_L$SeY most effectively increased the value of the n-6/n-3 ratio in the pancreas. The current results and our recent finding (Czauderna et al., 2004, 2007a, 2009a,b, 2010) documenting that dietary Se and/or CLA isomers fed to animals considerably increase the level of CLA isomers, non-CLA fatty acids containing conjugated double bonds (Ulbricht and Southgate, 1991; Czauderna et al., 2004) and PUFA, especially n-3 PUFA, improving the n-6/n-3 ratio in some internal organs and muscles of animals, is valuable information for nutritionists carrying out research to improve the nutritive value of food for human health.

CONCLUSIONS

The presented study demonstrated that the rat diet enriched in the CLA isomer mixture, selenite or selenized yeast affected the body weight gain, pancreas mass, feed conversion efficiency, as well as the concentration of fatty acids in the pancreas of rats. The rat diet enriched in CLAmix and HSeIV most effectively stimulated the body weight gain and pancreas mass. In adition, diets containing CLAmix and selenite or selenized yeast (regardless of the concentration of extra Se) increased the concentration of

PUFA and, more importantly, CLA isomers, PUFAn-3 and LPUFAn-3, as well as improved the PUFAn-6/PUFAn-3 ratio in the pancreas of rats.

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