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Influence of dietary CLA isomers and selenium compounds on the fatty acid and amino acid profiles in blood plasma of rats*

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ABSTRACT

The influence of diets enriched in CLA isomers and/or selenium on the concentration of fatty acids and amino acids in blood plasma was studied on 20 groups of 7-8 rats aged 8 weeks and with a body weight (BW) of about 201 g. Rats were fed a basal diet for 29 days or a diet enriched with a combination of 1 or 2% CLA isomer(s) and/or 2 ppm as selenate, 1.2% as selenized yeast.

Diets enriched in 1% of *t10c12*CLA and Se compounds caused the most efficient body weight gain (BWG) and feed conversion. Diets enriched in Se compounds only showed a tendency to decrease BWG and feed conversion efficiency compared with control rats. Diets with CLA isomers increased isomer concentrations in plasma with preferential accumulation *c9t11*CLA in comparison with *t10c12*CLA. Supplemental Se and CLA isomer mixture usually increased β-oxidation of *t10c12*CLA compared with supplementing the CLA isomer mixture only. Supplementing 1% of the CLA isomer mixture showed tendency to decrease the concentration of C16:0 and SFA in plasma but supplementing 1 or 2% of the isomer mixture with Se compounds tended to or significantly decreased C16:0 and SFA in plasma. The *t10c12*CLA isomer reduced, whereas *c9t11*CLA, stimulated the yield of Δ9-desaturation.

An increase of linoleic and linolenic acid concentrations was found in the plasma of rats fed the diet with CLA isomer(s) and Se sources compared with the control diet. Supplementing Se sources or CLA isomer(s) stimulated MUFA and PUFA accumulation in plasma due to stimulation of Δ9-, Δ6- and Δ4-desaturation and elongation of fatty acids. Supplementing CLA isomers increased the concentration of amino acids in plasma, while the interaction between supplemented *c9t11*CLA and *t10c12*CLA in rats resulted in reduction of the concentration of amino acids in plasma.

KEY WORDS: CLA isomers, selenate, high-selenized yeast, fatty acids, amino acids, blood plasma, rat

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INTRODUCTION

Recent investigations have documented that conjugated linolenic acid (CLA) isomers could exert many beneficial effects on human health thanks to their antiproliferative, antitumour, antiinflammatory, antiatherogenic, antidiabetogenic, and antiadipogenic properties (Wahle et al., 2004; De La Tore et al., 2006). A beneficial regulatory influence of CLA isomers on cytokine and immunoglobulin production, and on immune function was found. CLA isomers affected the metabolisms of lipids and eicosanoids, as well as of other fatty acids (Naumann et al., 2006). Our recent studies also clearly evidence that dietary selenium (Se) and CLA isomers influenced the concentration of mono- (MUFA) and polyunsaturated (PUFA) fatty acids, minerals and amino acids in laboratory animals (Czauderna et al., 2004; Korniluk et al., 2006; Niedźwiedzka et al., 2006a,b). The nutritional requirements of animals and humans should met by provision of ultra-trace nutrients like Se or essential fatty acids. In humans and animals, numerous disorders related to Se have been recognized, including liver necrosis, white muscle dystrophy, exudative diathesis, and cardiomyopathy (Arthur, 2003; Lyons and Jacques, 2004). The discovery that Se was an essential component of radical- or/and peroxide-metabolizing seleno-proteins (e.g., glutathione peroxidase) led to the hypothesis that physiologically advantageous supplementation with Se results in elevation of unsaturated fatty acid concentrations in animals. Recent investigations revealed that dietary selenate and high-selenized yeast increased the accumulation of CLA isomers and other PUFA in the bodies of rats (Czauderna et al., 2004a,b). Therefore, a protective effect of dietary Se (seleno-cysteine is an essential component of more than 25 seleno-proteins) on the deposition of CLA isomers and other unsaturated fatty acids in the body of mammals may also be plausible.

Considering the above evidence, we decided to carry out experiments to test the hypothesis that dietary CLA isomer(s), inorganic and organic Se (as selenate and high-selenized yeast) improve the fatty acid profile in blood plasma of rats. The influence of diets enriched in CLA isomer(s) and Se on the concentrations of free essential (E-AA) and non-essential (NE-AA) amino acids in plasma and protein E-AA and NE-AA amino acids in the liver and femoral muscles was also examined. On the basis of these investigations we intended to compare the efficacy of dietary selenate (Se^{VI}) and organic Se (Se-yeast) on the capacity for accumulation of CLA isomers, other unsaturated fatty acids and, in particular, E-AA in plasma, liver, and muscles.

MATERIAL AND METHODS

Animals and experimental design

The experimental protocol was approved by the Local Animal Care and Use Committee (The Agricultural University, Warsaw, Poland).

Ten groups of 7-8 female rats (Wistar, Ifz: BOA), at 8 weeks of age, each weighing 201±1 g at the beginning of the experiment, 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%. During a one-week preliminary period the animals were fed a standard Labofeed H diet produced by the Feeds and Concentrates Production Plant in Kcynia, (Poland) (Pastuszewska et al., 2000) given at a submaintenance level (9 g of diet daily per rat) to reduce body fat content. During that time the rats decreased their body weight by about 10% of their initial weight. Then for 29 days the rats were fed the experimental diets (Table 1) enriched with 1 or 2% of the mixture of CLA isomers (CLAmix), 1% of individual isomers (i.e. *c9t11* and *t10c12*), 2 ppm Se as Se^{VI} (Experiment I) or 1.2 ppm Se as Se-yeast (Experiment II). The composition of dietary CLA isomer(s) is presented in Table 1. The rations were adjusted each day to ensure an *ad libitum* feeding level. After day 29 rats were killed by CO_2 and the liver and femoral muscles removed and freeze-dried. Blood samples were collected into heparinized tubes kept in an ice bath and centrifuged at 1500-1700 *g* for 15 min (at 2-4°C). Muscle, liver and blood plasma samples were stored at -28°C until analysed for the concentrations of fatty acids and amino acids.

Reagents and chemicals

Sodium selenate (Na_2SeO_4) and amino acid (AA) standards were provided by Sigma (USA), whereas methanol, 99.9% acetonitrile and 95% heptane were HPLC grade and purchased from Lab-Scan (Ireland). The CLA isomer mixture (CLAmix), *c9t11*CLA and *t10c12*CLA isomers were supplied by Larodan Fine Chemicals AB (Sweden). Composition details (Table 1) and the purity of CLA isomer mixtures and individual isomers were examined using our Ag^+ -HPLC and GLC method (Czauderna et al., 2003, 2005).

All fatty acid (FA) standards, 50% BF_3 in methanol, o-phthaldialdehyde (OPA) and butylated hydroxytoluene were provided by SIGMA (USA) and Suppelco. Ethanethiol, tetrahydrofuran and sodium hypochlorite water solution (4% available Cl) were from Fluka. High-selenized yeast (Se-yeast) was donated by Sel-Plex (Alltech Inc., USA). Eighty-three per cent of the total Se content of Se-yeast represents Se in the form of Se-methionine incorporated into the proteins of *Saccharomyces cerevisiae* (Rayman, 2004). Other reagents,

Table 1. Dietary effects[1] of 1%, 2% CLA isomer mixture (CLAmix), *cis9trans11*CLA (*c9t11*), *trans10cis12*CLA (*t10c12*), selenate (Se^{VI}) and high-selenized yeast (Se-yeast) on body weight of rats after 7 days of adaptation[2], the body weight gain (BWG)[3] and the feed conversion efficiency (FCE)[4] of rats after 29 days feeding with experimental diets

Group	Experiment I Rats fed diets enriched in CLA isomer(s) and selenate(Se^{VI})						Experiment II Rats fed diets enriched in CLA isomer(s) and Se-yeast					
	Additives	Content[8]	Body weight, g initial	Body weight, g after 7 days	BWG g	FCE g/g	Additives	Content[8]	Body weight, g initial	Body weight, g after 7 days	BWG g	FCE G
Control[5]	-	-	200.1	184.9 (8)[9]	59.4[a]	0.1365	Control[5]	-	199.5	177.0 (8)[9]	61.1[a]	0.1221[a]
Se^{VI} or Se-yeast	selenate	2 ppm	200.1	185.3 (8)	52.8[b]	0.1220	Se-yeast	1.2 ppm	200.4	179.0 (8)	60.9	0.1215
1%CLA	CLAmix[6]	1%	200.5	184.4 (7)	54.8	0.1285	CLAmix[7]	1%	201.1	183.0 (7)	62.9	0.1258
c9t11	*c9t11*	1%	200.8	185.6 (7)	59.7	0.1359	*c9t11*	1%	200.6	177.5 (7)	67.5[b]	0.1337[b]
t10c12	*t10c12*	1%	200.9	184.4 (7)	54.1	0.1287	*t10c12*	1%	201.1	180.0 (7)	61.6[c]	0.1282
2%CLA	CLAmix[6]	2 ppm	200.4	183.3 (7)	56.8	01376	CLAmix[7]	2%	200.1	178.0 (7)	56.4	0.1227
1%CLA+ Se^{VI} or Se-yeast	CLAmix[6] +selenate	1% 2 ppm	200.2	181.9 (7)	56.1	0.1319	CLAmix[7] +Se-yeast	1% 1.2 ppm	202.6	180.0 (7)	63.9	0.1302
c9t11+ Se^{VI} or Se-yeast	*c9t11* +selenate	1% 2 ppm	199.6	184.2 (7)	55.5[a]	0.1289	*c9t11* +Se-yeast	1% 1.2 ppm	201.2	181.0 (7)	56.8[b]	0.1185[b]
t10c12+ Se^{VI} or Se-yeast	*t10c12* +selenate	1% 2 ppm	200.3	183.8 (7)	62.2[b]	0.1427	*t10c12* +Se-yeast	1% 1.2 ppm	200.3	178.0 (7)	68.8[ac]	0.1366[a]
2%CLA+ Se^{VI} or Se-yeast	CLAmix[6] +selenate	2% 2 ppm	200.2	182.8 (7)	58.4	0.1409	CLAmix[7] +Se-yeast	1% 1.2 ppm	204.1	182.0 (8)	60.4	0.1293

[1] means in columns with the same letter are significantly different: [A,B] -$P<0.01$; [a,b] - $P<0.05$. Analyses were performed by one Mann-Whitney U and two factorial ANOVA tests. Statistical analyses of simultaneously the CLA isomer(s) and Se^{VI} or Se-yeast treatments were performed applying two-factorial ANOVA analysis

[2] body weight of individually adapted rats after 7 days of submaintenance feeding (daily: 9 g/the standard diet per rat). Initial body weight of rats and after 7 days of adaptation did not statistically differ among group at the $P<0.1$ level

[3] after feeding for 29 days with experimental diet enriched in CLA isomer(s) and/or Se^{VI} or Se-yeast (2 ppm or 1.2 ppm, respectively)

[4] FCE: g body weight gain/g feed intake

[5] the concentrations of Se (as Na_2SeO_2), Zn, Fe, Mg and Ca in the standard Labofeed H diet found: 0.63, 137, 698, 1653 and 10683 µg/g, respectively

[6] the dietary CLA isomer mixture contains, %: *t11t13* - 2.9; *t10t12* - 5.1; *t*9*t*11 - 4.3; *t8t10* - 2.9; *c11t13* - 13.4; *c10t12* - 28.0; *c9t11* - 28.6; *c8t10* - 9.6; *c11c13* - 1.6; *c10c12* - 1.5; *c9c11* - 1.4; *c8c10* - 0.7. The ratio of the concentration of the isomers (*c9t11*/*t10c12*) in the dietary CLA isomer mixture: 1.0242. The composition of individual isomers: *c9t11* and *t10c12* - 95% of *c*9*t*11 and *t*10*c*12 isomer, respectively; *tt*CLA isomers - ca. 2%; no other fatty acids were detected

[7] the CLA isomer mixture contained: 1.94% *t,t*CLA isomers, 95.22% *c9t11*CLA and *t10c12*CLA, and 1.48% *c,c*CLA isomers and 1% linoleic acid (LA); the ratio of the *c9t11*CLA to *t10c12*; CLA contents in the CLA isomer mixture was 0.981 (i.e., 47.3 and 48.2% respectively). The composition of individual isomers: *c9t11*CLA and *t10c12*CLA - 98% of *c9t11*CLA and *t10c12*CLA, respectively; *t,t*CLA isomers - 0.2%; LA - 1% (Czauderna et al., 2003a, 2005)

[8] the concentration of CLA isomer(s) and Se in the rats' diets

[9] in parenthesis - number of rats in a group

includingdichloromethane (DCM), KOH, NaOH, Na_2SO_4 and concentrated HCl, were analytical grade and were purchased from POCh (Gliwice, Poland).

Chromatographic equipment

An alliance separation module (model 2690, Waters) with a Waters 996 photodiode array detector (DAD) and Waters 474 fluorescence detector (FD) was used for determination of the concentration of free amino acids (AAs) and fatty acids in assayed blood plasma samples.

The underivatized CLA isomers and other fatty acids containing conjugated double bonds (CFA) in blood plasma of rats fed all experimental diets were determined according to Czauderna et al. (2003). The derivatized non-CLA fatty acids (FAs) in plasma of rats fed the diets enriched in CLA isomer(s) and Se^{VI} (selenate) were determined using an HPLC system according to Czauderna and Kowalczyk (2001). Methylated non-CLA FAs in plasma of rats fed the diets enriched in CLA isomer(s) and high-selenized yeast (Se-yeast) were determined using long-capillary gas-liquid chromatography (GLC) with a flame-ionization detector (FID) (Czauderna et al., 2005).

The method of free amino acid (AAs) conversion to o-phthaldialdehyde (OPA)-derivatives (OPA-AAs) followed by reversed-phase HPLC separations and quantifications of OPA-AAs in plasma of rats fed the all experimental diets were as previously described (Czauderna et al., 2002; Niedźwiedzka et al., 2006c).

Statistical analysis

The results in Tables 1-6 are presented as means of 7-8 individually analysed rat body parameters, and liver, femoral muscle, and plasma blood samples. Statistical analyses of the effects of the CLA isomer(s) and/or Se (as Se^{VI} or Se-yeast) in the diets were conducted using the nonparametric Mann-Whitney U test for comparing pairs in an independent experimental group (single-factor analysis), while statistical analyses of the interaction between the CLA isomer(s) and Se (as Se^{VI} or Se-yeast) were performed using two-factorial ANOVA (CLA isomer(s) × Se). The statistical analyses were performed using the Statistica ver. 6 package (Statistica, 2002). Differences were considered significant at the $P<0.05$ or $P<0.01$ level, while tendencies, at the $P<0.1$ level. Statistical analyses of the interaction between the CLA isomer and Se were performed using two-factorial ANOVA analysis (the CLA isomer(s) × Se); *-$P<0.05$ and **-$P<0.01$ were considered statistically significant.

Table 2. The concentration of selected fatty acids and the sum of amino acids (ΣAA), essential (ΣE-AA), non-essential (ΣNE-AA) amino acids in blood plasma of rats fed the diets enriched in *cis9trans11*CLA (*c9t11*), *trans10cis12*CLA (*t10c12*) or/and Se^{VI} (selenate)

Group	Control	Se^{VI}	*c9t11*	*t10c12*	1%CLA[4]	*c9t11* +Se^{VI}	*t10c12* +Se^{VI}	[4]1%CLA +Se^{VI}	Significance of effect[1]						
									one factorial analysis				interaction		
Item[3]	µg/ml blood plasma[2]								Se	*c9t11*	*t10c12*	CLA	*c9t11* × Se	*t10c12* × Se	CLA × Se
C16:0	82.0	137.0	53.1	82.4	56.9	83.1	80.9	131.9	NS	NS	NS	NS	NS	NS	NS
SFA_{18+16}	133	223^{ab}	108^{a}	140	181	143	139^{b}	213	*	NS	NS	NS	NS	NS	NS
*c9*C18:1	19.5	37.4	22.0	17.9	20.2	24.0	36.7	19.6	NS	NS	NS	NS	NS	*	NS
Δ9index	0.128	0.143	0.169	0.114	0.100	0.147	0.110	0.121	NS	*	NS	NS	NS	NS	*
LA	24.7	42.0	28.0	32.6	30.9^{a}	42.1	54.1	37.4^{a}	NS	NS	NS	NS	NS	NS	NS
α-LNA	10.8	19.8	13.9^{a}	18.5	14.2	23.9^{a}	25.2	18.9	NS	NS	NS	NS	NS	*	*
c9t11	NQ	NQ	28.7	0.53	12.1	48.4	1.8	16.3	-	-	-	-	-	-	-
t10c12	NQ	NQ	0.94	31.2	10.0	1.7	33.0	12.7	-	-	-	-	-	-	-
*ct/tc*CLA	NQ	NQ	29.6	32.0	23.5	50.3	37.1	30.9	-	-	-	-	-	-	-
*tt*CLA	NQ	NQ	9.4	6.6	15.2	13.5	15.2	9.5	-	-	-	-	-	-	-
*cc*CLA	NQ	NQ	1.1	1.3	2.7	1.3	2.1	2.3	-	-	-	-	-	-	-
ΣCLA	NQ	NQ	42.3	43.6	45.6	69.5	62.1	48.2	-	-	-	-	-	-	-
SFA	136	235	116	146	101	145	323	150	NS	NS	NS	NS	NS	NS	NS
UFA	86.6	166	208	149^{a}	158	203	285^{a}	182	*	*	NS	NS	NS	*	NS
ΣFA	223	401	324	295	259^{a}	348	608	332^{a}	NS	NS	NS	NS	NS	NS	NS
ΣE-AA	337	377^{Aa}	415^{b}	392^{B}	324	327^{b}	307^{AB}	335^{a}	NS	**	**	NS	**	**	NS
ΣNE-AA	460	476^{A}	550^{B}	532	424	419^{B}	380^{A}	445	NS	*	**	NS	**	**	NS
ΣAA	797	853^{A}	965^{a}	924^{B}	748	746^{a}	687^{AB}	780	NS	**	**	NS	*	**	NS

[1] significance of effects: ** - P<0.01; * - P<0.05; NS - P≥0.05; interactions were analysed by two-factorial ANOVA test followed by one-factorial Mann-Whitney U analysis

[2] means in rows with the same letters are significantly different: ^{A,B} - P<0.01 and ^{a,b} - P<0.05; analyses were performed using one-factorial Mann-Whitney U analysis

[3] NQ - below the quantification limit. SFA_{18+16} - the sum of C18:0 and C16:0. ΣFA - the sum of SFA and UFA; UFA - the sum of MUFA and PUFA without CLA isomer Δ9index - (*c*9C16:1+*c*9C18:1)/(*c*9C16:1+*c9*C18:1+C16:0+C18:0); SFA - the sum of C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0 and C22:0

[4] value ratios of *c9t11*/*t10c12*: 1.209 and 1.277 for the groups 1%CLA and 1%CLA+Se, respectively

RESULTS AND DISCUSSION

Feed conversion efficiency, body weight gain of rats

It is well established that Se as inorganic Se (particularly selenate) is less effectively accumulated in the body of laboratory rats in comparison with dietary organic Se (e.g., Se-cysteine, methionine or Se-yeast) (Lyons and Jacques, 2004; Rayman, 2004). Therefore, in the current study, the concentration of Se as selenate in the rats' diets was higher than the dietary concentration of organic Se as high-selenized yeast (i.e. 2 ppm vs 1.2 ppm, respectively).

No macroscopic lesions or toxic symptoms of CLA isomers, selenate and Se-yeast were observed in the animals fed experimental diets. The diet containing 2 ppm of Se would not be toxic for rodents like rats or mice because only dietary inorganic Se compounds, selenite in particular, chronically consumed at a rate of more than 5 ppm can be hepatotoxic and teratogenic in humans and animals (Tapiero et al., 2003; Tinggi, 2003). The value of LD_{50} is about 5 mg Se/kg of body mass for rats, thus, this correspond to a diet containing ~50 ppm Se (i.e. 20 g of the Labofeed H diet enriched in 50 ppm Se per rat and day). In contrast to selenate and particularly selenite, Se-Met (the main Se-compound in Se-yeast) is less reactive because $tRNA_{Met}$ does not discriminate between Se-Met and methionine (Met). Therefore, the Se-Met residue in general proteins is a stable and safe-storage mode for Se in the body of rats fed the diet enriched in 1.2 ppm Se as Se-yeast.

In the current study, the influence of dietary CLA isomer(s) and/or Se (as Se^{VI} and Se-yeast) on the body weight gain (BWG) of rats and feed conversion efficiency (FCE) was confirmed as being the highest in animals fed the diet enriched simultaneously in *t10c12* and Se^{VI} or Se-yeast (Table 1). Interestingly, the addition of organic Se (as Se-yeast) to the diet with *t10c12* resulted in a more efficient increase of BWG (11.2%) of animals and FCE (11.9%) compared with BWG (4.7%) of rats and FCE (4.5%) fed inorganic Se (as selenate) in the diet containing *t10c12* (i.e. 68.8 g vs 62.2 g). In contrast, the addition of Se (as Se^{VI} and Se-yeast) to the diet enriched in *c9t11* tended to or statistically significantly decreased the BWG and FCE of rats, although the diet containing *c9t11* and Se-yeast resulted in the strongest reduction of BWG (-7.0%) and FCE (-2.95%). Consistently with these results, the diet containing selenate most efficiently reduced BWG (-11.1%) and FCE (-10.6%), whereas the diet containing only Se-yeast resulted in a minute decrease in BWG (-0.33%) and FCE (-0.49%). We also found a tendency towards decreased BWG of rats fed the diet enriched in 2% CLAmix. Similar results were also reported by Terpstra et al. (2002) and Wahle et al. (2004) in mice and rats. These studies and our recent investigations confirm that CLA isomers decrease the BWG of laboratory animals by increasing energy expenditure and also by increasing energy loss in the excreta (Czauderna et al.,

2004a,b; Niedźwiedzka et al., 2006a; Korniluk et al., 2006, 2007). Consistently, other investigation (Terpstra et al., 2002) reported an elevation in the concentration of insulin and a decrease in the level of leptin in blood plasma of mice fed a diet containing CLA isomers.

CLA isomer concentrations in plasma of rats

We found that feeding the diets with CLA isomer(s) increased the concentration of CLA isomer(s) in plasma, regardless of the geometrical configuration of CLA isomers (Tables 2, 3 and 5). However, detailed analysis of

Table 3. The concentration of fatty acids in blood plasma of rats fed for 4 weeks 2%CLA isomer mixture without or with Se^{VI} (selenate)

Group	Control	Se(VI)	2%CLA[4]	[4] 2%CLA +Se^{VI}	Significance of effect[1]	
					2%CLA	interaction
Item[3]	µg/g blood plasma[2]					2%CLA × Se
C16:0	82.0	137	61.7	92.4	NS	NS
SFA_{18+16}	133	223	108	147	NS	NS
*c9*C18:1	19.5	37.4[a]	14.2	25.4[a]	NS	NS
Δ9-index	0.128	0.143	0.116	0.147	NS	NS
LA	24.7	42.0	33.3	42.2	NS	NS
α-LNA	10.8	19.8	18.7	22.8	NS	NS
c9t11	NQ	NQ	24.3	25.5	-	-
t10c12	NQ	NQ	17.0	20.6	-	-
*ct/tc*CLA	NQ	NQ	44.1	49.0	-	-
*tt*CLA	NQ	NQ	13.3	16.4	-	-
*cc*CLA	NQ	NQ	3.0	4.0	-	-
ΣCLA	NQ	NQ	67.3	79.8	-	-
SFA	136	235	114	156	NS	NS
UFA	86.6	166	177	214	*	*
ΣFA	223	401	291	370	NS	NS
ΣE-AA	337	377[a]	351	314[a]	NS	*
ΣNE-AA	460	476	480[A]	405[A]	NS	NS
ΣAA	797	853	831	719	NS	NS

[1] significance of effects: **- P<0.01, *- P<0.05; NS-P≥0.05; interactions were analysed by two-factorial ANOVA test

[2] means in rows with the same letters are significantly different: [A,B] - P<0.01; [a,b] - P<0.05

[3] abbreviations for FA and other items see Table 2

[4] value ratios of *c9t11*/*t10c12*: 1.426 and 1.234 for the groups 2%CLA and 2%CLA+Se, respectively

Table 4. The concentration of fatty acids in blood plasma of rats fed the diets enriched in *cis9trans11*CLA (*c9t11*), *trans10cis12*CLA (*t10c12*) or/and Se-yeast (high-selenized yeast)

Group	Control	Se-yeast	*c9t11*	*t10c12*	1%CLA[3]	*c9t11* +Se-yeast	*t10c12* +Se-yeast	1%CLA[3] +Se-yeast	Significance of effect[1]						
									one factorial analysis				interaction		
Item	µg/ml blood plasma[2]								Se	*c9t11*	*t10c12*	CLA	*c9t11* x Se	*t10c12* x Se	CLA x Se
C16:0	114	95.2^{AB}	95.7	81.0	97.3	154^{A}	150	332^{B}	NS	NS	NS	NS	**	NS	**
SFA_{18+16}	253	226^{ABC}	221	205	235	381^{A}	370^{B}	640^{C}	NS	NS	NS	NS	**	*	**
*c9*C18:1	54.7	40.0^{ABa}	68.7	30.8^{a}	40.1	100^{A}	102	351^{B}	NS	NS	NS	NS	NS	NS	**
Δ9-index	0.184	0.162^{A}	0.224	0.140	0.151^{B}	0.204	0.180	0.348^{AB}	NS	NS	**	NS	NS	*	**
LA	167	139^{AB}	172	147	156	229^{A}	191	644^{B}	NS	NS	NS	NS	NS	NS	**
αLNA	31.9	25.1^{ABC}	26.8	27.2	30.1	40.2^{A}	48.3^{B}	139^{C}	NS	NS	NS	NS	*	NS	**
c9t11	NQ	NQ	29.2^{A}	1.3	14.5^{B}	127.6^{A}	4.8	76.5^{B}	-	-	-	-	-	-	**
t10c12	NQ	NQ	NQ	14.8^{B}	13.3^{C}	4.3	59.2^{B}	57.9^{C}	-	-	-	-	-	-	**
*ct/tc*CLA	NQ	NQ	30.0^{A}	17.4	27.9^{B}	139^{A}	71.6	134^{B}	-	-	-	-	-	-	**
*tt*CLA	NQ	NQ	1.68^{A}	4.1^{a}	0.71^{B}	4.2^{A}	1.36^{a}	6.8^{B}	-	-	-	-	-	-	**
*cc*CLA	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	-	-	-	-	-	-	**
ΣCLA	NQ	NQ	31.7^{A}	21.5	28.6^{B}	144^{A}	73.0	141^{B}	-	-	-	-	-	-	**
SFA	257	230^{ABCDE}	591^{A}	323^{B}	240	452^{C}	474^{D}	1334^{E}	NS	NS	NS	NS	NS	NS	**
MUFA	70.6	51.5^{ABab}	93.5	42.6^{a}	50.8	140^{A}	124^{b}	577^{B}	NS	NS	NS	NS	NS	NS	**
PUFA	408	347^{AB}	530	349	405	601^{A}	466	1689^{B}	NS	NS	NS	NS	*	NS	**
UFA	479	399	624	392	456	741	590	2266	NS	*	NS	NS	*	NS	**
ΣFA	735	628^{ABC}	1114	715	696	1193^{A}	1064^{B}	3600^{C}	NS	NS	NS	NS	NS	**	**

[1] significance of effects: **- P<0.01; *- P<0.05; NS - P≥0.05; interactions were analysed by two-factorial ANOVA test followed by one-factorial Mann-Whitney U analysis

[2] means in rows with the same letters are significantly different: ^{A,B} - P<0.01 and ^{a,b} - P<0.05; analyses were performed using one-factorial Mann-Whitney U analysis

[3] value ratios of *c9t11*/*t10c12*: 1.0903 and 1.3212 for the groups 1%CLA and 1%CLA+Se, respectively

abbreviations for FA and other items see Table 2

Table 5. The concentration of fatty acids in blood plasma of rats fed for 4 weeks 2% CLA isomer mixture without or with Se-yeast

Group	Control	Se-yeast	2%CLA[4]	2%CLA +Se-yeast[4]	Significance of effect[1]	
					2%CLA	interaction
Item[3]	μg/g blood plasma[2]					2%CLA × Se
C16:0	114	95.2[A]	119	142[A]	NS	**
C18:0	139	131[A]	164	218[A]	NS	*
SFA_{18+16}	253	226	283	360	NS	**
*C9*C18:1	54.7	40.0[a]	59	55[a]	NS	NS
Δ9-index	0.184	0.162	0.156	0.144	NS	NS
LA	167	139[A]	227	199[A]	NS	NS
αLNA	31.9	25.1[A]	43.4	48.3[A]	NS	*
c9t11	NQ	NQ	17.8[a]	43.1[a]	-	-
t10c12	NQ	NQ	14.8[A]	30.4[A]	-	-
*ct/tc*CLA	NQ	NQ	32.6[a]	73.5a	-	-
*tt*CLA	NQ	NQ	1.48	4.7	-	-
*cc*CLA	NQ	NQ	NQ	1.04	-	-
ΣCLA	NQ	NQ	34.1[a]	79.3[a]	-	-
SFA	257	230[A]	340[a]	509[Aa]	NS	*
MUFA	70.6	51.5[A]	75.8	144[A]	NS	NS
PUFA	408	347[A]	647	499[A]	NS	NS
UFA	479	399	723	643	NS	NS
ΣFA	735	628[A]	1063	1152[A]	NS	NS

[1] significance of effects: **- P <0.01, *- P<0.05; NS-P≥0.05; interactions were analysed by two-factorial ANOVA test

[2] means in rows with the same letters are significantly different: [A,B] - P<0.01; [a,b] - P<0.05

[3] abbreviations for FA and other items see Table 2

[4] value ratios of *c9t11*/*t10c12*: 1.2027 and 1.4178 for the groups 2%CLA and 2%CLA+Se, respectively

our results revealed a slight preference towards accumulation of *c9t11* in comparison with *t10c12* in the plasma of rats fed the diets enriched in 1 or 2% CLAmix, irrespective of the presence of Se (as Se^{VI} and Se-yeast). Indeed, the concentration of *t10c12* as well as *t10t12* isomers tended to be lower than *c9t11* and *t9t11* isomers in tissues of rats due to more efficient β-oxidation of *t10c12* and *t10t12* isomers than their *9,11* homologues (Alasnier et al., 2002; Czauderna et al., 2004a,b). Therefore, the value of the concentration ratios of *c9t11*/*t10c12* in the plasma of rats fed the diets enriched in 1 or 2% CLAmix, irrespective of the presence of Se (as Se^{VI} and Se-yeast), was higher compared with the value of the concentration ratios of these isomers in the dietary CLAmix (see data in Tables 2-5 vs Table 1). Addition of Se^{VI} to the diet enriched in 1% CLAmix stimulated β-oxidation of *t10c12* in plasma. On the other hand, the higher concentration of CLAmix in the diet enriched in Se^{VI} disturbed the

β-oxidation of *t10c12* in comparison with the β-oxidation of this isomer in the blood of rats fed the diet enriched in only 2% CLAmix (Tables 2 and 3). The different chemical forms of Se may have different metabolic roles (Tapiero et al., 2003; Suzuki, 2005), as observed in our previous studies (Czauderna et al., 2004a,b; Korniluk et al., 2006). Indeed, in our current investigation we found that dietary Se-yeast (a rich-source of seleno-methionine) significantly stimulated β-oxi-dation of *t10c12* regardless of the amount of CLAmix added to the diets (Tables 4 and 5). Moreover, as can be seen from the results summarized in Tables 2-5, the addition of organic Se as Se-yeast to the diets enriched in CLA isomer(s) resulted in significantly stronger stimulation of the accumulation of CLA isomer(s) in plasma in comparison with Se^{VI} supplied to the diets containing CLA isomer(s). Different chemical forms of dietary Se (as Se^{VI} or Se-yeast) could be attributed to the different influence of Se additives to the diets on the profile and yield of the accumulation of CLA isomers in plasma of rats. Indeed, selenate (Se^{VI}), unlike selenite (Se^{IV}), is metabolized in the liver to give selenide, seleno-diglutathione, seleno-cysteine (Se-Cys), etc. (Combs, 2004; Suzuki, 2005). Therefore, Se-Cys-proteins are the predominant Se-compound in blood plasma of rats fed diets enriched in Se^{VI}. On the other hand, dietary Se-yeast stimulated accumulation of Se-Cys-proteins and, particularly, seleno-methionine-proteins in the body of rats (Suzuki, 2005). Therefore, these changes in the profile and concentration of CLA isomer(s) could be attributed to the higher concentration of seleno-methionine-proteins in the plasma of rats.

Effect of CLA isomer(s) and Se on the concentration of non-conjugated fatty acids in plasma

The current study was designed to determine whether changes of the concentration of non-conjugated fatty acids depended upon the chemical form of dietary Se and the positional and geometrical structure of CLA isomers. As can be seen from the results summarized in Tables 2-5, the dietary 1% CLAmix showed a tendency towards decreasing the concentration of C16:0 and the sum of all assayed saturated fatty acids (SFA) in plasma. Unexpectedly, the diets enriched in 1 or 2% CLAmix and Se (as Se^{VI} or Se-yeast) tended or statistically significantly increased of the concentration of C16:0, $SFA_{C16:0+C18:0}$ as well as the sum of SFA in plasma. Likewise, adding Se-yeast to the diets containing 1 or 2% CLAmix revealed a similar effect on these saturated fatty acids, although the influence of dietary high-selenized yeast was considerably stronger (Tables 2 and 3 vs 4 and 5). Dietary Se-yeast more efficiently stimulated the accumulation these saturated fatty acids in plasma of rats fed the diets enriched in *c9t11* or *t10c12* compared with rats fed the diets containing Se^{VI} and *c9t11*or Se^{VI} and *t10c12*. On the other hand, the diet enriched in only Se-yeast showed a tendency towards a slight decrease in the

concentration of these saturated fatty acids in plasma, whereas dietary Se^{VI} tended to or statistically significantly increased the concentration of these fatty acids. The obtained results documented that inorganic Se as selenate stimulated the accumulation of saturated fatty acids in plasma, while Se-yeast, mainly as selenomethionine, lowered the yield of saturated fatty acid formation in rat plasma.

Dietary CLA isomers exert a variety of influences on the capacity of Δ9-desaturation of fatty acids (Belury, 2002; Wahle at al., 2004). Indeed, the value of the desaturase index (Δ9-index) as well as the concentration of *c9*C18:1 tended to decrease in the plasma of rats fed the diet enriched in *t10c12* or 1% CLAmix, regardless of the presence of Se^{VI} (Tables 2-5), whereas the addition of Se-yeast to the diet containing these isomers almost eliminated this effect of dietary *t10c12* (Table 4). This is in agreement with our previous studies (Czauderna et al., 2004a,b; Korniluk et al., 2006; Niedźwiedzka et al., 2006a) in which dietary *t10c12* also reduced the Δ9-desaturation activity and inhibited steaoryl-CoA desaturase mRNA expression and fatty acid synthesis in rats (Terpsta, 2004). Terpsta (2004) explained that a 12-double bond appears to be a key structure for inhibiting stearoyl-CoA desaturase activity, especially when coupled with a 10-double bond, however not with a 9-double bond (Belury, 2002). On the other hand, the diet containing *c9t11* tended to increase the value of the Δ9-index as well as the concentration of *c9*C18:1 in plasma, whereas addition of this isomer to the diets enriched in Se (as Se^{VI} and Se-yeast) tended to increase the concentration of *c9*C18:1 in plasma compared with the concentration of this isomer in plasma of rats fed the diets containing only *c9t11*. So, these results show that simultaneous addition of *c9t11* and Se (as Se^{VI} and Se-yeast) to the diets stimulated Δ9-desaturation activity or/and increased steaoryl-CoA desaturase mRNA expression in the body of rats.

We found that feeding Se as Se^{VI} and Se-yeast usually tended to increase, or significantly increased, the concentration of linoleic (LA) and linolenic (LNA) acids in plasma of rats fed the diets containing CLA isomer(s) and Se regardless of its chemical form (a positive interaction) (Tables 2-5). A significantly higher increase of the concentrations of LA and LNA was observed in plasma of rats fed the diets enriched in CLA isomer(s) and Se-yeast, despite the diet enriched in only Se-yeast tending to decrease the concentrations of LA, LNA as well as MUFA, PUFA and the sum of unsaturated fatty acids (UFA) as well as the sum of all assayed fatty acids (ΣFA) in plasma (Tables 4 and 5). A similar effect was observed in the liver, spleen and pancreas of rats fed the diet enriched in only Se-yeast (Korniluk et al., 2006, 2007). The positive correlation between the concentration of the sum of UFA (i.e. MUFA and PUFA) and simultaneous addition of CLA isomer(s) and Se (as Se^{VI} and Se-yeast) to the diet was observed in plasma of rats. As expected, the addition of Se-yeast together with CLA isomer(s) caused a significantly higher increase in the concentration of UFA in plasma than the addition of Se^{VI} and CLA isomer(s). These results are consistent with our recent

investigation in rats showing that the interaction between Se-yeast and CLA isomer(s) was also responsible for stimulating the accumulation of LA, LNA as well as other PUFA in spleen, pancreas and kidneys of rats fed the diets containing Se-yeast and CLA isomer(s) (Korniluk et al., 2006, 2007). The explanation for the interaction mechanism of the increase in the concentration of unsaturated fatty acids, particularly long-chain PUFA (Tables 4 and 5), is through the increase of the capacity of Δ9-, Δ6-, Δ4-desaturations and elongation of fatty acids. As a consequence of the above observations, feeding CLA isomer(s), particularly with Se (as Se^{VI} or Se-yeast), has been shown to usually increase the sum of all assayed fatty acids in plasma of rats.

Concentrations of amino acids in plasma, liver and femoral muscles of rats

Our recent investigations (Czauderna et al., 2004a,b; Niedźwiedzka et al., 2006b) as well as other studies (Alasnier et al., 2002; Terpstra et al., 2002; Wahle et al., 2004) indicated that CLA isomers decreased body fat, increased lean body mass and the amount of protein in the body of laboratory animals. In the presented study, the concentrations of amino acids in blood plasma (Tables 2 and 3), liver and femoral muscles (Table 6) were also affected by the diets enriched in CLA isomer(s). The diet containing individual CLA isomers increased the concentration of the sum of essential (ΣE-AA) and non-essential (ΣNE-AA) amino acids in plasma (Table 2). On the other hand, the addition of 1% of the CLA isomer mixture to the diet showed a tendency to decrease the concentration of these fatty acids in plasma. This result suggests that the interaction between dietary *c9t11* and *t10c12* in rats resulted in reducing the concentration of ΣE-AA and ΣNE-AA in plasma. Increasing the concentration of the CLAmix in the diet diminished this antagonistic effect of the isomer mixture in rats; consequently, the diet containing 2% CLAmix showed a tendency to slightly increase the concentration of these fatty acids (Table 3).

Addition of Se^{VI} to the diet enriched in individual isomers decreased the concentration of ΣE-AA and ΣNE-AA in plasma compared with rats fed the diet enriched in only the individual isomer (Table 2). Similarly, the antagonistic interaction between dietary Se^{VI}, *cc, tt, c9t11* and *t10c12* CLA isomers decreased the effect of individual CLA isomers and Se^{VI} (Tables 2 and 3) on the concentration of these amino acids. Consequently, the concentrations of ΣE-AA and ΣNE-AA in plasma were similar to the concentration of these fatty acids in the plasma of control rats.

The antagonism between dietary geometrical configuration of CLA isomer(s) and Se^{VI} was also reflected in the concentration of these fatty acids in the liver and femoral muscle (Table 6). The diet enriched in CLA isomer(s) and Se^{VI} resulted in a small numerical decrease in the concentration of ΣE-AA in the liver and muscle and in the

Table 6. Mean concentrations[1] of the sum of protein essential-(ΣE-AAs)[2], non-essential- (ΣNE-AA)[2] amino acids and the sum of all protein amino acids (ΣAA)[2] in femoral muscles and liver of rats fed the diet enriched in CLA isomer(s) and Se (as Se^{VI} and Se-yeast)

Group	Tissue	Se, μg/g[3]		ΣCLA, μg/g[3]		ΣE-AA, mg/g		ΣNE-AA, mg/g		ΣAA, mg/g	
		Se^{VI}	Se-yeast	Se^{VI}	Se-yeast	Se^{VI}	Se-yeast	Se^{VI}	Se-yeast	Se^{VI}	Se-yeast
Control	Liver	$4.48^{ABCDEFGHa}$	3.03^{ABCD}	0.07	-	230	319	228	220	458	775
	Muscles	-[4]	0.44^{ABa}	-	-	190	214	221	168	411	538
Se	Liver	5.77^{AIJb}	3.45^{DEFGH}	0.08	-	211	229	230	212	440	646
	Muscles	-	0.99^{ACDEb}	-	-	153	181	156	164	310	503
1%CLAmix	Liver	4.17^{a}	2.70^{A}	2.60^{a}	1.35^{a}	233	236	238	225	471	686
	Muscles	-	0.50^{a}	4.92^{Aa}	7.44^{A}	196	258	221	219	417	691
c9t11	Liver	3.91^{B}	2.71^{B}	2.60	1.71^{b}	222	178	227	195	449	561
	Muscles	-	0.50	6.19	8.92^{a}	144	221	164	139	308	500
t10c12	Liver	4.07^{C}	3.12	2.90	1.71	265	220	275	210	541	635
	Muscles	-	0.48	5.80^{b}	5.50^{b}	181	227	206	141	388	502
2%CLAmix	Liver	3.89^{D}	2.75^{C}	9.62^{ab}	1.93^{a}	226	240	237	227	463	687
	Muscles	-	0.58^{B}	9.31^{A}	12.5^{A}	145	241	178	143	323	523
1%CLAmix + Se	Liver	5.15^{Ej}	3.98^{E}	2.85	1.24	221	203	245	187	466	567
	Muscles	-	1.16^{C}	7.37^{a}	8.49	188	283	192	213	379	708
c9t11+Se	Liver	5.50^{F}	3.95^{F}	2.98	1.31^{b}	222	289	229	268	452	816
	Muscles	-	1.16^{D}	6.85	11.5^{a}	171	294	164	179	335	647
t10c12+Se	Liver	5.23^{Gb}	4.11^{G}	2.71	1.76	212	230	223	207	434	643
	Muscles	-	1.14^{b}	8.56^{b}	8.30^{b}	170	313	167	229	336	770
2%CLAmix + Se	Liver	5.12^{Hj}	4.22^{H}	7.99^{b}	1.84	226	247	234	238	459	722
	Muscles	-	1.20^{E}	1.74	10.1	177	303	168	229	344	463

[1] the concentrations of AAs analysed in pooled samples prepared by combination of all livers or muscles from rats fed the same diet

[2] the sum of essential (ΣE-AA), non-essential (ΣNE-AA) and all assayed (ΣAAs) amino acids determined as described by Czauderna et al. (2002) and Niedźwiedzka et al. (2006c)

[3] the concentration of Se and the sum of CLA isomers in the liver and femoral muscles of rats fed the diets enriched in CLA isomer(s) and Se (as Se^{VI} (Czauderna et al., 2004a,b) and Se-yeast (Koniluk et al., 2006, 2007)). Means in columns with the different letter are significantly different at $^{a,b}P<0.05$ or at $^{AB}P<0.01$; statistical analyses of the effects of the CLA isomers or Se were conducted using the non-parametric Mann-Whitney U test for comparing independent experimental groups, while statistical analyses of the simultaneous the CLA isomers and Se treatments were performed applying two-factorial analysis for comparison with the independent control group

[4] not measured

concentration of ΣNE-AA in muscles of rats fed the diet containing CLA isomer(s) and Se^{VI}. Similarly, a numerical decrease of the concentration of ΣE-AA was also observed in the liver of rats receiving the diets enriched in Se-yeast and CLA isomer(s). A similar effect, although weaker, was found in the liver of rats fed the diets containing pure CLA isomer(s), i.e. possessing smaller concentrations of *tt*CLA and *cc*CLA (Table 1). The current results confirm those of our recent investigations and other studies in rodents showing that dietary CLA isomer(s) increased liver weight (up to 50%); this effect on liver weight of CLA isomer(s) was mainly due to the increase the total lipid content per gram of liver in rats and mice (Terpstra et al., 2002; Czauderna et al., 2004a; Korniluk et al., 2006), while the total protein content in the liver decreased.

The data summarized in Table 6 confirm the results of other studies showing that CLA isomer(s) appear to increase the lean body mass and the amount of protein in the body of laboratory animals (Belury, 2002). Indeed, dietary CLA isomer(s), possessing a lower concentration of *tt*CLA, numerically stimulated the accumulation of ΣE-AA in muscles, while decreasing the sum of all amino acids (ΣAA) (Table 6). This effect on the concentration of ΣE-AA was stronger in muscles of rats fed the diets containing these CLA isomer(s) and Se-yeast. The obtained results suggest that dietary CLA isomers decreased the biosynthesis of NE-AA, so the mean concentration of ΣNE-AA in muscles numerically declined; this effect was stronger in the muscles of rats (Table 6) fed the diets enriched in CLA isomer(s) containing a higher level of *tt*CLA and *cc*CLA isomers (Table 1), irrespective of the presence of Se^{VI}.

CONCLUSIONS

As reported in other studies on rodents, the effectiveness of dietary CLA isomers in stimulating the body weight gain of rats and feed conversion efficiency was confirmed in our current study. In it, we also unexpectedly found that the strongest positive interactions on these parameters occurred between *t10c12* and Se, regardless of the latter's chemical form. Therefore, we hypothesize that these effects can be explained by the interaction between metabolite(s) of dietary Se^{VI} and Se-yeast (probably the same ones) and *t10c12* and/or its metabolite(s). More importantly, we suggest that dietary Se^{VI} and Se-yeast increased the capacity of β-oxidation of *t10c12* as well as *t10t12*; therefore in our current and previous studies, the *c9t11* isomer was preferentially accumulated in the body of rats and sheep, especially those fed the diets simultaneously enriched in the mixture of CLA isomers and selenium.

Moreover, we hypothesize that the interaction between dietary CLA isomer(s) and Se (as Se^{VI} or Se-yeast) stimulates the capacity of Δ9-, Δ6-, Δ4-desaturation and elongation of fatty acids.

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