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Dietary selenized yeast and CLA isomer mixture affect fatty- and amino acid concentrations in the femoral muscles and liver of rats

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ABSTRACT

The effect of diets enriched in CLA isomer mixture (CLAmix) and/or selenized yeast (Se-Y) on the concentration of fatty acids (FA), amino acids (AA), Se, Fe and Zn in the liver and femoral muscles was studied on 4 groups of 7-8 rats aged 8 weeks. Rats were fed the Labofeed H diet for 29 days (control group) or the diet enriched with 2% CLAmix with or without 1.2 ppm Se as Se-Y (the experimental groups). Feeding the CLAmix-enriched diet reduced the body weight of rats, while the diet containing CLAmix and Se-Y increased it in comparison with the control rats. The muscle concentrations of non-essential and essential amino acids, and in particular, sulphur amino acids (ΣS-AA) can be significantly increased, while the muscle concentration of total fatty acids (ΣFA), decreased by feeding the diet with Se-Y and CLAmix. Feeding the diet with Se-Y leads to significant lowering of the ratios of ΣS-AA, methionine, cysteine and taurine to Se concentrations in the liver and muscles as compared with the control group. Dietary Se-Y or CLAmix lead to a decrease in the concentrations of atherogenic and thrombogenic saturated fatty acids (SFA) in muscles. Feeding the diet with Se-Y and CLAmix increased the concentration of Se and the atherogenic and thrombogenic indexes in the liver and muscles. There were also higher concentrations of *cis9trans11*CLA and *t10c12*CLA in the muscles compared with the concentrations of these isomers in the liver of rats fed the diets enriched in only CLAmix. Deposition in the liver and femoral muscle of *trans10cis12*CLA was higher in both tissues than *cis9trans11*CLA. The diet with CLAmix, regardless of the presence of Se-Y, decreased the rate of elongation and Δ5-, Δ9-desaturase capacities of in the liver and muscle. The diet with CLAmix effectively increased the ratio of PUFAn-3/SFA,

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PUFAn-6/SFA, PUFAn-3/ΣFA and PUFAn-6/ΣFA in muscles. The interaction between Se-Y and CLAmix decreased the capacity of liver and muscle desaturation, i.e. these dietary additives reduced the ratio of unsaturated fatty acids to SFA in the liver and muscles.

KEY WORDS: selenized yeast, fatty acids, amino acids, CLA isomers, Se, liver, femoral muscles, rats

INTRODUCTION

The trace element selenium (Se) is a component of over 25 Se-enzymes (e.g., Se-protein P or thioredoxin reductase, isozymes of the glutathione-peroxidase (GPx) family: GPx-1, -2, -3, -4 and -6) involved in diverse metabolic processes (Schomburg et al., 2004). Therefore, Se has recently come to be considered an essential element in animal and human diets, and organic or inorganic forms of Se are commonly used as dietary additives for the treatment of Se deficiency (Surai, 2006; Boosalis, 2008). As the molecular physiology of Se-proteins/Se-enzymes became known, it became apparent that Se concentrations differentially influenced Se-protein expression in animals and humans (Raymond and Kristina, 2005). Nearly half of Se-proteins have been implicated in antioxidant functions, and Se-cysteine (Se-Cys) is essential in the active center of Se-proteins that carry out redox reactions (Tapiero et al., 2003; Suzuki, 2005). Indeed, these proteins act in synergy with tocopherols and provide a second line of defense against hydroperoxides, which can damage membranes and other cell structures (Tapiero et al., 2003). There are numerous studies showing that an adequate Se level in animal and human diets protects against peroxidation of unsaturated fatty acids (FA) and accumulation of carbonyl moieties on proteins produced by oxidative stress (Tapiero et al., 2003). Feeding rats dietary selenized yeast (Se-Y; 83% Se as Se-Met) (Rayman, 2004; Weiss and Hogan, 2005) results in induction of GPx activity, indicating that in these animals, part of the Se added to the diet is converted to Se-Cys, which is an essential chemical form of Se for the synthesis of Se-proteins possessing antioxidant properties (Whanger, 2004). Moreover, the principal physiological roles of half of Se-proteins are to maintain the appropriate metabolism of arachidonic acid and low concentrations of pre-oxides or free radicals within cells, thus decreasing oxidative stress in living organisms (Tapiero et al., 2003; Schweizer et al., 2005). GPx acts in synergy with tocopherol in the regulation of lipid peroxidation. Recent studies documented that phospholipid hydroperoxide GPx in particular, interacted more directly than cytosolic and mitochondrial GPx in protecting PUFA from peroxidation damage (Crespo et al., 1995; Tapiero et al., 2003). Moreover, a positive correlation was observed between concentrations of unsaturated FA and the dietary content of Se (Crespo et al., 1995; Tanguy et al., 2003; Yu et al., 2008).

CLA isomers play a principal role in many important physiological functions and may lead to changes in body composition, body fat reduction, overall energy expenditure or modulate immune function and inhibit tumorigenesis (Banni et al., 2004; King et al., 2004; Rainer and Heiss, 2004). Considering the above evidence, we investigated whether a diet enriched in Se-Y stimulated the accumulation of conjugated linoleic acid (CLA) isomers in rat livers and femoral muscles.

The objective of our studies was to determine the effects of Se-Y and CLA isomer mixture (CLAmix) on the profile of CLA isomers and other FA concentrations in the liver and femoral muscles of rats. Furthermore, the influence of the experimental diets on the concentrations of selected amino acids, Zn, Fe and Se in the liver and muscles was studied.

MATERIAL AND METHODS

Animals, diets and sampling

The investigation was carried out on 32 female rats (Wistar, Ifz: BOA), 8 weeks of age and with an initial body weight of 200±1 g. The rats were housed individually in plastic cages at a temperature of 22±1°C with a 12 h light-dark cycle and relative humidity of 50-60%. Each group comprised 7 or 8 rats. During a one-week preliminary period the rats were fed a standard Labofeed H diet (Table 1) according to the recommendations of Pastuszewska et al. (2000). This diet was offered at a sub-maintenance level (i.e. 8 g diet per rat and day) to reduce the body fat content in rats. Consequently, the body weight of animals decreased by about 15 g per rat during the preliminary period lasting 7 days. Finally, the animals were fed experimental diets supplemented with a CLA isomer mixture and/or Se (as high-selenized yeast; Sel-Plex, Alltech Inc., USA) *ad libitum* (Table 1). The dietary CLA isomer mixture contained: *trans,trans*CLA isomers (*tt*CLA), 1.94%; *cis9,trans11*CLA (*c9t11*CLA) and *trans10,cis12*CLA (*t10c12*CLA), 95.57%; *cis,cis*CLA isomers (*cc*CLA), 1.48%; and linoleic acid (LA) 1.0%. The ratio (R) of the *c9t11*CLA to *t10c12*CLA concentration in the CLA isomer mixture in the rats' diets was 0.981 (i.e. 47.3 and 48.2%, respectively). Feed intake and body weight were measured weekly. At the end of the 28-day experiment the animals were killed. Liver and femoral muscles were removed, weighed and frozen. Amino acids (AA), fatty acids (FA) as well as Se, Zn and Fe were analysed in liver and femoral muscles.

Table 1. Chemical composition (g/100 g diet) and the energy value of the Labofeed H diet (Pastuszewska et al., 2000) and Se-Y (high selenized yeast, Sel-Plex) (Rayman, 2004) and dietary CLA isomer mixture[1]

Item	Labofeed H[3] g/100 g diet	Item	Se-Y
Dry matter[2]	88.2 ± 0.9	Se	1.8 mg Se/g yeast
In dry matter			
crude protein	21.8 ± 1.3	sum of identified Se species	88.3% Se
lysine	1.31	Se-methionine	83.0% Se
methionine+cystine	0.76	Se-cysteine	5.0% Se
tryptophan	0.28	selenite	0.3% Se
threonine	0.87	fatty acids[4]	mg/g yeast
crude fibre	21.8 ± 1.3	C16:0	9.0
fat	3.0 ± 0.8	*Cis9*C16:1	4.1
ash	5.9 ± 0.6	C18:0	13.6
Energy value, MJ, ME/kg	13.9, mean from 3 samples	*Cis9*C18:1	11.3
		*Cis11*C18:1	0.8
		*Cis9cis12*C18:2 (LA)	14.7
		*Cis9cis12cis15*C18:3 (LNA)	0.16

[1] the CLA isomer mixture contained: 1.94% *t,t*CLA isomers, 95.57% *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%

[2] ingredients: maize, wheat, oat flakes, green meal, soyabean oilmeal, fishmeal, soya oil, vitamins; macro-elements (Na, K, Ca and P: 3.6, 8.3, 10.7 and 7.6 mg/g Labofeed H diet, respectively) and trace elements (Se as Na_2SeO_3, Cu, Zn, Mn, Fe, Mg: 0.63, 13.9, 98, 112, 698, 1653 μg/g Labofeed H diet, respectively)

[3] means from 9 samples

[4] main FA peaks (i.e. ~95% area of all FA peak areas in a GLC chromatogram

Reagents and analytical methods

Organic solvents were of HPLC grade and all chemicals were analytical grade. Dichloromethane (DCM), KOH, NaOH, Na_2SO_4 and conc. HCl were purchased from POCH (Gliwice, Poland). Methanol, acetonitrile (ACN) and n-heptane (99.5%, HPLC) were supplied by Lab-Scan (Ireland), while the CLA isomer mixture by Larodan Fine Chemicals AB (Sweden). Composition details and the purity of CLA isomer(s) were examined using our Ag^+-HPLC and GLC methods (Table 1) according to Czauderna et al. (2003a, 2005a). Fatty acid methyl ester standards, methionine (Met), cysteine (Cys) and 25% BF_3 in methanol were purchased from Supelco or Sigma (USA). The high-selenized yeast (Se-*Saccharomyces cerevisiae*) was donated by Sel-Plex (non-commercial yeast sample; Alltech In., USA). About 83% of the total Se content of high-selenized

yeast (Se-Y) represents Se in the form of Se-methionine incorporated into the proteins of *Saccharomyces cerevisiae* (Table 1). Water used for the preparation of chemical reagents and mobile phases was prepared using an Elix™ water purification system (Millipore). The mobile phases were filtered through a 0.45 μm membrane filter (Millipore).

Saponification and preparation of fatty acid methyl esters (FAME)

Liver and muscles were frozen, lyophilized, finely powdered and the obtained samples were stored at -20°C until analysis. The underivatized CLA isomers and other fatty acids (FA) containing conjugated double bonds (CFA) were determined using silver-ion exchange liquid chromatography (Ag^+-HPLC) according to Czauderna et al. (2003). All fatty acids (FA), including CLA isomers and CFA, in liver and muscles were saponified (Czauderna et al., 2007a) followed by gentle base- and acid-catalysed methylations (Czauderna et al., 2008). Finally, methylated fatty acids (FAME) were quantified using capillary gas-chromatography with mass spectrometry (GC-MS) according to Czauderna et al. (2008). Amino acid (AA) concentrations in all assayed biological samples were determined *via o*-phthaldialdehyde-derivatives according to Czauderna et al. (2002).

The concentrations of Se (*via* Se^{82}) in liver and muscles were analysed by the fluorimetric method of Rodriguez et al. (1994), while the level of Fe and Zn in these samples was determined by flame atomic absorption spectrometry (Czauderna et al., 2005b).

Analytical equipment

The analyses of all FAME were performed on a SHIMADZU GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica capillary column (120 m × 0.25 mm i.d. × 0.25 μm film thickness; SHIM-POL). The underivatized CLA isomers and other fatty acids containing conjugated double bonds (CFA) were analysed on an Ag^+-HPLC (Waters, USA). This system comprised two ion-exchange columns loaded with silver ions (250 × 4.6 mm i.d., Chrompack ChromSper 5 μm, Lipids, The Netherlands) and a Waters 996 photodiode array detector (Czauderna et al., 2005a). Concentrations of Fe, Cu and Zn in biological samples were determined on a PU9100X Atomic Absorption Spectrometer, UNICAM, Philips.

Statistical analyses

Results are presented as means±SD of individually analysed samples of liver and muscles. Statistical analyses of the effects of dietary Se and CLA isomer mixture were conducted using the non-parametric Mann-Whitney U test for

comparing independent experimental groups. Differences were considered significant at $^{a,b}P<0.05$ or $^{A,B}P<0.01$, while at $^{\alpha,\beta}P<0.1$ differences were taken as tendencies. Statistical analyses of the interaction between CLA isomer mixture and Se were performed using two factorial ANOVA analysis. For statistical analyses the program Statistica ver. 6 (2002; www.statsoft.pl) was used.

RESULTS AND DISCUSSION

The influence of dietary CLA isomers and Se on liver, muscles and body weight of rats and the concentration of elements and amino acids in liver and muscles. In the current study, no macroscopic lesions or pathological changes were found in the liver and femoral muscles or in any of other organs of rats fed the diets enriched in the CLAmix and/or Se-Y. Indeed, diets containing up to 2 mg Se per kg would not be toxic for rodents (McDowell et al., 2005). Only chronic consumption of inorganic Se compounds, selenate and especially selenite, at rates of more than 5 mg Se per kg diet can be hepatotoxic and teratogenic in humans and animals (Tapiero et al., 2003; Tinggi, 2003). The LD_{50} is ~5 mg Se per kg body weight for rats, which corresponds to about 50 mg Se per kg diet. Fortunately, in contrast to selenide and selenite, Se-Met is less reactive, and because *t*RNAMet does not discriminate between Se-Met and methionine, dietary Se-Met is incorporated into body protein in place of methionine (Tapiero et al., 2003; Weiss and Hogan, 2005). Consequently, Se-Met as a Se-Met residue in general proteins is a stable and safe-storage mode for Se in the body of animals and humans.

There were no differences ($P>0.05$) in the final body weight (BW) among the groups of rats fed the diets enriched in the CLAmix or/and Se-Y compared with the appropriate control groups (Table 2). However, BW was numerically lower (-1.6%) in the CLA group compared with the control group, while the Se-CLA diet most efficiently elevated BW (+3.5% and +1.8%) in the Se-CLA group compared with the CLA and the control groups, respectively. Moreover, dietary Se-Y and CLAmix most effectively increased the mass of the liver and the liver concentration sum of FA (ΣFA), as well as the Se concentration in the liver and muscles compared with all other groups. The diet containing only CLAmix caused an increase in the value of the ratio of Fe/Zn concentrations in both tissues compared with all other groups of rats. This finding confirms the opinion that dietary CLA isomers (especially *t10c12*CLA) enhanced the pro-oxidative activity of species present in rats. On the other hand, dietary Se-Y resulted in a decrease in the value of the ratio of Fe/Zn concentrations, supporting the mediatory effect of Se-Y (especially Se-methionine) on reducing the pro-oxidative capacity of species present in the body of animals.

Table 2. Dietary effects[1] of the high-selenized yeast (Se-Y) and 2% CLA isomer mixture (CLAmix) on the body weight of rats[2], the weights of liver and femoral muscles (DM[3] and fresh), and the amount (mg) of all determined fatty acids (ΣFA) and Se (μg) accumulated in these tissues after 29 days feeding with experimental diets

Group	Supplements	Content	Final body weight, g	Liver					Femoral muscles				
				DM g	fresh g	ΣFA mg	Fe/Zn[5]	Se μg	DM g	fresh g	ΣFA mg	Fe/Zn[5]	Se μg
Control	-	-	238.1 (8)[4]	2.850^{a}	9.115^{a}	180.4	6.491^{a}	8.632^{a}	0.9461	8.357	150.1	1.606^{ac}	0.413^{ab}
SE	Se-Y	1.2 μg Se/g diet	239.9 (8)	2.867^{b}	9.241	199.3	7.380	9.883^{c}	1.0514	8.441	142.2	1.177^{Ab}	1.040^{ac}
CLA	CLAmix	2%	234.3^{a} (7)	2.903	9.306	174.6	7.573^{a}	7.981^{b}	0.9983	8.418	136.8	2.204^{ab}	0.580^{cd}
Se-CLA	Se-Y CLAmix	1.2 μg Se/g diet 2%	242.4^{a} (8)	3.674^{ab}	10.434^{a}	214.4	6.562	15.474^{abc}	0.9902	8.404	143.4	2.108^{Ac}	1.187^{bd}

[1] means in columns with the same letter are significantly different: a - $P<0.05$ and α - $P<0.1$. Statistical analyses of simultaneously the CLA isomer mixture and Se (as Se-Y) treatments were performed applying two-factorial ANOVA analysis

[2] the body weight (g) of individually adapted rats after 7 days of sub-maintenance feeding. The initial body weights of rats (200±1 g) and after 7 days of adaptation did not (179 ± 2 g) statistically differ among the groups at the $P<0.1$ level

[3] DM - dry mass of livers and femoral muscles (i.e. lyophilized)

[4] in parenthesis the number of rats in the group

[5] the concentration ratio of Fe and Zn

The Se-CLA diet most efficiently increased the concentration of essential (+46%), non-essential (+39%) and sulphur amino acids (+138%) in muscles compared with the control diet as well as the CLA diet (+31%, +67% and +66%, respectively) and the SE diet (+72, +40 and +91%, respectively). The Se-CLA diet also most efficiently increased the concentration of taurine in the liver and muscles compared with all other groups, whereas the concentration of taurine was substantially lower in the liver and muscles of rats fed the diet enriched in Se-Y compared with rats fed other diets. Thus, the results from the current study confirm that dietary Se-methionine (i.e. the main Se-component of Se-Y) results in a decrease in the conversion yield of methionine (*via* taurine) to cysteine (Czauderna et al., 2007b), whereas the interaction between Se-Y and CLAmix stimulated the accumulation of methionine as well as the conversion yield of methionine to cysteine in muscles (Czauderna et al., 2007b).

The current trial has demonstrated that the muscle concentration of essential amino acids, sulphur amino acids in particular, can be significantly increased, while the muscle content of FA, decreased by feeding the Se-CLA diet. Therefore, this finding of our short-term pilot study constitutes valuable information for nutritionists carrying out studies to improve the performance of domestic animals *via* dietary Se-Y and CLA isomer mixture.

The influence of dietary CLA isomers and Se on the concentration of selected fatty acids in liver and muscles. Dietary Se-Y or CLAmix lead to a statistically insignificant decrease in the concentrations of atherogenic (A-SFA) and thrombogenic (T-SFA) saturated fatty acids in muscles, concomitantly these changes have a negligible influence on the values of the atherogenic (A-SFA index) and thrombogenic (T-SFA index) indexes (Table 3). Surprisingly, the Se-CLA diet resulted in an increase in the values of A-SFA and T-SFA indexes in the liver and muscles, while there were negligible changes in the concentrations of A-SFA and T-SFA in muscles and only a small increase of the T-SFA concentration in the liver.

The diet enriched in only CLAmix most effectively increased the concentration ratios of PUFAn-3/SFA, PUFAn-6/SFA, PUFAn-3/ΣFA and PUFAn-6/ΣFA in muscles. On the other hand, the Se-CLA diet resulted in a decrease in the values of these ratios in the liver. The concentrations of CLA isomers in muscles were ten-fold higher ($P<0.01$) than in the liver, regardless of the presence of extra Se in diets. Thus, the results of our pilot study of the concentrations of ΣS-AA, ΣE-AA, A-SFA, T-SFA, CLA isomers and the above values of fatty acid ratios in muscles of the CLA group rats is a valuable finding for nutritionists carrying out investigations to improve the performance of domestic monogastric animals *via* a dietary mixture of *cis9trans11*CLA and *trans10cis12*CLA (1:1, w/w).

The yield of the metabolism of *trans10cis12*CLA was higher in both tissues than of *cis9trans11*CLA. Moreover, our results show that *trans10cis12*CLA was

Table 3. The concentrations of unsaturated fatty acids and sulphur (ΣS-AA)[1], essential (ΣE-AA), non-essential (ΣNE-AA) amino acids[2] and atherogenic (A-SFA) and thrombogenic (T-SFA) indexes and the concentration ratio of selected fatty acids in the liver and muscles of rats fed diets enriched in Se-Y and/or CLAmix

Group	Taurine	ΣS-AA[3]	ΣE-AA	ΣNE-AA	A-SFA[4]	T-SFA[5]	A-SFA	T-SFA	PUFAn-3	PUFAn-3	PUFAn-6	PUFAn-6	SFA
	mg/g DM				index	index	mg/g	mg/g	SFA	ΣFA	SFA	ΣFA	ΣFA
Liver													
control	64.18	80.55	279.8	336.1	0.3468^{a}	0.4782^{a}	10.38	25.68^{a}	0.4826	0.2393^{a}	0.3524	0.1747	0.4961
SE	57.81	67.73	245.7	298.1	0.2906^{A}	0.4367^{b}	10.21	26.11	0.5176^{a}	0.2369	0.4091^{a}	0.1872^{a}	0.4577$^{\alpha}$
CLA	61.77	75.14	258.7	319.8	0.3423	0.5504	9.03$^{\alpha}$	26.59	0.4288	0.2282	0.3385	0.1802	0.5321
Se-CLA	66.90	49.27	265.6	344.9	0.4521^{Aa}	0.7183^{ab}	10.52$^{\alpha}$	28.32^{a}	0.3291^{a}	0.1867^{a}	0.2880^{a}	0.1634^{a}	0.5673$^{\alpha}$
Femoral muscles													
control	49.87	77.87	220.4	235.8	0.4158^{a}	0.4992^{a}	34.64$^{\alpha}$	40.48^{a}	0.2168	0.0771	0.7154	0.2543^{a}	0.3547
SE	44.21	97.14	187.1	233.6	0.4243	0.4977	30.01	34.36^{ab}	0.2126	0.0761	0.7158	0.2563	0.3582
CLA	45.56	112.18	246.4	196.2	0.4358	0.5243	29.57$^{\alpha}$	36.01	0.2321^{a}	0.0819	0.8071^{a}	0.2846^{a}	0.3527
Se-CLA	63.07	185.67	322.1	327.8	0.4863^{a}	0.5912^{a}	33.73	40.62^{b}	0.2013^{a}	0.0748	0.7091^{a}	0.2636	0.3723

[1] ΣS-AA - the concentration sum of taurine, methionine and cysteine

[2] all amino acids were analysed in pooled samples prepared by combination of the liver or muscle samples from each rats fed the same diet; the concentration of amino acids was determined by RP-HPLC procedure (Czauderna et al., 2002)

[3] the concentration sum of methionine (Met), taurine and cysteine

[4] atherogenic index = (C12:0 + 4*C14:0 + C16:0)/(MUFA + FAn-6 +FAn-3) (Ulbricht and Southgate, 1991)

[5] thrombogenic index = (C14:0 + C16:0 + C18:0)/0.5*MUFA + 0.5*FAn-6 + 3*FAn-3 + FAn-3/FAn-6) (Ulbrichtand and Southgate, 1991)

preferentially metabolized in the liver compared with the metabolic efficiency of this isomer in muscles (Table 4). The addition of Se-Y to the diet enriched in CLAmix resulted in further elevation of the metabolic yield of *trans10cis12*CLA in the liver, while slightly decreased that in muscles. Based on the concentration of α-linolenic acid (αLNA) in both tissues (see the elongase and Δ5-desaturase index in Table 4) we suggest that the diet enriched in CLAmix, regardless of the presence of Se-Y, decreased the elongation and Δ5-desaturase capacities in the liver and muscles. Similarly, the diet containing CLAmix, regardless of the presence of Se-Y, reduced the concentration of *cis9*MUFA in both tissues (Table 4). This is consistent with the reduction of the capacity of Δ9-desaturation in the liver, muscles and other organs of rats or rabbits (Czauderna et al., 2007a,b; Korniluk et al., 2007). Moreover, the results of feeding diets enriched in CLAmix with or without Se-Y demonstrate that these diets also decreased the capacity of Δ4-desaturation in muscles. Considering the above it seems reasonable to suggest that CLA isomers, especially *trans10cis12,* stimulated oxidation processes in mammals (Basu et al., 2000). Moreover in concert, dietary CLA isomers decreased the capacity of dehydrogenation processes like Δ4- or Δ9-desaturation in muscles, in particular. Moreover, CLA isomers may compete with certain fatty acids (i.e. substrates) at the level of desaturases and elongases, therefore influencing the formation yield of more unsaturated and longer chain fatty acids. Moreover, CLA isomers decreased the expression of the gene for stearoyl-CoA reductase, which is the initial Δ9-desaturation. The results reported here reinforce the finding of our previous studies with respect to a possible role for CLA isomers in influencing desaturation of selected fatty acids in kidneys, spleen and pancreas in rats (Korniluk at al., 2007). Recent studies also documented that these isomers reduced the capacity of other desaturases, i.e. Δ6-desaturase (Malcolm et al., 2008). Indeed, supplementation of CLAmix decreased the Δ6-desaturase capacity in the liver compared with the control rats (Table 4). Moreover, the current study demonstrated that the addition of Se-Y to the diet enriched in CLAmix amplified a decrease of Δ6-desaturase capacity in the liver and muscles compared with rats fed the control diet or the diet with CLAmix.

The use of Se-Y as an additive to the rats' diet led to significantly lower ratios of ΣS-AA, Met, Cys and taurine to Se concentrations in the liver and muscles of rats than in the control group (Table 4). Thus, our current investigation confirmed the Se-species antagonism towards sulphur-compounds in living organisms (Suzuki, 2005; Surai, 2006). Indeed, the main Se-component of Se-Y, Se-Met, can be incorporated into protein non-specifically in place of Met; excess incorporation of Se-Met into proteins can influence protein function if Se-Met replaces Met at the active site of an enzyme (Schrauzer, 2000). The extent of the incorporation of dietary Se-Met into protein depends upon the Met status of the animal: when Met is limiting, incorporation of Se-Met is increased. Moreover, when Met, cysteine or Se-cysteine are deficient, Se-Met conversion *via* Se-taurine into Se-cysteine and

Table 4. The concentrations of selected fatty acids, amino acids and the desaturase indexes in the liver and muscles of rats fed diets enriched in Se-Y and/or CLAmix

Group	CLA[1]		*cis9*-MUFA[2]	MUFA/SFA	MUFA/ΣFA	ΣFA[3]	Desaturase			ΣS-AA	Met	Cys	Taurine
	c9t11	*t10c12*					index [4]	index [5]	index [5]				
	μg/g		mg/g			mg/g DM	elong-Δ5	Δ6	Δ4	Se[6]	Se[6]	Se[6]	Se[6]
Liver													
control	-	-	5.017^{a}	0.1821	0.0893	63.28	0.6856	0.6249^{a}	0.8751	26.61^{A}	10.240$^{a\alpha}$	3.959$^{\alpha}$	21.17$^{\alpha}$
SE	-	-	5.442^{b}	0.2583^{Aa}	0.1182^{ab}	69.21	0.7115$^{\alpha}$	0.6093	0.8692	19.65^{a}	7.259$^{b\alpha}$	3.193^{a}	16.73^{a}
CLA	490^{a}	355 (1.381)[8]	2.490^{a}	0.0927^{A}	0.0487^{a}	60.56	0.6218$^{\alpha}$	0.5912	0.8842	27.30^{B}	9.091^{c}	3.987^{b}	22.48^{ab}
Se-CLA	679^{b}	397 (1.711)	3.246^{b}	0.1176^{a}	0.0668^{b}	58.30	0.6454	0.5874^{a}	0.8750	11.68^{aAB}	2.607^{abc}	1.421$^{ab\alpha}$	15.76$^{b\alpha}$
Femoral muscles													
control	-	-	44.8^{a}	0.878^{ab}	0.3123$^{a\alpha}$	58.3$^{\alpha}$	0.4405$^{\alpha}$	0.1106	0.4515$^{a\alpha}$	177.68^{ab}	36.57$^{a\alpha}$	9.158	114.3$^{ab\alpha}$
SE	-	-	38.0$^{\alpha}$	0.862	0.3087^{b}	34.6$^{\alpha}$	0.3880	0.1112	0.4394$^{\beta}$	98.3$^{\alpha}$	22.19^{abA}	6.063^{a}	44.7^{a}
CLA	7296	4610 (1.583)	28.2^{a}	0.644^{a}	0.2266^{ab}	136.7	0.3814	0.1108^{a}	0.3913$^{a\beta}$	192.8$^{a\alpha}$	53.42$^{bc\alpha A}$	13.766^{a}	78.5$^{\alpha}$
Se-CLA	7037	4836 (1.455)	31.5$^{\alpha}$	0.647^{b}	0.2409$^{\alpha}$	143.9	0.3480$^{\alpha}$	0.0981^{a}	0.4096$^{\alpha}$	155.3^{b}	34.06^{c}	8.328	52.7^{b}

[1] *tt*CLA isomer concentrations below detection limits (*t* - the abbreviation for *trans*)
[2] the concentration sum of *c9*C14:1, *c9*C16:1 and *c9*C18:1
[3] a sum of concentration of all assayed fatty acids (from C_6 to C_{24})
[4] the elongase and Δ5-desaturase index - the value of concentration ratio: *c11c14c17*C20:3n-3/(*c11c14c17*C20:3n-3 + *c9c12c15*C18:3)
[5] Δ6-desaturase index - the value of the concentration ratio: C20:4n-6/(C20:4n-6 + *c9c12*C18:2)
Δ4-desaturase index - the value of the concentration ratio: C22:6n-3/(C22:5n-3 + C22:6n-3)
[6] the concentration ratio of Se (μg/g) to ΣS-AA or to methionine (Met) or cysteine (Cys) or taurine, respectively (μg/g)
[7] below the detection limit (L_D)
[8] in parenthesis the values of the concentration ratio of *c9t11*CLA/*t10c12*CLA in the liver and muscles;
the concentration ratio of the *c9t11*CLA to *t10c12*CLA concentration in dietary CLA isomer mixture was 0.981

then incorporation (instead of cysteine) at the active site of GSHPx or other Se-enzymes is higher because of non-specific incorporation of Se-Met than selenite or selenate into body stores. Moreover, the addition of CLAmix to the diet enriched in Se-Y resulted in further decrease of these ratios in the liver compared with control rats, while slightly increasing them in muscles compared with the SE group of rats. Therefore, we suggest that CLA isomers, as well as their metabolites, amplified the antagonistic effect of Se-Met towards Met, cysteine and taurine, especially in the liver. On other hand, there was no noticeable interaction between and CLAmix (Se-Y × CLAmix) because the metabolic capacity of dietary CLA isomers and Se-Met was smaller in femoral muscles compared with the liver.

CONCLUSIONS

Dietary supplementation of a CLA isomer mixture, regardless the presence of extra selenized yeast, seems to be an efficient way of increasing the concentration of *cis9trans11*CLA and *trans10cis12*CLA in the liver and especially in femoral muscles of rats, as well as in other monogastric animals like rabbits, nutrias or pigs (King et al., 2004). The interaction between Se-Y and CLAmix results in a major increase in the Se concentration in the liver and especially in muscles; this increase is concomitant with increasing in the concentration of sulphur-, essential, and non-essential AA in muscles, thus protein synthesis and, consequently, rat body weight. The results reported here documented that the interaction between Se-Y and CLAmix resulted in decreased desaturation capacity in the liver and muscles, therefore, these dietary additives reduced the ratio of unsaturated to saturated fatty acid concentrations (i.e. (MUFA+PUFA)/SFA) in the liver and muscles.

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