Influence of dietary conjugated linoleic acid isomers and selenium on growth, feed efficiency, and liver fatty acid profile in rats

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ABSTRACT

The influence of dietary CLA isomer(s) and/or selenized yeast on the growth, concentration of CLA isomers and other fatty acids in the liver was investigated in rats. Plasma blood triacylglycerols (TAG), total cholesterol (TC), HDL and LDL cholesterol fractions in relation to dietary CLA isomer(s) and/or selenium (Se) were analysed. The experiment was performed on female rats (Wistar), 8 weeks of age and initial body weight of about 200 g. After a 1-week preliminary period, for 4 weeks the animals were fed a diet enriched in conjugated linoleic acid (CLA) isomer(s) and selenized yeast (2×2 experimental design). Dietary Se or/and CLA isomer(s) resulted in small changes in the spleen, heart, kidneys and brain, and increased liver weight. Administration of Se and *trans10cis12*CLA most efficiently increased the body weight gain of rats. CLA isomer(s) administered with or without Se elevated the CLA isomer(s) level in the liver. These results demonstrate that *trans,trans*CLA isomers are metabolized more slowly, while *cis,trans/trans,cis*CLA isomers, more rapidly to long-chain fatty acids containing a conjugated double bond. Enrichment of the diet in CLA isomer(s) with or without Se caused a reduction in the capacity of Δ9-, Δ6- and Δ5-desaturases in the liver, while dietary *trans10cis12*CLA or the CLA isomer mixture increased Δ4-desaturases. The contents of oleic acid, C20:4n-6, and C20:5n-3 decreased in the liver, whereas the level of C22:5n-3 and C22:6n-3 increased in the liver of rats fed the CLA isomer mixture. Individual dietary CLA isomers with or without Se increased the concentration of palmitic and stearic acids in the liver. All experimental diets increased the concentration of triacylglycerols in blood plasma, while *trans10cis12*CLA with or without Se usually decreased the concentration of total cholesterol, LDL, and HDL cholesterol.

KEY WORDS: conjugated linoleic acid isomers, selenium, cholesterol, liver, blood plasma, fatty acids, rats

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INTRODUCTION

Selenium (Se) is a trace bio-element essential for living organisms, occurring at about 10 mg Se/60 kg human body weight (Suzuki, 2005). A greater percentage of seleno-methionine (Se-Met) is incorporated non-specifically into animal proteins in place of methionine (Met), particularly efficiently in a low Met diet (Tapiero et al., 2003), as tRNAMet does not discriminate between Met and Se-Met. Stored Se-Met can be a reserve pool of Se and much evidence indicates that Se deficiency stimulates the metabolism of Se-Met to other chemical forms of Se like seleno-cysteine (Se-Cys) (Whanger, 2004; Suzuki, 2005). Half of Se-proteins have been implicated in antioxidant functions, and Se-Cys is essential in the active centres of Se-enzymes that carry out redox reactions, e.g., glutathione peroxidase (GPx), thyroid hormone deiodinase families, or thioredoxin reductase (Tapiero et al., 2003; Suzuki, 2005). GPx acts synergistically with tocopherol and provides a second line of defence against hydroperoxides, which can damage membranes and other cell structures (Tapiero et al., 2003). Recently it has been reported that an adequate Se level in animal and human diets protects against lipid peroxidation and accumulation of carbonyl moieties on protein produced by oxidative stress (Tapiero et al., 2003). Feeding diets with selenized yeast (Se-yeast; 60% Se as Se-Met (Rayman, 2004)) to animals results in effective induction of GPx activity, indicating that the animal converts this chemical form of Se to Se-Cys that is essential for the synthesis of Se-proteins (Whanger, 2004). Considering the above evidence, we decided to investigate whether a diet enriched in Se-yeast stimulated the accumulation of conjugated linoleic acid (CLA) isomers in rat livers. CLA isomers are believed to 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 (Banni et al., 2004; Rainer and Heiss, 2004). CLA isomers are also expected to inhibit tumorigenesis by interfering in the metabolism of some carcinogens (Wahle et al., 2004). Therefore, the objective of our studies was to determine the effects of Se-yeast on the CLA isomer and other fatty acid concentrations in the liver of rats. Furthermore, the influence of the experimental diet enriched in Se and/or CLA isomer(s) on the body gain of rats, organ weight and feed intake was also determined.

MATERIAL AND METHODS

Animals and treatments

Ten groups of female rats (Wistar, Ifz: BOA), 8 weeks of age with an initial body weight of about 200 g, were housed individually in plastic cages as described by Czauderna et al. (2004a,b). During a one-week preliminary period the animals

were fed a standard Labofeed diet (Se content less than 0.1 ppm) given at a submaintenance level (daily ~9 g of the diet per rat) to reduce their body fat (Table 1). In the experimental period lasting 4 weeks, rats were fed *ad libitum* with the Labofeed diet enriched with a mixture of CLA isomers, the *cis*-9,*trans-11*CLA (*c9t*11), or *trans-10,cis-12*CLA (*t10c12*) isomer and/or Se, as Se-yeast (Rayman, 2004); the Se-yeast was donated by Sel-Plex, Alltech Inc. (USA). At the end of the experiment the rats were killed, blood samples collected, femoral muscles and livers removed, weighed, and analysed for fatty acid and/or mineral element content.

Chemicals

All reagents were analytical grade, whereas organic solvents were HPLC grade. The CLA isomer mixture, the *cis-9,trans-11*CLA and *trans-10,cis-12*CLA isomers were supplied by Larodan Fine Chemicals AB (Sweden). The purity and composition details of the CLA isomer mixture were examined using our Ag^{+}-HPLC method (Czauderna et al., 2003a). n-Hexane (95%) was purchased from Lab-Scan (Ireland), while all fatty acid standards and other chemicals were provided by SIGMA (USA).

Saponification and fatty acid extraction

The liver sample was frozen, lyophilized and the obtained residue was stored at -20°C until analysis. The finely powdered liver sample (~50 mg) was placed in a vial and treated with a mixture of 2 ml of 2M KOH in water, 2 ml 1M KOH in methanol and 50 µl of BHT solution in methanol (20 mg/ml). The mixture in a closed vial was then vigorously vortexed and heated under argon at 95°C for 10 min, next cooled for 10 min at room temperature, and then sonicated for 10 min. The obtained solution was protected from the light and stored in a sealed vial at 22-25°C overnight. Then, 3 ml of water were added to the hydrolysate in the vial and the solution was again vigorously vortexed. The obtained solution was acidified with 4M HCl to pH 1-2 and free fatty acids (FAs) were extracted four times with 3 ml of dichloromethane (DCM). The lower DCM layer was dried with ~100 mg of Na_2SO_4. To avoid any loss of free FAs, extraction was repeated 4 times using 3 ml of n-hexane. Afterward, the upper n-hexane layer was combined with the DCM layer and the organic solvents were removed under a stream of argon. The concentration of assayed fatty acids was calculated from lyophilized liver samples.

Preparation of fatty acid methyl esters (FA-MEs)

To the residue, 2 ml of 2M NaOH in methanol and 50 µl of BHT solution in methanol (20 mg/ml) were added while mixing, then flushed with argon, and

reacted for 1h at 80°C. After cooling, 2 ml of 25% BF_3 in methanol were added to the reaction mixture, flushed with argon, and heated for 1h at 80°C. To the cooled reaction mixture 5 ml of water were added and then FA-MEs were extracted with 5 ml of n-hexane. The supernatant was transferred to a vial. Separation of FA-MEs was carried out by gas liquid-chromatography (GLC), while FA-MEs containing conjugated double bonds were also analysed using silver-liquid chromatography (Ag^+-HPLC) with photodiode array detection at 234 nm (Czauderna et al., 2003a).

Analytical conditions

The analyses of FA-MEs were performed on an Agilent 6890N GC equipped with a CP7489 fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Varian, USA) and FID, while split injection was performed using an Agilent 7683 autosampler; the Ag^+-HPLC equipment was as previously described (Czauderna et al., 2003a). The FA-MEs were determined using GLC and Ag^+-HPLC according to Czauderna et al. (2005).

Analysis of triacylglycerols, total, HDL and LDL cholesterol, and elements

The concentrations of triacylglycerols (TAG) in blood plasma were quantified using Ektachem DT-60 kits donated by Johnson and Johnson Clinical Diagnostics Inc (i.e. microfiltration of deposited samples on *Vitros*-Slides and then a series of specific reactions with active reagents on the slides to produce coloured compounds quantified at 555 nm, the intensity of the colour of these final products is proportional to the amount of TAG in assayed samples). Total cholesterol (TC), HDL and LDL cholesterol fractions in blood plasma were also determined using commercial *Vitros*-Slides test methodology (Johnson and Johnson Clinical Diagnostics Inc). Ten μl drops of a sample are deposited on the *Vitros*-Slide. The sample spreads evenly and diffuses into the reagent layers. TC or HDL cholesterol fractions in the assayed plasma samples undergo a series of reactions in a *Vitros*-Slide to produce coloured compounds. The intensity of these coloured products in analysed samples is proportional to the amount of TC (the detection at 555 nm) and HDL cholesterol (detection at 660 nm). The concentration of LDL cholesterol was calculated using the Friedwald equation (LDL cholesterol = TC – HDL cholesterol – VLDL cholesterol).

The concentration of Zn, Cu, Fe, Mg and Ca in lyophilized liver and femoral muscle samples was determined by flame (air-acetylene) atomic absorption spectrometry (PU9100X Atomic Absorption Spectrometer, UNICAM, Philips).

Statistical analyses

The data are presented as means. Statistical analyses of the effects of Se or the CLA isomers in the diets were conducted using the nonparametric Mann-Whitney U test for comparing independent experimental groups, while statistical analyses of the interaction between the CLA isomer and Se were performed using two-factorial ANOVA analysis (the CLA isomer x Se; the experimental design 2×2). The Statistica ver. 6 and Excel 2003 programs were used in the statistical analyses. Differences were considered significant at the $P<0.05$ or $P<0.01$ level.

RESULTS AND DISCUSSION

The relationships between the experimental diets and feed intake, organ and body weight gain of rats are summarized in Table 1. In the current study, similarly as in our previous investigations (Czauderna et al., 2004b), no lesions or symptoms of Se-yeast and CLA isomer intoxication were found in rats fed experimental diets. In addition, after only 4 weeks of feeding experimental diets there were no considerable differences in the weight gain of rats and their organs among the groups. This indicates that any substantial differences in lipid and protein levels were not due to experimental diet intake and that the level of dietary organic Se was not toxic. The diet enriched in 1.2 ppm of Se would not be toxic for rats as only chronic feeding of Se compounds at a rate of more than 5 ppm can be hepatotoxic and teratogenic in animals and humans (Tapiero et al., 2003; Czauderna et al., 2004b). As can be seen from Table 1, feeding only *t10c12*CLA (Group *t10c12*) and the higher CLA isomer mixture dose (2%) with or without Se to rats numerically or significantly reduced feed intake. These results are supported by several other studies, which have indicated that the higher CLA isomer mixture dose as well as *t10c12*CLA reduces feed intake, body weight gain of rats and increases energy expenditure (Czauderna et al., 2003b; Park et al., 2005). These results are supported by several other studies, which have indicated that due to its geometric and positional structure, the *t10c12* isomer in particular is the most potent CLA isomer in terms of antiobesity activity. Indeed, the hormones noradrenaline and adrenaline were significantly higher in experimental animals gavaged with the CLA isomer mixture, and data suggest that CLA isomers, especially *t10c12*CLA, enhance sympathetic nervous activity that leads to increased energy metabolism and eventual reduction of adipose tissue weight (Belury, 2002). Czauderna et al. (2003b) and Degrace et at. (2003) also reported that feeding rats or mice a diet supplemented with CLA isomers, particularly with the *t10c12* isomer, increases fatty acid β-oxidation in the fat

Table 1. Effects of CLA isomer mixtures, individual CLA isomers without Se ($_{Se}$) and with Se supplemented ($_{+Se}$) diets[1] on feed intake, and initial body mass of rats[2], body mass of rats after one week adaptation, body weight gain (BWG) of rats after whole experiment period[3], feed conversion efficiency (FCE)[4], fresh liver, spleen, heart, kidneys and brain masses

Group	Supplement	Content	Feed[5] intake g	Body weight initial g	Body weight after one week[6], g	BWG of rats g	FCE g/g	Liver g/100 g body[7]	Liver mass g	Spleen g	Heart g	Kidneys g	Brain g
Control	-	-	500.4^{A,B}	199.5	177.0 (8)	61.1^a	0.1221^a	3.82^{ABabc}	9.1^{ABab}	0.59	0.77	1.80^A	1.74
Se	Se	1.2 ppm	501.1	200.4	179.0 (8)	60.9	0.1215	3.83	9.2	0.45	0.77	1.76	1.67
1%CLA	CLA isomers	1%	500.1	201.1	183.0 (7)	62.9	0.1258	4.03^a	9.9^a	0.52	0.80	1.73	1.73
c9t11	c9t11CLA	1%	504.8	200.6	177.5 (7)	67.5^b	0.1337^b	4.00^b	9.8^b	0.51	0.78	1.88	1.77
t10c12	t10c12CLA	1%	480.4	201.1	180.0 (7)	61.6^c	0.1282	4.18^A	10.1^A	0.52	0.78	1.92	1.74
2%CLA	CLA isomers	2%	459.6^A	200.1	178.0 (7)	56.4	0.1227	3.97	9.3	0.53	0.76	1.75	1.74
1%CLA+Se	CLA isomers + Se	1% 1.2 ppm	490.9	202.6	180.0 (7)	63.9	0.1302	4.02	9.8	0.56	0.76	1.85	1.68
c9t11+Se	c9t11CLA + Se	1% 1.2 ppm	479.4	201.2	181.0 (7)	56.8^b	0.1185^b	3.91	9.3	0.51	0.74	1.73	1.73
t10c12+Se	t10c12CLA + Se	1% 1.2 ppm	503.7	200.3	178.0 (7)	68.8^{ac}	0.1366^a	4.17^B	10.3^B	0.57	0.78	1.97^A	1.77
2%CLA+Se	CLA isomers + Se	2% 1.2 ppm	467.1^B	204.1	182.0 (8)	60.4	0.1293	4.29^c	10.4	0.55	0.77	1.82	1.75

[1] means in columns with the same letter are significantly different: A,B - $P<0.01$; a,b - $P<0.05$
[2] in parenthesis the number of rats in the group
[3] 29 days feeding with experimental diet enriched in CLA isomers and/or Se (1.2 ppm; 1.2 ppm = 1.2 μg/g)
[4] FCE: g body weight gain/g feed intake
[5] feed intake in 29 days feeding with experimental diets enriched in CLA isomers and/or Se
[6] body mass of individually adapted rats after one week of submaintenance feeding (in parenthesis - number of rats in a group). Initial body weight of rats and after one week adaptation did not differ statistically differ among group at the P<0.1 level
[7] liver masses derived from fresh liver weight of rats normalized to 100 g of a rat body

pad and in skeletal muscles (Alasnier et al., 2002), but not in the liver. The physiological responses to the CLA isomers in diets for rats suggest modulation of eicosanoid metabolism followed by elongation and Δ5-desaturation in the rat liver (Gnädig et al., 2001). Our data are consistent with these findings, thus feeding the CLA isomer mixture or the *t10c12* isomer, particularly simultaneously with Se, increased liver weight probably due to accumulation of fatty acid metabolites in this organ (Table 1). Moreover, a recent study (Simon et al., 2000) has shown that the content of protein in the liver of experimental animals fed CLA isomers increased. Another study also indicates that feeding *t10c12*CLA resulted in dramatic enlargement of the liver (Degrace et al., 2003). Indeed, at the peroxisomal level, considerable proliferation of small peroxisomes takes place together with increased capacity for β-oxidation of long-chain fatty acids (Terpsta et al., 2003). At the mitochondrial level, a greater part of activated fatty acids was directed from triacylglycerol esterification towards oxidation.

Interestingly, *t10c12*CLA is much more effective at lowering feed intake than the *c9t11*CLA isomer (Akahoshi et al., 2003), *t10c12*CLA appeared to be the effective isomer for modulating gene expression in, e.g., cultured 3T3-L1 pre-adipocytes (Choi et al., 2001). Consequently, no decrease in feed intake was observed when the diet was enriched only in the *c9t11* isomer, while administration of this isomer numerically increased body weight gain and feed conversion efficiency (FCE) compared with control rats. *t10c12*CLA in the diet enriched in Se most efficiently increased the body weight gain (BWG) of rats, liver and kidneys weight and FCE, whereas the feed intake was similar to that by the control rats. Thus, our current results are in agreement with our previous studies on the interaction between *t10c12*CLA and inorganic Se (selenate), which also stimulated BWG, FCE, and liver weight in experimental rats (Czauderna et al., 2003b, 2004b).

Investigations into the effect of experimental diets on rat organ weight gain (Table 1) have demonstrated that CLA isomers with or without Se have no consistent influence on the weight of the spleen, heart, brain or kidneys (with the exception of the diet enriched in *t10c12*CLA and Se) and the concentration of Zn, Fe and Ca in femoral muscle. Similarly to these results, all experimental diets resulted in inconsistent and negligible changes of the concentration of Zn, Cu, Fe and Mg in the liver (Table 2). Interestingly, the CLA isomers numerically elevated the concentration of Ca in the liver and the concentration of Fe in muscles; this effect was stronger as the concentration of CLA isomers in the diet increased. Our results are consistent with previous studies showing that CLA isomers mediated bone formation (Belury, 2002). In addition, the stimulating effect of CLA isomers on the accumulation of Ca in the liver is stronger if the diet is enriched in Se.

Table 2. The concentration of Zn, Cu, Fe, Mg and Ca in liver and muscles (µg/g of DM[1]) of rats fed CLA isomers and Se

Group	Type of Sample	Zn	Cu[2]	Fe	Mg	Ca
Control	Liver	102	5.90	660	594	78
	Muscle	49	-	79	1616	199
Se	Liver	96	5.93	705	589	81
	Muscle	50	-	59	1606	187
1%CLA	Liver	94	5.24	618	581	81
	Muscle	52	-	89	1638	213
c9t11	Liver	96	5.40	613	584	82
	Muscle	53	-	63	1672	234
t10c12	Liver	100	5.17	659	584	88
	Muscle	49	-	66	1630	198
2%CLA	Liver	93	4.94	700	596	87
	Muscle	48	-	107	1636	198
1%CLA+Se	Liver	109	4.75	665	594	90
	Muscle	53	-	67	1544	255
c9t11+Se	Liver	119	7.06	699	618	92
	Muscle	52	-	68	1648	287
t10c12+Se	Liver	98	5.00	661	603	88
	Muscle	47	-	77	1624	197
2%CLA+Se	Liver	105	7.41	688	603	97
	Muscle	50	-	106	1622	202

[1] dry mass of liver and muscles
[2] the level of Cu in femoral muscles is below the quantification limit

The effect of the diets on the concentration of CLA isomers and other fatty acids in the liver

The current study also investigated the relationship between the experimental diets and the concentration of CLA isomers and other fatty acids in the liver (Table 4). As expected, diets containing CLA isomer(s), irrespective of the presence of extra Se, resulted in considerable accumulation of CLA isomer(s) in the liver and affected the concentration of fatty acids in rat liver compared with the control rats. Our current results (Tables 3 and 4) are in agreement with the observations of Alasnier et al. (2002) and our previous studies (Czauderna at al., 2003b, 2004b) in which *t10c12*CLA and *t10t12*CLA are more efficiently driven through β-oxidation in cells of the liver, muscles, kidneys or adipose tissue than their *9,11* homologues. Therefore, the value of ratio ($R_{c9t11CLA/t10c12CLA}$) of these isomers in the administered CLA isomer mixture (R=0.981) was lower compared with the

Table 3. The concentration of fatty acids in liver of rats fed for 4 weeks *cis9trans11*CLA (*c9t11*), *trans10cis12*CLA (*t10c12*) or/and Se[1]

Group / Item	Control	Se	c9t11	t10c12	c9t11 + Se	t10c12 + Se	Significance of effects[1]			Interaction	
							Se	c9t11	t10c12	c9t11 × Se	t10c12 × Se
			µg/g liver								
c9C16:1	416	416	389	140	329	227	NS	NS	**	NS	NS
C18:0	14466	15082	16109	16091	16389	17050	NS	NS	**	NS	NS
C18:0+C16:0[2]	21516	22596	23786	22702	23367	24953	NS	NS	NS	NS	NS
c6+c7C18:1	1825	1500	1330	1315	1677	1398	NS	*	NS	NS	NS
c9C18:1	3351	2777	2762	2380	1685	2233	NS	NS	**	NS	**
c11C18:1	842	895	945	892	691	771	NS	NS	**	NS	NS
LA	7586	8073	6759	7220	6003	7854	NS	*	NS	NS	NS
αLNA	1088	1258	824	1020	669	1213	NS	*	NS	NS	NS
c9t11CLA	0[6]	0	457	162	448	128	-	**	NS	-	-
t10c12CLA	0	0	76	537	47	805	-	NS	**	-	*
ct/tcCLA[3]	0	0	773	926	890	1252	-	**	**	NS	NS
ttCLA	0	0	476	740	412	497	-	**	**	NS	NS
ccCLA	0	0	0	48	3	7	-	-	*	NS	NS
Total CLA	0	0	1249	1714	1305	1756	NS	**	**	NS	NS
C20:4n-6	13367	12943	8961	9585	9078	8754	NS	**	NS	NS	NS
C20:5n-3	3102	3090	2218	1995	2123	1889	NS	*	**	NS	NS
C22:5n-3	1235	1296	672	1439	801	1141	NS	**	NS	NS	NS
C22:6n-3	2917	2478	1896	2459	1726	2505	NS	*	NS	NS	NS
Δ9-index[5]	0.1491	0.1238	0.1170	0.0745	0.1039	0.0897	*	*	**	NS	**

[1] significance of effects: **- P<0.01; *- P<0.05; NS - P>0.05
[2] the sum of stearic and palmitic acid
[3] other CLA isomers possessing conjugated *cis* and *trans* (*ct*) or *trans* and *cis* (*tc*) double bonds
[4] non-CLA fatty acids containing conjugated double bonds (i.e. metabolites of the CLA isomers)
[5] Δ9-desaturase index: (*cis*-9C16:1 + *cis*-9C18:1)/ (*cis*-9C16:1 + *cis*-9C18:1 + C16:0 + C18:0)
[6] below the quantification limit (3 × the detection limit)

Table 4. The concentration of fatty acids in liver of rats fed for 4 weeks the mixture of CLA isomers (1% or 2%) or/and Se

Group	Control	1%CLA	2%CLA	1%CLA+Se	2%CLA+Se	Significance of effects[1]		interaction	
Item						1%CLA	2%CLA	1% CLA × Se	2% CLA × Se
			μg/g liver						
$c9$C16:1	416	234	117	177	147	**	**	NS	NS
C18:0	14466	15811	14149	13918	16982	*	*	NS	NS
C18:0+C16:0	21516	22830	20798	20208	23518	NS	NS	NS	NS
$c6$+$c7$C18:1	1825	2675	2535	793	687	NS	NS	NS	NS
$c9$C18:1	3351	2157	1764	1288	1855	**	**	NS	NS
$c11$C18:1	842	744	676	1432	714	NS	**	NS	NS
LA	7586	7643	7790	4824	7239	NS	NS	NS	NS
αLNA	1088	1258	1065	699	1028	NS	NS	*	NS
$c9t11$CLA[3]	0	363	525	253	434	**	**	**	*
$t10c12$CLA[3,4]	0	(1.16)314	(1.18)446	(1.16)219	(.90)484	*	**	NS	NS
$c,t/t,c$CLA[3,5]	0	854	1035	533	1099	**	**	NS	NS
ttCLA[3]	0	491	861	703	740	**	**	NS	NS
ccCLA[3]	0	0	30	0	3	-	NS	NS	NS
Total CLA[3]	0	1345	1926	1236	1842	**	**	NS	NS
C20:4n-6	13366	10877	2637	8799	10303	NS	NS	NS	NS
C20:5n-3	3102	2327	2422	2070	2074	*	**	NS	NS
C22:5n-3	1235	1330	1349	1118	1171	NS	NS	NS	NS
C22:6n-3	2917	3117	4084	2372	2992	NS	*	NS	NS
Δ9-index[2]	0.1491	0.0948	0.0828	0.0678	0.0785	**	*	NS	NS

[1] significance of effects: **- P<0.01; *- P<0.05; NS - P>0.05

[2] Δ9-desaturase index: (cis-9C16:1 + cis-9C18:1)/(cis-9C16:1 + cis-9C18:1 + C16:0 + C18:0)

[3] the concentration of the CLA isomer(s) were determined using Ag⁺-HPLC method (Czauderna et al., 2003a)

[4] in parenthesis - a value of a content ratio of $c9t11$ to $t10c12$ isomer. Dietary CLA isomer mixture contains: 1.96% tt isomers, 96.19% $c9t11$ and $t10c12$CLA isomers and 1.50% cc isomers; the ratio of content of $c9t11$ to $t10c12$ isomer in dietary CLA isomer mixture: 0.981

[5] the sum of all detected ct/tc CLA isomers

ratio of these isomers in the liver of rats fed the CLA isomer mixtures (R = 1.16 – 1.18; Table 4). The presence of Se in the diets enriched to a higher level of CLA isomers (2%) considerably increased the accumulation of $t10c12$CLA in the liver. Consequently, the value of $R_{c9t11CLA/t10c12CLA}$ in the liver of rats fed the 2% CLA isomer mixture and Se was significantly lower compared with the value of the ratio of these isomers in the dosed CLA isomer mixture and in the liver of rats fed the diets with only CLA isomer(s). The present results are in agreement with our previous studies in which dietary selenate also decreased the yield of preferential β-oxidation of $t10c12$CLA (Czauderna et al., 2004a,b), however, dietary organic Se more efficiently inhibited the capacity of β-oxidation of this isomer (Tables 3 and 4) compared with inorganic Se.

The current study clearly indicates that *trans,trans*CLA isomers are preferentially incorporated into the liver, whereas there is partial discrimination in the accumulation of *cis,cis* and *cis,trans/trans,cis*CLA isomers. The lower accumulation of *cis,trans/ trans,cis* and *cis,cis* is the result of rapid metabolism, β-oxidation (particularly of the *t10c11*CLA isomer) and unfavourable geometrical configuration. In contrast, more efficient accumulation of *trans,trans*CLA isomers could be due to their slower metabolism and β-oxidation as well as preferred geometrical insertion into membrane phospholipids.

As expected, feeding the CLA isomer mixtures (1 or 2%) or individual CLA isomers, particularly *t10c12*CLA, irrespective of the presence of Se, resulted in decreasing the content of oleic acid (*cis-9*C18:1) and *cis-9*C16:1 in the liver. This is consistent with our previous results (Czauderna et al., 2003b, 2004a, b) showing that the CLA isomers, especially *t10c12*CLA, are responsible for the change in the levels of monounsaturated and saturated fatty acids in organs from animals fed CLA isomers due to decreasing the Δ9-desaturation of FAs such as C16:0 or C18:0 (Alasnier et al., 2002). Recent studies (Alasnier et al., 2002; Kang et al., 2004) have demonstrated that CLA isomers, particularly *t10c12*CLA, caused a reduction in Δ9-desaturase capacity, inhibited stearoyl-CoA desaturase mRNA expression and fatty acid synthesis (Belury, 2002). Consequently, the value of the Δ9-desaturase index in the liver of rats fed a diet enriched in CLA isomers decreased compared with that in the liver of control rats (Tables 3 and 4). The experimental diet enriched only in Se also caused a reduction in Δ9-desaturase capacity, inhibited stearoyl-CoA desaturase mRNA expression (Table 3).

The addition of organic Se (mainly as Se-Met) to the diet resulted in decreasing the concentration of *cis-9*C18:1 in rat liver. Our current results are in agreement with our previous studies in which inorganic Se (as selenate) also efficiently decreased the concentration of *cis-9*C18:1 in the liver, femoral muscles and perigonadal fat of experimental rats (Czauderna et al., 2003b, 2004a,b). Se supplementation to the diet enriched in the CLA isomer mixture (1 and 2%) or *c9t11*CLA resulted in a

numerical decrease in the concentrations of linoleic (C18:2n-6; LA) and linolenic (C18:3n-3; αLNA) acids in the liver compared with rats fed with the CLA isomer mixtures or *c9t11*CLA (Tables 3 and 4). On the other hand, *c9t11*CLA in the diet most efficiently decreased the concentration of LA and αLNA in the liver compared with the control group or rats fed the diet enriched in only *t10c12*CLA (Table 3). These results indicate that the individual CLA isomers, particularly *c9t11*CLA, statistically or numerically decreased Δ6-desaturase capacity, while, surprisingly, the mutual interaction between dosed *t10c12*CLA and *c9t11*CLA tended to slightly increase the capacity of this desaturase. Thus, our current study is in agreement with our previous investigation (Czauderna et al., 2004b) in which the individual CLA isomers decreased Δ6-desaturase capacity, whereas the mixture of the CLA isomers increased the capacity of this desaturase in the liver of rats.

Detailed investigations of the concentration of stearic and palmitic acids in the rat liver reinforce the findings of this study and of Banni et al. (2004) showing that the *c9t11* isomer and *t10c10*CLA, in particular, decrease the yield of *cis-9* fatty acids formation (Table 3). Consequently, the concentrations of these saturated fatty acids are higher in the liver of rats fed the diet enriched in these isomers, irrespective of the presence of Se, compared with the controls. However, feeding the CLA isomer mixture (1 or 2%) with or without the presence of Se resulted in an inconsistent effect of the administered CLA isomer mixture on the concentration of stearic and palmitic acids in the liver. We hypothesize that this irregular effect of these additives to the rat diet is due to interactions between CLA isomers as well as dosed Se (Table 4).

In the present study, feeding the CLA isomer mixtures (1 or 2%) with or without Se affected the concentration of C20:4n-6, C20:5n-3, C22:5n-3 and C22:6n-3 in the rat liver (Table 4), whereas administration of individual CLA isomers, regardless of the addition of Se, usually numerically or significantly decreased the concentration of these polyunsaturated fatty acids in the rat liver (Table 3). Surprisingly, feeding the CLA isomer mixture (1 and 2%) with or without Se decreased numerically or significantly the concentration of only C20:4n-6 and C20:5n-3 in the liver of experimental rats. The diet enriched in the CLA isomer mixture (1 and 2%) increased the concentration of C22:5n-3 and C22:6n-3 in the liver and this effect was stronger as the concentration of the CLA isomer mixture in the diet increased (i.e. to 2%). Addition of Se to the diet enriched in the CLA isomer mixture reduced the observed influence of an isomer mixture on the concentration of these fatty acids; consequently the concentration of C22:5n-3 and C22:6n-3 in the liver was slightly lower compared with the concentrations in the liver of the control group or rats fed only the CLA isomer mixtures (Table 4). This influence of added Se is weaker as the concentration of the CLA isomer mixture in the diet is higher (2%). Therefore, we suggest that the diet enriched in the mixture of the

CLA isomers increased the yield of the elongation of C20:5n-3 and the capacity of Δ4-desaturase in the liver in comparison with the control group. In contrast, the CLA isomer mixture, irrespective of the presence of Se, caused a decrease in the concentration of C20:4n-6 and C20:5n-3 in the liver, therefore we conclude that administering the CLA isomer mixture to the diet reduced the capacity of Δ5-desaturase. In addition, supplementing the individual CLA isomers, regardless of the presence of Se, usually numerically or significantly decreased the yield of the elongation of C18:4n-3, and C18:3n-6, and the capacity of Δ6- and Δ5-desaturases in the liver. On the other hand, the diet enriched in only Se had a negligible influence on the concentration of C20:4n-6, C20:5n-3, C22:5n-3 and C22:6n-3 in the liver compared with the control group.

Table 5. The concentration of triacylglycerols (TAG), total (TC), HDL[1] fraction of cholesterol and LDL[2] fraction of cholesterol in blood plasma of rats feed CLA isomer(s) or Se

Group[3]	TAG	Cholesterol			LDL/HDL	TC/HDL
		total	HDL	LDL		
		mmol/l				
One-factorial ANOVA analysis						
Control	0.988[ab]	1.963	1.286	0.676	0.526	1.526
Se	1.015	1.937	1.246	0.698	0.560	1.555
1%CLA	1.672[a]	2.010	1.340	0.744	0.555	1.500
C9t11	1.327[b]	1.843	1.199	0.740	0.617	1.537
t10c12	0.940	1.846	1.250	0.600	0.480	1.477
2%CLA	1.178	1.841	1.184	0.657	0.555	1.555
Two-factorials ANOVA analysis (the CLA isomer × Se)						
1%CLA+Se	1.420	2.035	1.276	0.679	0.532	1.595
Intrraction[4]	NS	NS	NS	NS	NS	NS
c9t11CLA+Se	1.183	2.096	1.233	0.870	0.706	1.700
Interaction[4]	NS	NS	NS	NS	NS	NS
t10c12CLA+Se	1.564	1.825	1.099	0.672	0.611	1.661
Interaction[4]	NS	NS	NS	NS	NS	NS
2%CLA+Se	1.447	1.811	1.191	0.620	0.521	1.521
Interaction[4]	NS	NS	NS	NS	NS	NS

[1] high-density lipoprotein
[2] light-density lipoprotein
[3] means in columns with the same letter are significantly different: [a,b] -P<0.05
[4] interaction: data were analyzed by two-factorials: the CLA isomer(s) × Se; statistical significance was declared at * - P<0.05 and ** - P<0.01; NS - not significant (ANOVA test)

The results of the dose-response changes in triacylglycerols (TAG) and cholesterol fraction concentrations in blood plasma by different forms of additives are summarized in Table 5. It was found that there was a numerical decrease the

concentration of total cholesterol (TC) and the HDL cholesterol fraction in plasma of rats fed the diet enriched in Se only. Indeed, there is recent evidence that excess Se supplementation (5 µg/kg diet) significantly decreases TC in rabbits (Vinson et al., 1998). In our study, dietary *c9t11*CLA and *t10c12*CLA numerically decreased the concentration of TC and HDL cholesterol, whereas supplementing *t10c11*CLA resulted in the most efficient decrease in the LDL concentration. The diet enriched in the higher concentration of the CLA isomer mixture most efficiently decreased the concentration of TC and HDL cholesterol in blood plasma of rats. The mixture of CLA isomers at a dose of 1% numerically or significantly increased the concentration of HDL and LDL cholesterol as well as the content of TCG and TC. On the other hand, administering *t10c12*CLA as well as the mixture of the CLA isomers at a dose of 2% decreased the concentration of the HDL and LDL fractions in blood plasma. Surprisingly, in contrast to the recent results of Tricon et al. (2004), in our current study administering *t10c12*CLA revealed a tendency towards lowering the LDL:HDL concentration ratio as well as the value of the TC: HDL ratio as compared with the control group and all other experimental diets. Furthermore, in our study, the diet with *t10c12*CLA most efficiently numerically decreased the concentration of TCG in plasma compared with the control group. The possible explanation is that *t10c12*CLA treatment stimulated fatty acid oxidation, reduced body fat accretion with concurrent increases in lean tissue deposition (Belury, 2002). This is valuable information for nutritionists in the context carrying out research to improve the nutritional quality of food for human health.

CONCLUSIONS

The current study revealed that in rats, during feeding diets enriched in CLA isomer(s) with or without Se for 4 weeks, a major increase in the concentration of CLA isomer(s) occurred in the liver. Dietary *t10c12*CLA and Se significantly enhanced the body weight accretion of rats, the concentration of CLA isomer(s) in the liver and muscles, as well as feed conversion efficiency. Adding 1.2 ppm Se with or without the CLA isomer(s) increased the content of the essential element (Se) in the body of experimental animals (without toxicity), thus, supplementing Se with or without the CLA isomer(s) improves the nutritional quality of products derived from animals to a greater degree. The supplemented CLA isomer mixture elevated the liver concentration of n-3 long-chain polyunsaturated fatty acids (i.e. C22:5n-3 and C22:6n-3) and this effect was stronger as the level of CLA in the diet increased. Addition of Se to the diets containing the CLA isomer(s) at the dose of 1% documented a tendency toward increasing the values of the plasma LDL:

HDL and TC:HDL ratios. Moreover, the diet enriched in only Se also resulted in increases in the values of these ratios and the concentration of LDL cholesterol and TAG.

These results constitute valuable information for nutritionists in the context of carrying out research to improve the nutritional quality of food for domestic animals and human health.

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