Dietary conjugated linoleic acid isomers and selenite or selenized yeast affect fatty acid concentration in the kidneys of rats*

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ABSTRACT

The influence of diets enriched in a mixture of conjugated linoleic acid (CLA) isomers (CLAmix) and/or selenium as Na₂SeO₂ (SeIV) or selenized yeast (SeY) on feed intake, kidneys' weight and the concentrations of fatty acids (FA) in the kidneys of rats was investigated. The study was performed on 80 female Wistar rats (Hsd Brl Han: WIST), 8 weeks of age with an initial body weight of 195.7±0.8 g. Each group numbered 8 rats. During the 7-day preliminary period the rats were fed a standard Labofeed H diet at a sub-maintenance level. Next, for 6 weeks the rats were fed ad libitum on the experimental diets supplemented with CLAmix, 0.2 ppm Se or 0.5 ppm Se as SeIV (SeIV or _HSeIV, respectively) and SeY (_LSeY or _HSeY, respectively). The rats were sacrificed at the end of the six-week experimental period. The diets enriched in CLAmix or Se (as SeIV or SeY) showed a negligible effect on kidneys' weight, although the diets containing simultaneously CLAmix and Se numerically increased kidneys' weight compared with the control animals. The addition of CLAmix to the diet, regardless of the higher level of SeY, showed a tendency to decrease in feed intake and decreased the concentrations of saturated (SFA), mono- (MUFA) and polyunsaturated (PUFA) fatty acids in comparison with the control rats. Dietary SeIV or SeY, irrespective of the concentration of extra Se, decreased the concentration of unsaturated fatty acids (UFA) and the sum of all assayed fatty acids (Σ FA) in the kidneys. The diet containing CLAmix and Se as "SeIV or "SeY decreased the concentrations of SFA, MUFA and PUFA in the kidneys compared with the control rats. On the other hand, the addition of $_{\rm L}$ SeIV or, especially, $_{\rm H}$ SeY to the diet enriched in CLAmix resulted in an increasing in the concentrations of SFA, MUFA and PUFA in the kidneys compared with the control group and rats fed the diets containing CLAmix, SeIV or SeY. The diet enriched in CLAmix and HSeY most effectively stimulated the accumulation of CLA isomers with the exception of cis11trans13CLA in the kidneys in comparison with other diets. Dietary CLAmix decreased the

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capacity of Δ 9-desaturase in the kidneys compared with the control rats. The addition of SeIV or SeY to the diet increased the capacity of Δ 4-desaturase, while the diet containing CLAmix with or without Se as SeIV or SeY increased the capacity of Δ 4- and Δ 5-desaturase in the kidneys compared with rats fed the diet with extra Se or the control diet. The present studies documented that dietary CLAmix resulted in decreasing in biosynthesis of fatty acids in the kidneys, while the diet with CLAmix and _HSeY most significantly stimulated the accumulation of SFA, MUFA and PUFA and long-chain PUFAn-3 and PUFAn-6 in the kidneys.

KEY WORDS: rats, kidneys, selenium, conjugated linoleic acid isomers, fatty acids

INTRODUCTION

The kidneys serve the body as a natural filter of the blood, and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium; the kidneys also are responsible for the reabsorption of water, glucose and amino acids (Walter, 2004). The kidneys also produce hormones including calcitrol, renin and erythropoietin. The kidneys receive blood from the renal arteries, right and left, which branch directly from the abdominal aorta. Despite their relatively small size, the kidneys receive approximately 20% of the cardiac output (Al-kahtani et al., 2004).

Considering the above, it is reasonable to hypothesize that some additives to diets revealed influence on the concentration of physiologically important fatty acids (FA) in the kidney tissue. In our recent studies we found the influence of dietary conjugated linoleic acid (CLA) isomers or selected Se compounds on the accumulation of fatty acids and amino acids in the liver, pancreas, spleen, brain adipose tissues and muscles of rats (Czauderna et al., 2007, 2010a,b,c). Indeed, the addition of CLA isomers to rats' diets reduced body fat accretion, cholesterol ester synthesis and hepatocytic apolipoprotein B secretion (Javadi et at., 2007; Park, 2009). CLA isomers' influence on body fat reduction is suggested to be the result of multiple mechanisms: by reducing lipid accumulation in adipose tissues and/or adipocytes differentiation, by increasing energy expenditure, by increasing adipocyte apoptosis, by modulating adipokines and cytokines, such as leptin, TNF-a, adiponectin, or interleukins, and by stimulating FA β -oxidation in muscles (Park and Pariza, 2007).

Similarly, Se has been established as an essential microelement that is important in many biochemical and physiological processes (Wysocka et al., 2003; Navarro-Alarcon and Cabrera-Vique, 2008). Se exerts its biological effect through several selenoproteins of which there may be more than 30 in mammalian systems. In general, selenoproteins serve as enzymes that catalyse redox reactions (Han et al., 2009). Examples are the glutathione peroxidases (GPx) which protect against oxidative stress. Specifically, Se-dependent GPx enzyme recycles glutathione, reducing lipid peroxidation by catalysing the reduction of peroxides, including hydrogen peroxide (Navarro-Alarcon and Cabrera-Vique, 2008; Navas-Acien et al., 2008). Our early studies using rats found that the level of CLA isomers and other polyunsaturated fatty acids (PUFA) in a liver or femoral muscles was positively correlated with the selenite (SeIV), selenate or selenized yeast (SeY) content in diets (Czauderna et al., 2004, 2011). So, we hypothesized that dietary SeIV or SeY increased concentrations of CLA isomers and other PUFA in the kidneys of rats. Indeed, these fatty acids (FA) are components of membranes of kidney cells. Therefore, this paper's objective was to evaluate the impact of different chemical forms and amounts of SeIV or SeY added to a diet enriched with CLA isomers (CLAmix) on the fatty acid (FA) profile in kidneys of rats.

MATERIAL AND METHODS

Animals, housing, diets, experimental design and sampling

The experiment was carried out on 80 female rats (Wistar, Hsd Brl Han: WIST), 8 weeks of age and with an initial body weight of about 195.7±0.8 g. The animals were housed and handled in accordance with protocols approved by the Local Animal Care and Use Committee. The animals were housed individually in plastic cages at a temperature of $22\pm1^{\circ}$ C with a 12 h light-dark cycle and relative humidity of 50-60%. Each group comprised eight rats. The rats were fed the standard Labofeed H diet produced by the Feeds and Concentrates Production Plant in Kcynia (Poland), according to ISO 9001 Standards since 1998 (Czauderna et al., 2010a). For the current study, the standard Labofeed H diet was enriched in 0.2 µg Se/g diet as selenite (Table 1). During the 7-day preliminary period the rats were fed a standard Labofeed H diet offered at sub-maintenance level (9 g per day per rat) to reduce the rats' body fat; the average body weight of individually adapted rats was 179.0±6.1 g (Table 2). Next, the rats were fed ad libitum for 6 weeks the experimental diets supplemented with 1.5% CLA isomer mixture (CLAmix), 0.2 µg Se/(g diet) (, Se) or 0.5 µg Se/(g diet) (, Se) as selenite (, SeIV and "SeIV) or selenized yeast ("SeY and "SeY) (Table 2). Feed intake and body weight of rats were measured weekly. The rats were killed at the end of the six week experiment. The kidneys were removed, weighed and frozen. All kidneys were analysed individually. The concentrations of all assayed FA were calculated based on freeze-dried kidneys samples.

Reagents, chemicals and analytical methods

All chemicals were of analytical grade and organic solvents were of HPLC

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or GC grade. Dichloromethane (HPLC grade), KOH, NaOH, Na₂SO₄, chloroform and conc. HCl were purchased from POCH (Gliwice, Poland). Methanol (HPLC grade) and n-heptane (99%, GC) were supplied by Lab-Scan (Ireland),

Item	Content
Composition of the basal diet ² , g/100 g diet	
dry matter ³	88.2 ± 0.9
crude protein	21.8 ± 1.3
lysine	1.31
methionine and cysteine	0.76
tryptophan	0.28
threonine	0.87
crude fibre	3.85
crude fat	3.0 ± 0.8
ash	5.9 ± 0.6
N total	3.77
N protein	3.17
P total	0.75
mono-carbohydrates	5.75
starch	30.3
metabolizeable energy ⁴ , MJ/kg	13.9
Composition of selenized yeast (SeY)	
total Se, mg/g DM	1.8
identified Se species, % of total Se	88.3
selenomethionine, % of total Se	83.0
Se-cysteine, % of total Se	5.0
sodium selenite, % of total Se	0.3
fatty acids, mg/g DM ⁵	
C16:0	9.0
<i>cis9</i> C16:1	4.1
C18:0	13.6
<i>cis9</i> C18:1	11.3
<i>cis11</i> C18:1	0.8
cis9cis12C18:2 (linoleic acid; LA)	14.7
cis9cis12cis15C18:3 (aLNA)	0.16

Table 1. Chemical composition and energy content of the basal diet¹ and the selenized yeast (SeY)

¹ means of 9 samples; ² ingredients of the basal diet: maize, wheat, oat flakes, green meal, soyabean oilmeal, fish meal, soya oil, vitamins, per kg diet: IU: vit. A 10 096, vit. D₃ 2000; mg: vit. E 86.1, vit. K1 3, vit. B₁ 15.7, vit. B₂ 16, vit. B₆ 5.24, vit. B₁₂ 81, biotin 0.2, folic acid 3.03, nicotinic acid 79.3, pantothenic acid 25.5; g: choline 2.02; minerals (contained per g DM diet; mg: Na 3.6; K 8.3; Ca 10.68; P 7.6 and trace elements, contained per kg DM diet: Se as Na₂SeO₃ 0.2; Cu - 13.9; Zn 98; Mn 112; Fe 698; Mg 1653; ³ the concentration of main fatty acids in the basal diet, mg per kg DM diet: C8:0 37; C10:0 6; C12:0 11; *cis9cis12cis15*C18:3 (αLNA) 8; *cis6cis9cis12*C18:3 (γLNA) 715; C14:0 11; *cis9cis12*C18:2 (LA) 429; C16:0 250; *cis9*C18:1 187; *cis6*C18:1 112; C18:0 89; C20:0 11; C22:0 2.4; Σ saturated fatty acids (SFA) 417; Σ polyunsaturated fatty acids (PUFA) 1499; Σassayed fatty acids (ΣFA) 1915; ⁴ the mean from 3 samples; ⁵ main fatty acid peaks (i.e. ~95% of peak area of all fatty acids in SeY determined by GC-MS

ner mixture (CLAmix) and Se as selenite (SeIV) or selenized yeast (SeY) on fresh kidney	of mono- (MUFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA) and	s (g) of rats after 6 weeks feeding with experimental diets ¹
2. The effects of diets enriched in 1.5% CLA isomer mixture (CL	; feed intake (g) and the concentration of the sum of mono- (MUF	tyed fatty acids (ΣFA) in the freeze-dried kidneys (g) of rats after
Table 2	weight,	all assa

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		Kidneys	Total feed	Adapted	Final body	OTA 5	ATTEA	DITEA	T TE A	NE 4
Group	Additives	weight	intake ²	body weight ³	weight ⁴	SFA	MUFA	PUFA	UFA	2FA
I	per g ulei	mg		ас				mg/g		
Control		1704^{a}	742ª	180.4	230.4	20.0^{a}	$15.8^{a\beta}$	39.0	54.8	74.8ª
SelV	0.2 µg Se as SeIV	$1666^{a\alpha}$	755	176.9	230.4ª	18.6^{a}	15.2^{α}	34.0 ^α	49.2	67.8^{A}
SeIV	0.5 µg Se as SeIV	1740	767	180.1	237.2	20.3	$15.5^{\rm b}$	34.5	50.0^{By}	70.3 ^a
SeY	0.2 µg Se as SeY	1775	763	179.2	237.3	18.2	$13.1^{\beta c}$	32.3	45.4	63.6
SeY	0.5 µg Se as SeY	1642^{β}	740	177.3	232.9	19.1°	15.0^{d}	36.6^{b}	51.5 ^a	70.6^{A}
CLA	1.5% CLAmix	1752	$719^{a\alpha}$	181.7	233.6	17.7^{Aa}	11.9ae	34.1^{pac}	46.0^{ab}	63.7^{Ba}
L ^{SeIV} _{CLA}	0.2 μg Se as SeIV 1.5% CLAmix	1767 ^a	728	180.3	235.0	25.1 ^{αbX}	22.2 ^{Aa}	69.3 ^{abax}	91.4 ^{abx}	116.5 ^{Ax}
H _H SeIV _{CLA}	0.5 μg Se as SeIV 1.5% CLAmix	1720	743	177.0	237.0 ^a	19.8 ^b	10.9^{Ab}	28.9	39.87	59.6α
$_{\rm L}{\rm SeY}_{\rm CLA}$	0.2 µg Se as SeY 1.5% CLAmix	1739	772 ^a	178.3	234.3	19.6 ^x	10.2°	29.9	40.1	59.6
${}^{\rm H}{\rm SeY}_{\rm CLA}$	0.5 μg Se as SeY 1.5% CLAmix	1742 ^β	719	177.4	233.0	34.3 ^{AcX}	46.6 ^{deX}	138.3 ^{bcX}	184.9^{abX}	219.2 ^{ABX}
interactic feeding f	In columns sharing the mass of CLAmix x_L Se ε for 6 weeks (the stand	ie same lette: and CLAmix ard Labofeed	r are signific x _H Se (Se as s I H diet conta	antly different: ^a selenite or SeY), ained 0.2 mm Se	a,bP<0.05 and A significant at *)	$^{B}P<0.01; ^{a}$ P<0.05 and 3 the body v	^β P<0.1 dift ^X P<0.01, r veight (a)	cerences wer espectively; of individua	e taken as ² total feed Ilv adanted	tendencies; intake after rats after 7

days of sub-maintenance feeding: ⁴the average body weight (g) of rats feeding for 42 days with the experimental diets enriched in CLAmix or/and Se as SeIV or SeY; ⁵ the concentration sum of all assayed saturated fatty acids including long-chain saturated fatty acids (L-SFA) and odd-chain

saturated fatty acids (Odd-SFA)

while the CLA isomer mixture (2.1% *tt*CLA, 7.1% *c11t13*CLA, 40.8% *c9t11*CLA, 41.3% *t10c12*CLA, 6.7% *c8t10*CLA and 2.0% *cc*CLA; *t* and *c* -abbreviations for *trans* and *cis*) by Industrial Chemistry Research Institute (Warsaw, Poland). The concentration ratio ($R_{c9t11}CLA, 10e_{12}CLA$) of *c9t11*CLA to *t10c12*CLA in the dietary CLA isomer mixture was 0.988. The selenized yeast (Se-*Saccharomyces cerevisiae*) was donated by Sel-Plex (non-commercial yeast sample; Alltech Inc., USA). About 83% of the total selenium content of the selenized yeast (SeY) represents Se in the form of seleno-methionine (Se-Met) incorporated into the proteins of *Saccharomyces cerevisiae* (Table 1) (Wysocka et al., 2003; Rayman, 2004; Weiss and Hogan, 2005). Fatty acid methyl ester (FAME) standards, 25% BF₃ in methanol and sodium selenite were purchased from Supelco and Sigma (USA). Water used for the preparation of mobile phases and chemical reagents was prepared using an ElixTM water purification system (Millipore).

All of the fatty acid standards and reagents, saponification and FA methylation methods were as described previously (Czauderna et al., 2009).

Chromatographic equipment

The analyses of all FAME in processed kidneys samples were performed on a SHIMADZU GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica capillary column (120 m \times 0.25 mm i.d. \times 0.25 µm film thickness; SHIM-POL), quadrupole mass selective (MS) detector (Model 5973N) and injection port. Helium as the carrier gas operated at a constant pressure (223.4 kPa) and flow rate of 1 ml/min. Injector and MS detector temperatures were maintained at 200 and 240°C, respectively. The total FAME profile in a one µl sample at a split ratio of 10:1 was determined using the column temperature gradient programme as previously described (Czauderna et al., 2009). FAME identification was validated based on electron impact ionization spectra of FAME and compared with authentic FAME standards and NIST 2007 reference weight spectra library.

Statistical analysis

Results are presented as means of 8 individually analysed kidneys samples. Mean values in columns having the same superscripts are significantly different at ^{a,b}P<0.05 and ^{A,B}P<0.01, while differences at ^{a,β}P=0.1 are indicated as tendencies. These one-factorial statistical analyses of the effects of SeY or the CLAmix in the diets were conducted using the non-parametric Mann-Whitney U test for comparing independent experimental groups. Statistical analyses of interactions

between CLAmix and Se as LSeIV, HSeIV, SeIV, SeY or SeY (i.e. $CLA \times SeIV$, CLA $\times SeIV$, SeIV, CLA $\times SeIV$, SeIV, SeIV,

RESULTS AND DISCUSSION

The effects of experimental diets on kidneys weight and feed intake. Mammalian kidneys have a dominant role in controlling both the concentration of body fluids and their volume. Fortunately, in the current study, no macroscopic lesions or pathological changes were found in the kidneys as well as in other internal organs of rats fed diets enriched in 1.5% CLAmix regardless of the presence of Se as SeIV or SeY. Moreover, there was no negative influence on body weight of rats fed the diets enriched in the lower (0.2 μ g Se/g diet) and higher (0.5 μ g Se/g diet) levels of Se regardless of the chemical form of added Se compounds (Table 2). Indeed, signs of selenosis were observed for diets containing $\sim 5 \mu g$ Se per g diets, which is a level ~100 times the contribution of Se to typical mammal diets (Navarro-Alarcon and Cabrera-Vique, 2008). Only chronic feeding of inorganic Se compounds at a rate of more than 5 µg Se per g diets can be hepatotoxic and teratogenic in animals and humans (Tapiero et al., 2003). Moreover, our previous studies documented that only 2 µg Se as selenite per g diets decreased the body weight of rats, although no macroscopic lesions and toxic symptoms of this diet were observed in rats fed this diet (Czauderna et al., 2003, 2004).

As can be seen from the results summarized in Table 2, the addition of CLAmix to the diets, irrespective of the presence of extra Se as SeIV or SeY, increased the kidneys' weight in comparison with the control group, although these differences were no statistically significant. Concomitantly, these diets increased also the body weight gain (BWG) of rats (Czauderna et al., 2010a). Interestingly, the diets containing , SeIV or "SeY numerically decreased the weight of kidneys compared with the control rats; moreover, the diets enriched in SeIV or SeY, regardless of the concentration of extra Se, also resulted in a decreasing in the weight of the brain, liver and spleen of examined animals compared with the control group, while the diets containing SeY or SeIV (i.e. SeY, SeY and especially SeIV) increased BWG of rats (Czauderna et al., 2009, 2010a,b,c). Therefore, we suggest that dietary SeIV or SeY revealed specific effects on some internal organs of rats. The addition of CLAmix to the diet, regardless of the higher level of SeY, showed a tendency to decrease in feed intake in comparison with the control rats (Table 2). On the other hand, other experimental diets resulted in inconsistently small changes in feed intake compared with the control group.

The effects of experimental diets on concentrations of saturated and monounsaturated fatty acids in the kidneys. It was found that supplementation of the diet enriched in CLAmix decreased (P < 0.05) the concentration sum of saturated (SFA) and monounsaturated (MUFA) fatty acids in the kidneys compared with the control group (Table 2). Moreover, detailed analysis of above results documented that dietary CLAmix decreased (P < 0.05) the concentration of myristic acid (C14:0; Table 3), the sum of atherogenic (A-SFA), thrombogenic (T-SFA) SFA (Ulbricht and Southgate, 1991) (Table 4) as well as long-chain (L-SFA) and odd (Odd-SFA) SFA in the kidneys (Table 5). Moreover, the serious decrease in the concentrations of mono- and polyunsaturated FA in the kidneys (Table 2) explains higher values of atherogenic (A_{index}) and thrombogenic (T_{index}) indexes in the kidneys than in the control group (Table 4). The diet enriched in 1.5% CLAmix had a similar effect on the concentrations of A-SFA and T-SFA in the brain, spleen, femoral muscles and abdominal fat of rats (Czauderna et al., 2009, 2010a,c). Thus, our current results are consistent with studies of Alasnier et al. (2002), Bauman et al. (2003) and Czauderna et al. (2004) in which diets supplemented with CLA isomers increased FA β -oxidation in the fat pad and skeletal muscles but not in the liver of mice and rats. The addition of the lower amount of SeIV or SeY to the diet resulted in a numerically decreasing in the concentrations of SFA, A-SFA, T-SFA, L-SFA and MUFA, including trans-FA (t11C18:1), in the kidneys compared with the control group (Tables 2-5). Considering the above, we suggest that the lower amount of SeIV or SeY, like CLAmix, in the diet stimulated SFA β-oxidation in the kidneys.

On the other hand, the accumulation of saturated fatty acids in the kidneys stimulated the addition of CLAmix to the diet enriched in SeIV or SeY; there are statistically significant CLAmix \times SeIV and CLAmix \times SeY interactions (P<0.01) on the concentration of SFA in the kidneys. There are also the statistically significant CLAmix \times_{H} SeY interactions on the concentrations of L-SFA, Odd-SFA, MUFA and c9C18:1 in the kidneys (Tables 3 and 5). Thus, it can be concluded that the simultaneous addition of CLAmix and "SeY to the diet stimulated elongation of fatty acids and decreased the β -oxidation of fatty acids in the kidneys. In consequence, the concentrations of all assayed FA (Σ FA), including long-chain polyunsaturated fatty acids (LPUFA) and L-SFA (Tables 2, 4 and 5), increased in the kidneys of rats fed the diet containing CLAmix and "SeY. Interestingly, the significant increase in the concentrations of mono- and polyunsaturated FA in the kidneys explains lower values of A_{index} and T_{index} in the kidneys of rats fed the diets containing CLAmix and "SeY in comparison with the control group (Table 4). Similarly, the simultaneous addition of CLAmix and selenate to the rats' diet resulted in increasing in the concentration of Σ FA in the kidneys, pancreas, heart, femoral muscles, abdominal fat and blood plasma (Czauderna et al., 2003, 2004; Niedźwiedzka et al., 2006). Moreover, the diet enriched in CLAmix and

ffects of 1.5% CLA isomer mixture (CLAmix) and Se as selenite or SeY on the concentration of C14:0, cis9C18:1 (c9C18:1),	accenic acid; <i>tIIC</i> 18:1), conjugated linoleic acid (CLA) isomers, <i>cis9cis12C</i> 18:2 (LA), <i>cis9cis12cis15C</i> 18:3 (aLNA),	C20:4 (AA) and values of $\Delta 9$ -desaturase index ($\Delta 9_{miner}$) and ratios of $c9tIICLA$ to $t10c12CLA$ in the kidneys of rats after 6	h experimental diets
Table 3. Dietary effects of 1.5%	trans11 C18:1 (vaccenic acid)	cis5cis8cis11cis14C20:4 (AA) i	weeks feeding with experiments

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Group	С14:0 ug/g	<i>c9</i> C18:1 mg/g	$\Delta 9_{index}^{-1}$	<i>t</i> 1/C18:1	<i>c9t11</i> CLA	t10c12 CLA	<i>clltl3</i> CLA	$ttCLA^2$	ΣCLA^3	$\frac{c9t11^4}{t10c12}$	LA	αLNA	AA
	0	0				3n	g/g					mg/g	
Control	435	11.4	0.420^{ab}	ı ع	I	ı	ı	$40^{a\alpha}$	40	I	18.3	2.52	15.0
SelV	412	10.7^{a}	0.421	ı	ı	ı	ı	$81^{\mathrm{A}\alpha}$	81 ^A	ı	16.6^{a}	2.74	11.8^{α}
SeIV	$438^{\rm a}$	10.5	0.397 ^a	ı	ı	ı	ı	97^{aB}	97 ^в	ı	16.3	2.73ª	12.2^{β}
LSeY	315^{a}	9.1	0.383^{b}		ı	ı		63	$63^{\rm C}$	ı	15.3	2.36^{a}	11.7
SeY	411^{A}	10.2^{A}	0.402^{d}	ı	ı	ı	ı	83	83 ^D	ı	17.4^{d}	2.96^{b}	12.9ª
CLA	355^{Bb}	9.0^{Bb}	$0.364^{\rm acef}$	5.7^{ABCD}	471^{AB}	264^{Aa}	44.8^{AB}	$45^{Cbc\gamma}$	$824^{E\alpha}$	1.785 ^{aβya}	16.6^{b}	1.95 ^{Aβ}	12.1 ^{yõb}
SelV	810^{abx}	17.4^{abx}	0.443°	3.9^{A}	$831^{\rm Aa}$	399^{ab}	1.42^{Aa}	14^{Ab}	$1260^{A\alpha X}$	2.083^{a}	$33.9^{\rm abcx}$	4.37^{B}	25.3^{wyx}
^H SelV _{CLA}	421	8.7	0.328 ^{ax}	$2.7^{\rm B}$	549 ^a	$262^{\rm b}$	3.55^{Ba}	$8^{\rm BC}$	850 ^B	2.097ª	15.3°	1.48^{a}	9.5 ^{p6}
$^{\rm L}{ m SeY}_{ m CLA}$	376α	8.1	0.314°	1.7 ^c	486	230	ı	8°	742 ^c	2.110^{β}	15.4	1.54^{a}	10.5
$\operatorname{SeY}_{\operatorname{CLA}}$	1320^{ABX}	36.1^{ABx}	0.554^{dfX}	44.1 ^D	2389 ^в	1139 ^A	ı	$115^{\gamma X}$	3791 ^{dex}	2.097	65.3^{dX}	8.56^{AbX}	50.6^{abX}
$\frac{1}{\Delta 9-desal}$	urase ind	ex = (c9C)	16:1+ <i>c9</i> C1 ed CLA is	8:1)/(<i>c9</i> C18 omers: ⁴ the	:1+c9C1	8:1+C16:0 ation ratio	+C18:0); (R) of <i>cis</i>	² the conc	entration s LA to <i>tran</i>	um of <i>tran</i> s10cis12CI	<i>is, tranns</i> C	LA isome io of <i>c9t1</i>	rrs; ³ the /CLA to

t10c12CLA in CLA mix added to the diets was 0.988; ⁵ below the quantification limit (L_Q)

enic SFA (A-SFA)	rombogenic (T _{index}	I assayed FA ($\Sigma F/$	
ntration of atherog	nic $(A_{indev})^5$ and th	FA and PUFA to a	
nite) on the conce	alues of atheroge	6/n-3), PUFA to S	
Se or _H Se (as sele	PUFAn-6 ⁴ , and v	6 to PUFAn-3 (n-	
(CLAmix) and ¹	PUFAn-3 ³ , and L	6/Ln-3), PUFAn-	cimental diets
A isomer mixture	n-3, PUFAn-6, L	DUFAn-3 (Ln-	eeding with exper
fects of 1.5% CL.	(T-SFA) ² , PUFA	s of LPUFAn-6 to	ts after 6 weeks f
ble 4. Dietary eff	ombogenic SFA	lexes, and rations	he kidneys of ra

Group	A-SFA	A.	T-SFA	Ţ	PUFAn-3	LPUFAn-3	PUFAn-6	LPUFAn-6	<u>Ln-6</u>	<u>0-u</u>	<u>PUFA</u>	<u>PUFA</u>
-	mg/g	Index	mg/g	Index		mg	/g		Ln-3	n-3	SFA	2FA
Control	12.5^{aba}	0.260	19.4^{a}	0.495	5.32	2.79	33.7	15.4	5.44 ^{ao}	6.39^{ab}	1.94	0.516
SelV	$11.5^{\rm b}$	0.262	18.2^{a}	0.497	5.10	2.36	28.8^{a}	12.2 ^α	5.17^{b}	5.72 ^A	1.82	0.500^{A}
SelV	12.2	0.273^{A}	19.8	0.521^{A}	5.44^{a}	2.71 ^a	29.0	12.6^{a}	4.70^{aA}	5.38^{Ba}	1.70^{a}	0.489
SeY	10.7^{a}	0.258^{B}	17.7	0.519^{B}	4.84^{b}	2.49^{Λ}	27.4	12.1	$4.85^{B\alpha}$	5.69°	1.77^{b}	0.505
SeY	$11.5^{\beta c}$	0.249^{a}	18.6^{b}	0.470^{a}	5.68°	2.71^{b}	30.8^{a}	13.4^{b}	4.94	5.46^{Cb}	1.91°	0.516^{a}
CLA	11.0^{adA}	0.268^{Cbc}	17.4^{acd}	0.533^{CDb}	$4.33^{A\alpha}$	2.38^{cda}	$28.9^{\rm bc}$	12.4^{Byc}	5.65 ^{By}	7.58ª	1.92^{ABd}	$0.534^{\rm bca}$
$_{1}$ SeIV $_{0.4}$	17.2^{bd}	0.247	$24.8^{\circ\circ}$	0.453	8.51	4.13	59.5 ^{ab}	25.6^{ab}	6.01^{b}	7.14^	2.53	0.571^{A}
SelV	12.6	0.352^{AC}	19.5	0.743^{AC}	3.05^{a}	$1.57^{\rm ac}$	25.0	$9.6^{a\gamma}$	6.24^{AB}	9.62^{Ba}	1.46^{Aa}	0.486^{b}
LSeY _{CLA}	11.9	0.326^{Bbx}	18.9	0.697 ^{BDx}	3.24^{ba}	$1.71^{A\alpha}$	25.9	10.5	$6.24^{\rm By}$	8.61°	1.52^{Bb}	0.500^{a}
${}^{\rm H}{}_{\rm CLA}$	24.1 ^{cA}	0.163^{acX}	32.2^{bd}	0.315^{abx}	17.79 ^{AcX}	9.23 ^{bdX}	116.8 ^{acX}	$51.4^{\rm bcX}$	5.42	6.52 ^c	3.72^{cdX}	0.606 ^{acx}
¹ the conc	centration s	um of C12:	0, C14:0 a	ind C16:0								
2 the cond	antration a	m of C14.	0.016.0	18.0 Pu								

une concentration sum of C14:0, C10:0 and C18:0

³ the concentration sum of *cllcl4c17*C20:3, *c8cllcl4c17*C20:4, *c5c8cllcl4c17*C20:5, *c7c10c13c16c19*C22:5 and *c4c7c10c13c16c19*C22:5 ⁴ the concentration sum of *cllc1*4C20:2, *c8cllc1*4C20:3, *c5c8cllc1*4C20:4, *c13c1*6C22:2 and *c7c10c13c1*6C22:4

⁵ the atherogenic index = (C12:0+4*C14:0+C16:0)/(MUFA+PUFAn-6+PUFAn-3) (Ulbricht and Southgate, 1991)

• the thrombogenic index = (C14:0+C16:0+C18:0)/(0.5*MUFA+0.5*PUFAn-6+3*PUFAn-3+PUFAn-3/PUFAn-6) (Ulbricht and Southgate, (1991)

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Group	<i>c</i> 11C20:1	EPA	DPA	DHA	Elongase	$\Delta 4_{2}$	$\Delta 5$ 3	$\Delta 6$	L-SFA ⁵	odd-SFA ⁶	
-		8n	g/		Index	Index	Index	- Index	ŝ'n	<u>8/8</u>	
Control	10.3^{abcd}	881	591^{aca}	1296	0.401^{a}	0.681^{Aabca}	0.977^{Aab}	ı	167^{δ}	239	
SelV	34.0^{Aa}	804	420^{a}	1071^{a}	0.343	0.719^{Ba}	0.976^{B}	ı	141^{a}	236^{α}	
SelV	32.6^{Bb}	1040^{A}	466^{Ab}	1143^{α}	$0.310^{a\alpha}$	0.708^{Ca}	0.972^{AC}	ı	178^{B}	262^{β}	
SeY	$25.8^{\rm Fc}$	904^{B}	422^{cd}	1103	0.318^{b}	0.721^{Db}	0.976^{D}	ı	150	236^{γ}	
SeY	32.3 ^d	945^{b}	474°	1232 ^b	0.334	$0.722^{\rm Ec}$	$0.974^{\rm Eb}$	ı	$157^{\rm b}$	250^{A}	
ËLA	$22.7^{\text{CDE}\alpha}$	$686^{\rm acd}$	$408^{\alpha\beta\gamma\delta}$	1181^{Byc}	$0.373^{b\beta}$	0.744^{ABde}	0.985^{a}	$0.0383^{\mathrm{AB}\alpha}$	$103^{\alpha\beta\delta c\gamma\epsilon}$	182^{B}	
SelV	2.3^{ACX}	1156	655×	2287^{abx}	0.361	$0.772^{B\beta}$	0.988^{B}	0.0178^{A}	$46^{a\alpha x}$	$125^{\omega x}$	
SelV	4.2^{BDX}	331^{Aax}	$289^{Ab\beta}$	897^{w}	0.466^{ab}	$0.757^{\rm C}$	0.991^{CX}	0.0131^{B}	43^{BBx}	161^{β}	
SeY	$5.5^{\rm EFx}$	$346^{\rm Bc}$	$300^{d\gamma}$	1036	$0.464^{\rm bbx}$	0.773^{Dd}	0.991^{Dx}	0.0361^{a}	235 [°]	197^{γ}	
SeV	30.8^{a}	2707^{bdX}	$1450^{e\delta X}$	4852^{bcX}	0.349	0.768^{Ee}	0.985^{E}	0.0247	$306^{\text{bcy}_{\text{X}}}$	1719^{ABX}	
¹ the elon	gase index = c	:7c10c13c16	c19C22:5/(c)	7c10c13c16c1	9C22:5 + c5c	8 <i>c11c14c17</i> C2	20:5)				
² ∆4-desai	urase index =	c4c7c10c13	c16c19C22:6	5/(c4c7c10c13	<i>c16c19</i> C22:6	+ c7c10c13c1	6c19C22:5)				
³ ∆5-desai	urase index =	c5c8c11c14	C20:4/(<i>c5c8c</i>	c11c14C20:4 -	+ <i>c8c11c14</i> C2	(0:3n-6)					
⁴ Δ6-desai	urase index =	c6c9c12c15	:C18:4/(<i>c6c9</i> ¢	:12c15C18:4 -	+ <i>c9c12c15</i> C	18:3)					
5+40 0000	antrotion ann		LCO bus O.CC	Ģ							

⁵ the concentration sum of CZ0:0, CZ2:0 and CZ4:0 ⁶ the concentration sum of C11:0, C13:0, C15:0, C17:0 and C21:0 $_{\rm H}$ SeY also stimulated accumulation of Σ FA in the liver, pancreas, blood plasma and abdominal fat of rats in comparison with the control group (Czauderna et al., 2007, 2009, 2010a,b,c, 2011).

Recent investigations (Alasnier et al., 2002; Bauman et al., 2003; Czauderna et al., 2004) have found that after CLA isomers (especially t10c12CLA) were fed to rats or mice, the content of stearyl-CoA desaturase gene 1 mRNA in the liver decreased by 45-75%. Therefore, a decrease in Δ 9-desaturation is a major reason for modification of monounsaturated fatty acids, especially c9C18:1, in animals fed diets enriched in CLA isomers (Tables 2 and 3). The substrate: product relationship for $\Delta 9$ -desaturase is further supported by detailed analysis of values of the $\Delta 9$ -desaturase index (Table 3). Interestingly, the diets enriched in SeIV or SeY also numerically decreased the concentration of c9C18:1 and usually MUFA and values of the Δ 9-desaturase index in the kidneys. Similarly, diets enriched in SeIV or SeY also decreased the concentration of c9C18:1 and values of $\Delta 9$ -desaturase index in the liver, femoral muscles, brain, pancreas and abdominal fat of examined rats (Czauderna et al., 2007, 2009, 2010a,b,c, 2011). Moreover, our recent investigation documented that a diet enriched in selenate (2 μ g Se/g diet) also decreased the concentration of c9C18:1 and the value of Δ 9-desaturase index in the liver, kidneys, femoral muscles and spleen of rats (Czauderna et al., 2004). Surprisingly, the addition of CLAmix to the diet enriched in the higher concentration of SeY significantly stimulated the accumulation of MUFA, including especially c9C18:1 and c9C16:1, in the kidneys in comparison with the control group. Indeed, the yield of $\Delta 9$ -desaturation as well as the value of Δ 9-desaturase index significantly increased in the kidneys of rats fed the diet enriched in CLAmix and "SeY; there are the statistically significant interactions at P<0.01 (Tables 2 and 3). Similarly, the diet containing CLAmix and Se as SeY or SeIV (regardless of their concentration) increased the accumulation of c9C18:1 as well as MUFA in the abdominal fat (Czauderna et al., 2010a) and blood plasma (Czauderna et al., 2010c) in comparison with the control group or rats fed the diet enriched in SeIV or SeY. Thus, the current study and our previous studies documented that simultaneously dietary CLAmix and "SeY (the positive interaction) most efficiently stimulated the Δ 9-desaturation in the kidneys as well as in abdominal fat and plasma of rats (Czauderna et al., 2010a,c).

The effect of experimental diets on concentrations of CLA isomers in the kidneys. The experimental results regarding the concentration of additives in the diets as well as the influence of the examined diets on the concentration of CLA isomers in the kidneys of rats are summarized in Table 3. As expected, the addition of CLAmix to diets, regardless of the presence of SeIV or SeY, resulted in an increasing in concentrations of *c9t11*CLA, *t10c12*CLA, *c9t13*CLA and *tt*CLA in the kidneys in comparison with the control rats or rats fed the diets

containing only extra SeIV or SeY. The diets containing CLAmix and Se as SeIV or SeY statistically significantly (P < 0.01) increased the concentration of the sum of all assayed CLA isomers (Σ CLA) in the kidneys compared with the control rats or rats fed the diets with SeIV, SeIV Thus, the current results support our recent studies, in which dietary CLAmix, irrespective of presence of selenate, SeIV or SeY, significantly (P<0.01) increased the concentration of CLA isomers in femoral muscles and internal organs of rats (Czauderna et al., 2003, 2004, 2007, 2009, 2010a,b,c, 2011; Niedźwiedzka et al., 2006). Detailed analysis of these results revealed that the addition of "SeY to the diet containing CLAmix statistically significantly increased the concentration of c9t11CLA, t10c12CLA, ttCLA and Σ CLA in the kidneys compared with rats fed the diet with CLAmix (P<0.01), as well as rats fed the diets containing CLAmix and Se as , SeIV, "SeIV or , SeY. Similarly, the diet enriched in CLAmix and , SeIV increased the concentration of c9t11CLA, t10c12CLA and Σ CLA in the kidneys compared with rats fed the diet enriched only in CLAmix. On the other hand, the addition of "SeIV or SeX to the diet containing CLAmix usually numerically decreased the concentration of CLA isomers in the kidneys in comparison with rats fed the diet with CLAmix. Interestingly, the addition SeIV or SeY to the diet containing CLAmix statistically significantly (P<0.01) reduced the accumulation of *c11t13*CLA in the kidneys compared with rats fed the diet with only CLAmix. Surprisingly, *tt*CLA isomers are detected in the kidneys of rats fed the control diet as well as the diets containing Se as SeIV or SeY. These results suggest that examined rats practice coprophagia as bacteria in rats' large-intestine can produce ttCLA isomers. Thus, our current results are in agreement with our previous studies which also revealed that CLA isomers were quantitatively detected in femoral muscles and perigonadal fat of rats fed a control diet, i.e. without CLA isomers (Czauderna et al., 2003). Interestingly, the diets enriched in SeIV or SeY numerically increased the accumulation of *tt*CLA isomers in the kidneys compared with the control group. On the other hand, the addition of CLAmix to the diets containing SeIV, SeIV or SeY revealed a competitive effect (a negative interaction) on the incorporation of *tt*CLA isomers in the kidneys. Consequently, these diets resulted in a decreasing in *tt*CLA isomers in the kidneys compared with the control rats or rats fed the diets containing SeIV, SeY or CLAmix.

Detailed analysis of results documented that t10c12CLA is faster metabolized in the kidneys than c9t11CLA. Therefore, the concentration ratio of c9t11CLA/ t10c12CLA in the kidneys of rats fed the diets enriched with the CLAmix were, regardless of the presence of SeIV or SeY, higher compared with the ratio (R_{c9t11}CLA/ t10c12CLA) of these isomers in the CLA isomer mixture added to the diets (i.e. 1.785 - 2.110 vs 0.988; Table 3). Thus, our current results are in agreement with results of Alasnier et al. (2002) and our previous investigations (Czauderna et al., 2003,

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2004) in which the t10c12CLA and t10t12CLA were also more efficiently driven through β-oxidation in the cells of the liver, kidneys, adipose tissue or femoral muscles of rats than their 9,11 homologues of CLA. Moreover, our present studies documented that the addition of SeIV or SeY to the diet enriched in CLAmix increased the concentration ratio (R) of c9t11CLA/t10c12CLA in the kidneys in comparison with the R_{c9t11CLA/t10c12CLA} value in the kidneys of rats fed the diet containing only CLAmix. Therefore, it can be concluded that interaction between CLAmix and Se as SeIV or SeY, irrespective of the concentration of extra dietary Se, increased the efficiency of β -oxidation of t10c12CLA and t10t12CLA in the kidneys. The current studies are in agreement with our previous investigation in which the diet containing CLAmix and Se as SeY or SeIV also increased (the positive interaction) the β -oxidation of *t10c12*CLA in the liver, femoral muscles, spleen and abdominal fat of rats (Czauderna et al., 2009, 2010a,b,c, 2011). On the other hand, selenate (a stronger oxidant than selenite) added to the diet with CLAmix diminished the efficiency of β -oxidation of *t10c12*CLA and *t10t12*CLA in the kidneys, liver, plasma or femoral muscles of rats compared with rats fed the diet containing only CLAmix (Czauderna et al., 2003, 2004; Niedźwiedzka et al., 2006).

The effects of experimental diets on concentrations of polyunsaturated fatty acids in the kidneys. The present studies documented that the addition of CLAmix and/or Se as SeIV or SeY to the diet had significant effect on concentrations of PUFA in the kidneys of rats. Indeed, as can be seen from results summarized in Tables 3, 4 and 5, the diet enriched in CLAmix numerically decreased the concentrations of c9cl2C18:2 (LA), c9cl2cl5C18:3 (aLNA), c5c8c11c14C20:4 (AA), c5c8c11c14c17C20:5 (EPA), c7c10c13c16c19C22:5 (DPA) and c4c7c10c13c16c19C22:6 (DHA) in the rats' kidneys. Furthermore, this additive in the rats' diet decreased the concentration sum of PUFA. PUFAn-3. LPUFAn-3, PUFAn-6 and LPUFAn-6 in the kidneys. Considering also the influence of dietary CLAmix on the concentration of SFA, MUFA, unsaturated FA (UFA) and Σ FA (Table 2), we argue that the CLAmix added to the rats' diet stimulated the β-oxidation of saturated and unsaturated fatty acids in the rats' kidneys. Indeed, our recent studies also indicate that feeding the CLA isomers resulted in enlargement of the liver (Czauderna et al., 2003). At the peroxisomal level, considerable proliferation of small peroxisomes takes place together with increased capacity for β -oxidation of long-chain FA; at the mitochondrial level, a greater part of activated fatty acids in animals' body was directed from triacylglycerol esterification towards oxidation (Alasnier et al., 2003). According to the above, our detailed investigations also demonstrated that the addition of CLAmix to the diet decreased the value of the elongase index in the kidneys and pancreas (Czauderna et al., 2010b) in comparison with the control rats (Table 5). Unexpectedly, dietary CLAmix numerically increased values of the ratio of LPUFAn-6/LPUFAn-3 and PUFAn-6/PUFAn-3 in the kidneys (Table 5) as well as in the liver, spleen and blood plasma (Czauderna et al., 2010c, 2011). Moreover, the addition of SeIV or SeY to the diet containing CLAmix usually increased the values of these ratios in the kidneys compared with rats fed the diet containing only CLAmix. Considering the above, we suggest that the addition of CLAmix to the rats' diet preferentially stimulated the accumulation of polyunsaturated FA n-6 and, probably, the catabolism of polyunsaturated FA n-3 in the kidneys.

On the other hand, the addition of $_{\rm L}$ SeIV or $_{\rm H}$ SeY to the diet enriched in CLAmix stimulated the accumulation of LA, α LNA, AA, EPA, DPA and DHA in the rats' kidneys, pancreas (Czauderna et al., 2010b) and blood plasma (Czauderna et al., 2010c) in comparison with rats fed the diet containing the single additive as well as the control rats. Accordingly, these diets increased also the concentration sum of PUFA, PUFAn-3, LPUFAn-3, PUFAn-6, LPUFAn-6 as well as the ratio of PUFA/SFA and PUFA/ Σ FA in the kidneys; there are statistically significant CLAmix x $_{\rm H}$ SeY interaction effect (P<0.01) on the concentrations of these fatty acids in the kidneys (Tables 2-5) and liver (Czauderna et al., 2011).

The addition of SeIV or SeY to the diet, regardless of the concentration of extra SeIV or SeY, decreased the ratio of PUFAn-6/PUFAn-3 and LPUFAn-6/LPUFAn-3 in the kidneys, liver (Czauderna et al., 2009, 2011), pancreas (Czauderna et al., 2010b) and blood plasma (Czauderna et al., 2010c) compared with the control rats. Considering the above, we argued that dietary SeIV or SeY more effectively stimulated the catabolism of PUFAn-6 and LPUFAn-6 than PUFAn-3 and LPUFAn-3 in these tissues of rats. As can be seen from results summarized in Table 5, dietary SeIV or SeY increased the yield of Δ 4-desaturation in the kidneys and femoral muscles (Czauderna et al., 2009, 2011) compared with the control group. On the other hand, these dietary additives decreased the yield of Δ 5-desaturation in the kidneys, while the simultaneous addition of CLAmix and Se as SeIV or SeY lowered the capacity of this desaturase compared with rats fed the diet with only CLAmix. On the other hand, the addition of CLAmix to the diet enriched in SeIV or SeY enlarged the capacity of Δ 4- and Δ 5-desaturases in the kidneys compared with rats fed the diet containing CLAmix, SeIV or SeY.

CONCLUSIONS

Diets containing selenite, selenized yeast (up to 0.5 mg total dietary Se/kg diet) and CLA isomers (up to 1.5%) can be regularly used to increase the concentration of Se and CLA isomers in monogastric farm animals without adversely influencing performance. The results documented that the CLAmix and _HSeY treatment most significantly stimulated the accumulation of saturated, mono- and polyunsaturated

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fatty acids in the kidneys of rats. In accordance with physiological properties of CLA isomers, CLAmix added to the rats' diet resulted in the decreasing in biosynthesis of fatty acids in this internal organ.

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