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Selenite, selenized yeast, or conjugated linoleic acid isomers supplemented to the diet influence the fatty acid profile in the spleen and blood plasma of rats*

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ABSTRACT

The effect of dietary conjugated linoleic acid (CLA) isomers (CLAmix), Na_2SeO_3 (SeIV) or selenized yeast (SeY) on spleen weight, contents of CLA isomers and other fatty acids (FA), and efficiency of fatty acid desaturation in the spleen and blood plasma of rats were investigated. The study was performed on 80 female Wistar rats, 8 weeks of age with an initial body weight of 195.4±0.8 g. Each group numbered 8 rats. For 6 weeks the rats were fed *ad libitum* the Labofeed H diet supplemented with 1.5% CLAmix, 0.2 ppm Se as sodium selenite ($_L$SeIV), selenized yeast ($_L$SeY), or 0.5 ppm Se as SeIV ($_H$SeIV) or SeY ($_H$SeY). The addition of CLAmix to the diet with $_L$SeIV or $_H$SeIV increased the weight of the spleen in comparison with rats fed the diet with SeIV or the control group. The diets enriched in CLAmix, $_H$SeY, or the diet with CLAmix and SeY (as $_L$SeY or $_H$SeY) decreased spleen weight. The diet containing CLAmix, with or without Se, as SeIV or SeY, reduced the sum of fatty acids (ΣFA) in the spleen, while increasing it in the plasma. The diet with CLAmix and $_H$SeY most efficiently increased the content of CLA isomers, especially *c9t11*CLA, in the spleen and plasma. *C9t11*CLA in the spleen was metabolized more efficiently than *t10c12*CLA, while in plasma *t10c12*CLA was metabolized faster than *c9t11*CLA. The diet containing CLAmix with or without SeIV or SeY, significantly decreased the contents of *c9*C18:1, *c11*C18:1, and sums of saturated fatty acids (SFA), mono-(MUFA) and polyunsaturated fatty acids (PUFA) in the spleen, whereas the diet containing CLAmix and/or $_H$SeY increased the concentrations of these fatty acids in plasma. The diet enriched in CLAmix or $_H$SeY decreased the ratios of PUFA/SFA, MUFA/SFA and PUFA/ΣFA in the spleen and plasma. The addition of SeIV or SeY to the diet with CLAmix usually slightly decreased these ratios in the spleen and plasma in comparison with the diet

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containing CLAmix. Our results indicate that the diet with CLAmix more significantly stimulated the metabolism of PUFA and MUFA in the spleen than of SFA. The addition of SeIV or SeY to the diet with CLAmix increased the metabolic efficiency of PUFA and MUFA in the spleen.

KEY WORDS: selenized yeast, selenite, CLA isomers, fatty acids, spleen, blood plasma, rats

INTRODUCTION

The spleen is an important internal organ found in practically all vertebrate animals and plays a crucial role in regard to red blood cells as well as the immune system (Gilmore, 2006; Perkins, 2007). It removes old red blood cells, holds a reserve of blood in case of haemorrhagic shock, and also recycles iron. This organ synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation. Additionally, the spleen also clears bacteria and is important for proper immune function, especially in fighting bacteria. Other important functions of the spleen include erythropoiesis, storage of red blood cells and other formed elements, as well as production of opsonins, properdin, and tuftsin (Brender et al., 2005; Harold, 2010). Interestingly, the cells that consumption larger amounts of Se are immune cells, erythrocytes and platelets (Yusuf et al., 2002; Navarro-Alarcon and Cabrera-Vique, 2008; Slavik et al., 2008). Moreover, Se is needed for the proper functioning of the immune system. On the other hand, a low Se concentration in food is associated with a decrease in immune efficiency as well as poor growth and impairment of animal production (Navarro-Alarcon and Cabrera-Vique, 2008). Animal investigations have also documented that isomer mixtures of conjugated linoleic acid (CLA) as well as individual *cis9trans11*CLA (*c9t11*CLA) and *trans10cis12*CLA (*t10c12*CLA) isomers might have a beneficial influence on the immune system (Jørgensen et al., 2010) and possess antiproliferative, antitumour, antiinflammatory and antiatherogenic properties (De La Tore et al., 2006; Naumann et al., 2006).

Solid evidence based on epidemiological studies conducted in the last 50 years shows positive relationships between Se and CLA isomer dietary intake and the effectiveness of immune functions in living organisms (Yu et al., 2002, 2008; Park and Pariza, 2007; Navarro-Alarcon and Cabrera-Vique, 2008). In addition, recent investigations have shown that dietary supplementation with CLA isomers and various Se-sources affected the concentration of fatty acids in the spleen, blood, and other tissues in examined animals (Czauderna et al., 2007a, 2009, 2010a,b; Jørgensen et al., 2010). Importantly, long-chain polyunsaturated fatty acids (LPUFA), especially n-3LPUFA, showed a positive influence on the immune

system in living organisms. Changes in the concentration of these fatty acids in animal tissues depended on the chemical forms of Se and/or CLA isomers in a diet (Yu et al., 2008).

Considering the above, we hypothesized that addition of Se (as selenite or organic Se) and CLA isomers to a rat diet affected the level of LPUFA in the spleen and blood of rats. Therefore, the aim of the current study was to investigate the influence of a diet enriched in CLA isomer mixture (CLAmix) and different doses of sodium selenite (SeIV) or selenized yeast (SeY) on the fatty acid profiles in the spleen and blood plasma of rats. Selenized yeast has been shown to be a highly bioavailable source of Se (Rayman, 2004).

MATERIAL AND METHODS

Animals, housing, diets, sampling and analytical methods

Ten groups of female rats (Wistar, Hsd Brl Han: WIST), 8 weeks of age with an initial body weight of 195.4±0.8 g were housed individually in plastic cages and fed the control and experimental diets as described in detail in our previous publication (Czauderna et al., 2010a).The rats were housed and handled in accordance with protocols approved by the Local Animal Care and Use Committee (the Agricultural University of Warsaw, Poland). Briefly, after a 7-day preliminary period, for 6 weeks the rats were fed *ad libitum* the standard Labofeed H diet (Czauderna et al., 2009) supplemented with 1.5% CLA mix (Czauderna et al., 2010a), 0.2 ppm Se as sodium selenite ($_{L}$SeIV) or selenized yeast ($_{L}$SeY) or 0.5 ppm Se as SeIV ($_{H}$SeIV) or SeY ($_{H}$SeY). SeIV, SeY and/or CLAmix mixed with a finely powdered standard Labofeed H diet. The rats were killed at the end of the six-week experiment. Spleens were removed, weighed, and frozen, blood samples were collected and processed as described in our previous publication (Niedźwiedzka et al., 2007).

Lipids in spleen (~25 mg) and blood plasma (100 µl) samples were saponified (Czauderna et al., 2009) followed by gentle base- and acid-catalysed methylations of free fatty acids (Czauderna et al., 2007b). Tissue fatty acids were quantified as methyl esters using capillary gas chromatography with a quadrupole mass selective detector as previously described (Czauderna et al., 2010a).

Chemicals, chromatographic equipment and statistical analysis

All of the fatty acid standards and reagents, saponification (Czauderna at al., 2009) and methylation (Czauderna et al., 2007b) methods, as well as chromatographic equipment were as previously described (Czauderna et al., 2010a).

Results are presented as means of individually analysed samples of fresh spleen and blood plasma. Statistical analyses of the effects of dietary CLA isomer mixture and selenite or selenized yeast (i.e. SeIV or SeY) on the concentration of fatty acids and enzyme indexes were conducted using the non-parametric Mann-Whitney U test for comparing independent experimental groups. Statistical analyses of the interaction between CLAmix and Se (as SeIV or SeY) were performed using two-factorial ANOVA. For statistical analyses the program Statistica ver. 6 (Statistica by StatSoft, 2002) was used.

RESULTS AND DISCUSSION

The effect the experimental diets on the concentration of CLA isomers in the spleen and blood plasma of rats. In the current study, no macroscopic lesions or pathological changes were found in the spleen or any of the other organs of rats fed diets enriched in the CLAmix and/or Se (as $_{L}$SeY, $_{H}$SeY, $_{L}$SeIV or $_{H}$SeIV). As can be seen from the results (Table 1), the addition of CLAmix to the diet enriched in SeIV, irrespective of the concentration of extra SeIV, increased the weight of the spleen in comparison with rats fed the diet containing only SeIV or with the control group. On the other hand, the diets enriched in CLAmix or $_{H}$SeY, or the diet containing CLAmix and SeY (as $_{L}$SeY or $_{H}$SeY), decreased spleen weight. In comparison with the control diets, the other experimental diets showed a small and inconsistent influence on the spleen weight.

Supplementing CLAmix to the diet reduced the total concentration of all assayed fatty acids (ΣFA) in the spleen compared with the control (Table 1). Moreover, the addition of SeIV or SeY to the diet enriched in CLAmix enhanced reduction of the concentration of ΣFA in the spleen. The addition of $_{H}$SeY to the diet also tended to reduce the concentration of ΣFA. Similarly, the addition of selenate to the rat diet decreased the concentration of ΣFA in the spleen (Niedźwiedzka et al., 2006). On the other hand, the higher dietary SeIV content stimulated the accumulation of ΣFA in the spleen.

In plasma, the addition of 1.5% CLAmix to the diet stimulated the accumulation of ΣFA, which is in agreement with that of Niedźwiedzka et al. (2006) who reported that diets enriched in 1% or 2% CLAmix also increased the concentration of ΣFA in plasma. Moreover, as can be seen from the present study, the addition of $_{H}$SeY to the diet, regardless of the presence of CLAmix, stimulated the accumulation of ΣFA in plasma (Table 1).

The addition of CLAmix to the diet significantly increased ($P<0.01$) the concentration of *c9t11*CLA and *t10c12*CLA in the spleen and blood plasma of

Table 1. Dietary effects of 1.5% CLA[1] isomer mixture (CLAmix) and selenite (SeIV) or selenized yeast (SeY) on fresh spleen masses and the concentration of CLA isomers, the sum of all assayed fatty acids (ΣFA) and values of the ratio of UFA/ΣFA in the spleen and blood plasma of rats after 6 weeks feeding with experimental diets[2]

Group	Additives	Spleen mass[3] mg	Spleen						Blood plasma					
			c9t11 CLA µg/g	*t10c12* CLA µg/g	*c11t13* CLA µg/g	ΣCLA isomers µg/g	ΣFA mg/g	UFA/ΣFA	*c9t11* CLA µg/g	*t10c12* CLA µg/g	*c11t13* CLA µg/g	ΣCLA isomers µg/g	ΣFA µg/g	UFA/ΣFA
Control[4]	-	495	-[5]	-	-	-	$44.7^{aa\beta}$	$0.69^{A\alpha}$	-	-	-	-	$461^{a\,\alpha}$	0.57
$_{L}$SeIV	0.2 ppm SeIV	481^{α}	-	-	-	-	49.0	0.71^{B}	-	-	-	-	483	0.56^{α}
$_{H}$SeIV	0.5 ppm SeIV	496^{a}	-	-	-	-	72.5^{α}	$0.72^{C\alpha}$	-	-	-	-	493	0.55^{A}
$_{L}$SeY	0.2 ppm SeY	490	-	-	-	-	47.2	0.68^{D}	-	-	-	-	462	0.58^{B}
$_{H}$SeY	0.5 ppm SeY	461	-	-	-	-	28.4^{β}	0.27	-	-	-	-	566^{α}	0.53
CLA	CLAmix	466	$40.2^{A\alpha}$ r=0.746[6]	$53.9^{Aa\alpha}$	$5.11^{AB\alpha}$	99.2^{Aa}	21.0^{a}	0.46^{Aab}	3.72 r=2.818[7]	1.32	-	5.04	639^{abAB}	0.52
$_{L}$SeIV$_{CLA}$	0.2 ppm SeIV CLAmix	530^{α}	38.7 r=1.052[6]	36.8^{α}	2.01^{α}	77.5	18.1	0.46^{B}	2.85 r=1.952[7]	1.46	-	4.31	485^{bx}	0.49^{α}
$_{H}$SeIV$_{CLA}$	0.5 ppm SeIV CLAmix	577^{a}	10.9^{AX} r=0.551[6]	19.8^{AX}	0.28^{Ax}	31.0^{Ax}	16.4^{X}	0.41^{CaX}	3.55 r=2.139[7]	1.66	0.064^{α}	5.29^{α}	480^{A}	0.50^{A}
$_{L}$SeY$_{CLA}$	0.2 ppm SeY CLAmix	462	17.0^{α} r=0.773[6]	22.0^{ax}	0.30^{Bx}	39.4^{aX}	15.4^{y}	0.41^{Db}	3.47 r=2.210[7]	1.57	0.112	5.20	514^{Bx}	0.53^{B}
$_{H}$SeY$_{CLA}$	0.5 ppm SeY CLAmix	475	51.2 r=0.994[6]	51.5	4.59	107.3	18.9	0.46^{X}	4.48 r=2.448[7]	1.83	0.108^{α}	6.42^{α}	582	0.52

[1] the composition of dietary CLA isomer mixture (CLAmix): 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. The concentration ratio (r = *c9t11*CLA/*t10c12*CLA) of *c9t11*CLA to *t10c12*CLA in the dietary CLA isomer mixture was 0.988; [2] means in columns sharing the same letter are significantly different: $^{a,b}P<0.05$ and $^{A,B}P<0.01$; $^{\alpha,\beta}P<0.1$ differences were taken as tendencies; interactions of CLAmix x $_{L}$Se and CLAmix x $_{H}$Se (Se as SeIV or SeY), significant at $^{x}P<0.05$ and $^{X}P<0.01$, respectively; [3] average pancreas fresh masses; [4] the standard Labofeed H diet contained 0.2 ppm Se as selenite; [5] below a quantification limit (i.e. 3 x the detection limit); [6] the concentration ratio of *c9t11*CLA/*t10c12*CLA in the spleen; [7] the concentration ratio of *c9t11*CLA/*t10c12*CLA in blood plasma

rats compared with the control rats and animals fed the diets enriched in SeIV or SeY. On the other hand, inclusion of CLAmix to the diet increased ($P<0.01$) the concentration of *c11t13*CLA only in the spleen. Feeding CLAmix with Se as $_{L}$SeIV, $_{H}$SeIV, or $_{L}$SeY resulted in decreasing in the concentration of CLA isomers in the spleen. Considering above, we suggest that dietary SeIV, especially $_{H}$SeIV, has a *pro*-oxidant effect on the CLA isomers in the spleen. The pro-oxidative properties of sodium selenite are known from *in vitro* and *in vivo* experiments (Spallholz, 1997; Terada et al., 1999; Boldižárová et al., 2005). Consequently, the combined addition of CLAmix and $_{H}$SeY to the diet showed a high but not significant increase of the concentration of *c9t11*CLA in the spleen (+27%) and blood plasma (+21%). This diet particularly efficiently increased the total concentration of all assayed CLA isomers (+27%) in plasma. Moreover, the diet with the combined addition of CLAmix and Se as SeIV or SeY stimulated the accumulation of *t10c12*CLA and *c11t13*CLA in plasma compared with the diet enriched in only CLAmix.

Our results indicate that the ratio of the concentrations of *c9t11*CLA to *t10c12*CLA in the spleen of rats fed the diet enriched with the CLAmix was lower compared with the ratio of these isomers in the CLA isomer mixture added to the diets (i.e. 0.746 vs 0.988; see Table 1). Our present results are in agreement with our previous study (Niedźwiedzka et al., 2006), in which *t10c12*CLA and *t10t12*CLA were also less efficiently metabolized than *c9t11*CLA in the spleen of rats fed a diet with 1 or 2% CLA isomer mixture. Thus, our current and previous studies clearly document that *c9t11*CLA is more efficiently metabolized than *t10c12*CLA or *t10t12*CLA in the spleen. Our findings are not in agreement with those of Alasnier et al. (2002) and our previous studies (Niedźwiedzka et al., 2006; Korniluk et al., 2007; Czauderna et al., 2007a, 2009, 2010a,b) showing that the *t10c12* and *t10t11*CLA isomers were also more efficiently metabolized in other organs and tissues (e.g., the liver, pancreas, brain, kidneys, heart, adipose tissues, blood plasma and muscles) than the *c9t11* isomer. Our results documented that the spleen is a unique internal organ possessing a more efficient *c9t11*CLA metabolism than other internal organs, adipose tissue, blood plasma, and muscles. On the other hand, in the current study, it was found that the metabolic efficiency of isomer *t10c12* in plasma was higher than that of *c9t11*CLA, which is in agreement with the results of Alasnier et al. (2002) and our studies on the liver, heart, muscle, kidney, pancreas and brain of rats (Niedźwiedzka et al., 2006; Korniluk et al., 2007; Czauderna et al., 2010a,b).

Interestingly, our current study documented that the *c9t11*CLA/*t10c12*CLA ratio in the spleen and plasma is affected by the addition of SeIV or SeY to the diet containing CLAmix. As can be seen from the results summarized in Table 1, the addition of $_{L}$SeIV to the diet containing CLAmix increased the metabolic efficiency of *t10c12*CLA compared with the diet enriched in only CLAmix. On the other

hand, the addition of the higher amount of SeY the diet with CLAmix stimulated the accumulation of *c9t11*CLA in the spleen and plasma. Supplementing $_{H}$SeIV or $_{L}$SeY diets with CLAmix significantly reduced the concentration of *c9t11*CLA and *t10c12*CLA in the spleen, although isomer *c9t11* is metabolized more efficiently than *t10c12*CLA. In plasma, these additives in the diet with CLAmix slightly reduced the efficiency of isomer *t10c12* metabolism, while somewhat increasing the rate of *c9t11*CLA metabolism, although the ratio of *c9t11*CLA to *t10c12*CLA was lower than in rats given the diet with CLAmix.

The effect the experimental diets on the concentration of saturated, mono- and polyunsaturated fatty acids in the spleen and blood plasma of rats. The diet enriched in CLAmix, regardless of the presence of SeIV or SeY, significantly decreased the concentration of *c9*C18:1, *c11*C18:1 as well as the total concentration of all assayed monounsaturated fatty acids (MUFA) in the spleen compared with the control group or rats fed the diet containing $_{L}$SeIV, $_{H}$SeIV, or $_{L}$SeY in particular (Table 2). This effect could be related to reduction of Δ9-desaturation capacity in the spleen of rats fed the diet containing CLAmix, regardless of the presence of SeIV or SeY. In contrast, dietary SeIV, irrespective of its concentration in the diet, increased the Δ9-desaturation index in the spleen and plasma, while dietary SeY, especially, $_{H}$SeY decreased the value of this index. The present results support our previous studies on rats fed a diet containing 1.2 ppm Se as selenized yeast (SeY) with or without CLA isomer mixture (Korniluk et al., 2007; Niedźwiedzka et al., 2007), which also documented that the diet with 1.2 ppm as SeY decreased the Δ9-desaturation index in the spleen of rats and blood plasma.

Unexpectedly, the diet containing CLAmix stimulated the accumulation of *t11*C18:1 in the spleen and plasma compared with the control group and rats fed the diet with SeIV or SeY (Table 2). Moreover, the addition of $_{L}$SeIV or $_{H}$SeY to the diet with CLAmix resulted in more efficient accumulation of *t11*C18:1 in the spleen and plasma than the diet containing only CLAmix. Considering the above, we suggest that the presence of *t11*C18:1 in the spleen and plasma documents coprophagia of rats, as well as that some CLA isomers added to the rat diet are precursors of accumulated *t11*C18:1.

The splenic concentration of myristic acid (C14:1), as well as the total concentration of all assayed saturated fatty acids (SFA) having a detrimental effect on animal and human health, decreased with CLAmix and $_{H}$SeY treatments. Similarly, the addition of SeIV or SeY to the diet enriched in CLAmix decreased the concentration of these fatty acids in the spleen. Unexpectedly, supplementation of SeIV to the diet increased the concentration of C14:0 and SFA in the spleen and plasma. In addition, $_{H}$SeY treatment and supplementation of CLAmix and Se (as SeIV or SeY) increased the concentration of these fatty acids in plasma.

Table 2. Dietary effects of 1.5% CLA isomer mixture (CLAmix) and selenite (SeIV) or selenized yeast (SeY) on the concentration of selected monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) and Δ9-desaturase index ($\Delta 9_{index}$) in the spleen and blood plasma of rats after 6 weeks feeding with experimental diets[1]

Group	Spleen							Blood plasma						
	C14:0 μg/g	*t11*C18:1 μg/g	*c*9C18:1 mg/g	*c11*C18:1 μg/g	MUFA mg/g	$\Delta 9_{index}$	SFA mg/g	C14:0 μg/g	*t11*C18:1 μg/g	*c*9C18:1 μg/g	*c11*C18:1 μg/g	MUFA μg/g	$\Delta 9_{index}$	SFAμg/g
Control	169^{a}	-[2]	8.1^{ABa}	1313^{ABC}	10.9^{Aa}	0.395^{A}	13.9^{AB}	2.37^{a}	-	$30.7^{\alpha\beta\gamma}$	3.80^{α}	$37.5^{a\alpha\beta}$	0.148^{α}	194^{Aa}
$_{L}$SeIV	234	-	9.3^{C}	1530^{D}	12.9^{B}	0.432^{B}	14.1^{C}	3.36	-	37.2^{α}	4.41	$45.1^{\alpha\beta}$	0.163	209^{b}
$_{H}$SeIV	345^{ab}	-	13.9^{Da}	2095^{AE}	19.2^{Ca}	0.445^{C}	20.2^{AD}	2.54	-	39.2^{β}	4.84^{a}	48.6^{γ}	$0.166^{\alpha a}$	218
$_{L}$SeY	197	-	7.7^{E}	1389^{F}	10.9^{D}	0.364^{D}	15.0^{E}	2.38	-	30.3	4.27	37.8	0.149^{b}	188^{B}
$_{H}$SeY	77	-	2.7^{A}	321^{B}	9.4^{b}	0.242^{α}	8.6^{F}	2.93	-	39.6^{γ}	4.81^{α}	48.4^{a}	0.143	259^{A}
CLA	85	$2.17^{a\alpha}$	$2.6^{B\alpha}$	$476^{CGH\alpha}$	$3.6^{Ac\alpha}$	0.195^{Ab}	$11.3^{BGab\alpha}$	$3.31^{a\alpha\beta}$	-	42.6^{δ}	4.59	49.4^{δ}	0.131	$297^{ac\alpha\beta}$
$_{L}$SeIV$_{CLA}$	128	2.65	2.9^{C}	$409^{D\alpha}$	3.8^{B}	0.249^{B}	9.6^{Ca}	2.91	0.366^{A}	32.8	3.84	41.3	0.135	234^{bcx}
$_{H}$SeIV$_{CLA}$	84^{bx}	0.16^{a}	2.1^{Dx}	291^{EGX}	$2.6^{C\alpha x}$	0.183^{C}	9.6^{DbX}	2.59^{α}	0.066^{A}	32.6	3.50^{a}	38.8^{γ}	0.128^{a}	$237^{\alpha x}$
$_{L}$SeY$_{CLA}$	81	0.52^{α}	$2.1^{E\alpha}$	305^{FH}	2.6^{Dc}	0.195^{D}	9.0^{EGX}	2.79^{β}	0.075^{B}	31.7^{δ}	3.63	37.9^{δ}	0.123^{b}	$238^{B\beta}$
$_{H}$SeY$_{CLA}$	110	4.18	2.8^{X}	443^{X}	3.6^{b}	$0.232^{\alpha bX}$	$10.1^{F\alpha}$	3.22	0.174^{B}	38.0	4.60	47.1	0.131	274^{x}

[1] means in columns sharing the same letter are significantly different: $^{a,b}P<0.05$ and $^{A,B}P<0.01$; $^{\alpha,\beta}P<0.1$ differences were taken as tendencies; interactions of CLAmix x $_{L}$Se and CLAmix x $_{H}$Se (Se as SeIV or SeY), significant at $^{x}P<0.05$ and $^{X}P<0.01$, respectively; [2] below a quantification limit, LQ (i.e. LQ = 3 × the detection limit)

Changes in saturated fatty acid concentrations in the spleen and plasma are consistent with changes in the atherogenic and thrombogenic properties of the spleen and blood plasma. Indeed, the addition of CLAmix or $_{H}$SeY to the diet decreased the concentrations of atherogenic (A-SFA) and thrombogenic (T-SFA) saturated fatty acid in the spleen, and increased them in plasma (Table 3). Supplementing CLAmix and Se (as SeIV or SeY) to diets decreased the levels of these fatty acids in the spleen, while increasing them in plasma. Additionally, the diet containing the higher level of SeIV stimulated the accumulation of A-SFA and T-SFA in the spleen and plasma.

An inverse relationship between concentrations of A-SFA and T-SFA in the spleen and blood plasma and values of atherogenic (A_{index}) and thrombogenic (T_{index}) indexes in these tissues was found (Table 3). In fact, the addition of $_{H}$SeY or CLAmix to the diet or combined supplementation with CLAmix and Se (as SeIV or SeY) of diets increased A_{index} and T_{index} values in the spleen and plasma. Additionally, the larger amount of SeIV in the diet increased the A_{index} and T_{index} values in plasma. Increased A_{index} and T_{index} values in the spleen have been associated with a decrease in the concentration of PUFA, particularly PUFAn-3 (Table 4). In contrast, more efficient accumulation of A-SFA and T-SFA in plasma is responsible for the increase in the A_{index} and T_{index} values. Indeed, changes in the concentrations of PUFA, PUFAn-3, and PUFAn-6/PUFAn-3 ratio in plasma (Table 4) are usually small and not responsible for increasing A_{index} and T_{index} values (Ulbricht and Southgate, 1991).

Rats fed the diet enriched in CLAmix with or without Se (as SeIV or SeY) showed decreased concentrations of linoleic (LA), α-linolenic (αLNA), arachidonic (AA), docosapentaenoic (DPA) and docosahexanoic (DHA) acids as well as the total concentration of the assayed PUFA in the spleen compared with the control rats (Tables 3 and 4). Additionally, these diets reduced spleen concentrations of PUFAn-3 and long-chain PUFAn-3 (LPUFAn-3) and LPUFAn-6, while increasing the PUFAn-6/PUFAn-3 ratio with the exception of the diet containing only CLAmix. In contrast, the diet enriched in $_{L}$SeY, and especially $_{H}$SeIV, considerably increased spleen concentrations of the polyunsaturated fatty acids mentioned above and decreased the PUFAn-6/PUFAn-3 ratio. Surprisingly, the addition of the higher amount of SeY to the diet decreased the spleen concentrations of these fatty acids and increased the PUFAn-6/PUFAn-3 ratio. It appears that the highly bioavailable chemical form of dietary Se at the higher dose (i.e. 0.5 ppm Se as SeY) exhibited some pro-oxidative. It has been suggested that larger doses of dietary selenized yeast may lead to higher continuous release of selenomethionine from body deposits into the free amino acid pool with a subsequently higher production of H_2Se, which has pro-oxidative properties (Boldižárová et al., 2005).

Table 3. Dietary effects of 1.5% CLA isomer mixture (CLAmix) and selenite (SeIV) or selenized yeast (SeY) on values of atherogenic (A_{index})[1] and thrombogenic (T_{index})[2] indexes and the concentration of atherogenic SFA (A-SFA)[3], thrombogenic SFA (T-SFA)[4], linoleic acid (LA), linolenic acid (αLNA), arachidonic acid (AA; *c5c8c11c14*C20:4) in the spleen and blood plasma of rats after 6 weeks feeding with experimental diets[5]

Group	Spleen							Blood plasma						
	A-SFA mg/g	T-SFA mg/g	A_{index}	T_{index}	LA mg/g	αLNA µg/g	AA mg/g	A-SFA µg/g	T-SFA µg/g	A_{index}	T_{index}	LA µg/g	αLNA µg/g	AA µg/g
Control	9.5^{AB}	13.7^{AB}	0.326^{A}	$0.412^{A\alpha\beta}$	12.3^{Aab}	$477^{AB\alpha}$	$5.66^{ABab\alpha}$	$99^{\alpha ab}$	$192^{\alpha AB}$	0.376^{α}	0.557	139^{a}	15.6	50.2
$_{L}$SeIV	9.9^{C}	13.9^{C}	0.307^{B}	$0.357^{B\alpha}$	12.7^{B}	1006^{C}	6.47^{a}	107	206^{a}	0.396	0.584	138	13.8	50.7
$_{H}$SeIV	14.8^{AD}	19.9^{AD}	0.305^{C}	0.340^{C}	19.5^{Ca}	1420^{AD}	9.73^{b}	113^{α}	217^{α}	0.416^{a}	0.629^{A}	138	14.8	45.0
$_{L}$SeY	10.0^{E}	14.8^{E}	0.333^{D}	0.383^{D}	11.6^{D}	578^{E}	7.02^{AC}	91^{c}	186^{C}	0.342^{A}	0.489^{B}	129	13.7	53.8
$_{H}$SeY	3.9^{a}	8.6^{F}	0.344	0.590^{β}	8.2^{bc}	251^{α}	1.52^{α}	132^{a}	257^{A}	0.438	0.614^{α}	146	19.6	59.4
CLA	$`6.6^{Bb\alpha\beta}$	$11.2^{BGHa\alpha}$	0.929^{AEF}	1.075^{Aeab}	$3.6^{AEb\alpha}$	146^{Ba}	1.67^{BDEFc}	151^{bdef}	$295^{Bbc\beta}$	0.455^{α}	0.673	171^{abc}	18.3^{α}	59.3
$_{L}$SeIV$_{CLA}$	$6.0^{C\alpha}$	9.5^{Ca}	0.769^{B}	1.295^{BaX}	$3.2^{B\alpha}$	194^{C}	0.90^{cX}	111^{dx}	230^{abx}	0.473	0.660	92^{b}	12.1	47.5
$_{H}$SeIV$_{CLA}$	$5.8^{D\beta X}$	9.5^{DGX}	0.903^{CEX}	1.434^{CbX}	2.7^{Cbx}	58^{Dax}	1.03^{Dx}	113^{ex}	232^{ex}	0.481^{a}	0.718^{A}	111^{c}	12.9	49.5
$_{L}$SeY$_{CLA}$	5.5^{Eb}	8.9^{EHX}	0.917^{DFX}	1.535^{DEX}	2.5^{DE}	81^{E}	0.83^{CEX}	115^{cf}	$235^{C\beta}$	0.432^{A}	0.630^{B}	130	11.2^{α}	62.3
$_{H}$SeY$_{CLA}$	6.2^{a}	$10.0^{F\alpha}$	0.757^{X}	1.212^{X}	3.3^{c}	187	1.12^{FX}	142	267^{x}	0.482	0.683^{α}	147	15.0	57.0

[1] the atherogenic index = (C12:0+4*C14:0+C16:0)/(MUFA+PUFAn-6+PUFAn-3) (Ulbricht and Southgate, 1991); [2] 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); [3] the concentration sum of: C12:0, C14:0 and C16:0; [4] the concentration sum of C14:0, C16:0 and C18:0; [5] means in columns sharing the same letter are significantly different: $^{a,b}P<0.05$ and $^{A,B}P<0.01$; $^{\alpha,\beta}P<0.1$ differences were taken as tendencies; interactions of CLAmix x $_{L}$Se and CLAmix x $_{H}$Se (Se as SeIV or SeY), significant at $^{x}P<0.05$ and $^{X}P<0.01$, respectively

Table 4. Dietary effects of 1.5% CLA isomer mixture (CLAmix) and selenite (SeIV) or selenized yeast (SeY) on the concentration of *c7c10c13c16c19*C22:5 (DPA), *c4c7c10c13c16c19*C22:6 (DHA), n-3LPUFA[1] ($_{n-3}$LPUFA), n-6LPUFA[2] ($_{n-6}$LPUFA), n-3PUFA ($_{n-3}$PUFA) and values of the n-6PUFA/n-3PUFA ratio (n-6/n-3) in the spleen and blood plasma of rats after 6 weeks feeding with experimental diets[3]

Group	Spleen							Blood plasma						
	DPA µg/g	DHA µg/g	PUFA mg/g	$_{n-3}$LPUFA mg/g	$_{n-6}$LPUFA mg/g	$_{n-3}$PUFA mg/g	n-6/n-3	DPA µg/g	DHA µg/g	PUFA µg/g	$_{n-3}$LPUFA µg/g	$_{n-6}$LPUFA µg/g	$_{n-3}$PUFA µg/g	n-6/n-3
Control	799^{ABa}	223^{ABa}	19.8^{Aa}	1.24^{ABa}	$5.89^{AB\alpha}$	$7.16^{AB\alpha\beta}$	1.75	2.92^{abA}	5.56^{a}	225	19.5	51.0	85.5	1.63^{a}
$_{L}$SeIV	724^{C}	275^{C}	21.9^{B}	1.49^{C}	$6.74^{C\alpha}$	$8.67^{C\alpha}$	1.50^{A}	3.62^{c}	6.47	225	21.6^{α}	51.5	86.7	1.60^{A}
$_{H}$SeIV	1396^{Da}	426^{Da}	33.0^{Ca}	2.30^{Da}	10.05^{D}	12.97^{AD}	1.53^{a}	3.84	6.51	222	23.1^{a}	45.8^{α}	83.4	1.67
$_{L}$SeY	1112^{E}	354^{C}	21.1^{D}	1.85^{E}	7.22^{E}	9.06^{E}	1.30^{b}	$9.29^{a\alpha}$	7.07	231	29.8	56.3	98.2	1.34^{ab}
$_{H}$SeY	115^{A}	$30^{A\alpha}$	10.2^{b}	0.18^{A}	1.54^{A}	1.92^{B}	2.11	5.53^{b}	7.27	254	27.5	60.4^{α}	106.9	1.37
CLA	329^{BFHGI}	$100^{BEFb\beta}$	6.1^{AEFb}	0.51^{BFGHb}	$1.74^{BFGI\beta}$	$2.25^{FGHI\beta}$	$1.66^{BC c\alpha}$	8.47^{Ade}	10.14^{abc}	$286^{\alpha\beta a}$	30.4^{b}	61.3	108.6^{α}	1.60^{c}
$_{L}$SeIV$_{CLA}$	147^{CFX}	46^{CEX}	4.6^{Bb}	0.22^{CFx}	0.94^{CFX}	1.29^{CFx}	2.52^{ABx}	13.13^{c}	7.19^{bx}	197^{α}	31.1^{α}	56.1	91.9	1.11^{Ac}
$_{H}$SeIV$_{CLA}$	168^{DHX}	59^{DbX}	4.1^{CEX}	0.25^{DGX}	1.05^{DGx}	1.31^{DGX}	$2.09^{a\alpha}$	3.57^{dx}	6.91^{cx}	200^{a}	19.1^{abx}	50.8	82.1	1.38
$_{L}$SeY$_{CLA}$	123^{EGx}	46^{CFx}	3.7^{DF}	0.18^{EH}	0.85^{EI}	1.08^{EH}	2.40^{Cbx}	$3.55^{\alpha ex}$	7.82^{x}	232^{β}	20.8^{x}	63.5	95.6^{α}	1.39^{bx}
$_{H}$SeY$_{CLA}$	191^{IX}	$68^{\alpha\beta X}$	5.1^{b}	0.30^{bX}	$1.16^{\beta X}$	1.57^{IX}	2.20^{c}	4.81^{x}	8.43	253	23.7	58.8	97.1	1.55

[1] the concentration sum of *c11c14c17*C20:3, *c8c11c14c17*C20:4, *c5c8c11c14c17*C20:5, *c7c10c13c16c19*C22:5 and *c4c7c10c13c16c19*C22:5; [2] the concentration sum of *c11c14* C20:2, *c8c11c14*C20:3, *c5c8c11c14*C20:4, *c13c16*C22:2 and *c7c10c13c16*C22:4; [3] in columns sharing the same letter are significantly different: $^{a,b}P<0.05$ and $^{A,B}P<0.01$; $^{\alpha,\beta}P<0.1$ differences were taken as tendencies; interactions of CLAmix x $_{L}$Se and CLAmix x $_{H}$Se (Se as SeIV or SeY), significant at $^{x}P<0.05$ and $^{X}P<0.01$, respectively

Table 5. Dietary effects of 1.5% CLA isomer mixture (CLAmix) and selenite (SeIV) or selenized yeast (SeY) on ratio values of PUFA/SFA, PUFA/ΣFA, MUFA/SFA and indexes of [1]elongase, [2]Δ4-, [3]Δ5- and [4]Δ6-desaturases the spleen and blood plasma of rats after 6 weeks feeding with experimental diets[5]

Group	Spleen							Blood plasma						
	PUFA/SFA	PUFA/ΣFA	MUFA/SFA	Elongase index	$\Delta4_{index}$	$\Delta5_{index}$	$\Delta6_{index}$	PUFA/SFA	PUFA/ΣFA	MUFA/SFA	Elongase index	$\Delta4_{index}$	$\Delta5_{index}$	$\Delta6_{index}$
Control	1.42^{A}	0.44^{A}	$0.79^{A\alpha}$	0.882	0.22	0.973^{ABCDa}	0.043^{Aabcd}	1.16^{α}	0.49^{α}	0.193^{α}	0.763	$0.66^{ab\alpha}$	0.989^{α}	0.016^{α}
$_{L}$SeIV	1.55^{B}	0.45^{B}	0.91^{B}	0.865	0.23	0.972^{AE}	0.022^{Ba}	1.08	0.47	0.216	0.786	0.64	0.987^{A}	0.032^{A}
$_{H}$SeIV	1.64^{C}	0.46^{C}	$0.95^{C\alpha}$	0.855^{a}	0.23	0.976^{a}	0.009^{A}	1.02^{a}	$0.45^{\alpha a}$	0.222^{a}	0.752	0.63^{c}	0.982^{α}	0.029^{a}
$_{L}$SeY	1.40^{D}	0.45^{D}	0.73^{D}	0.853	0.24^{a}	0.972^{B}	$0.020^{b\alpha}$	1.23^{A}	0.50^{A}	0.202^{A}	0.797	0.43^{Aa}	0.985	0.055^{α}
$_{H}$SeY	1.18	0.14	1.09	0.884	0.21^{α}	0.986^{C}	0.033^{c}	0.98	0.45	0.187	0.752	0.57^{b}	0.991^{B}	0.021^{b}
CLA	$0.54^{Aab\alpha}$	0.29^{AE}	$0.32^{A\beta}$	0.895^{α}	0.23	0.978^{Dbce}	$0.127^{d\beta e\gamma\delta}$	0.96^{α}	0.45^{b}	0.167^{α}	0.764	$0.55^{\alpha\beta d}$	$0.990^{CD\gamma c}$	0.028^{BCDc}
$_{L}$SeIV$_{CLA}$	$0.48^{B\alpha}$	0.25^{B}	0.39^{B}	0.921	0.24	0.983^{Eb}	$0.077^{B\beta}$	0.84	0.41	0.177^{x}	0.798	0.35	0.977^{Acx}	0.096^{AB}
$_{H}$SeIV$_{CLA}$	0.43^{Cax}	0.25^{C}	$0.27^{C\beta x}$	0.933^{a}	0.26	0.989	0.056^{e}	0.85^{a}	0.42^{ab}	0.164^{a}	0.794	$0.66^{c\beta x}$	0.979^{D}	0.057^{Ca}
$_{L}$SeY$_{CLA}$	0.41^{Db}	0.24^{DE}	0.29^{D}	0.943	0.28^{a}	0.991^{cX}	$0.067^{\alpha\gamma}$	0.98^{A}	0.45^{A}	0.159^{Ax}	0.847	0.69^{AdX}	0.985^{γ}	0.104^{c}
$_{H}$SeY$_{CLA}$	0.51^{X}	0.27^{X}	0.36	0.914^{α}	0.26^{α}	0.981^{eX}	0.117^{δ}	0.92	0.44	0.172	0.791	0.64^{X}	0.977^{BcX}	0.085^{Db}

[1] the elongase index = *c7c10 c13c16c19*C22:5/(*c7c10c13c16c19* C22:5+*c5c8c11c14c17*C20:5)

[2] Δ4-desaturase index = *c4c7c10c13c16c19*C22:6/(*c4c7c10c13c16c19*C22:6+*c7c10c13c16c19*C22:5)

[3] Δ5-desaturase index = *c5c8c11c14*C20:4/(*c5c8c11c14*C20:4+*c8c11c14*C20:3n-6)

[4] Δ6-desaturase index = *c6c9c12c15*C18:4/(*c6c9c12c15*C18:4+*c9c12c15*C18:3)

[5] in columns sharing the same letter are significantly different: $^{a,b}P<0.05$ and $^{A,B}P<0.01$; $^{\alpha,\beta}P<0.1$ differences were taken as tendencies; interactions of CLAmix x $_{L}$Se and CLAmix x $_{H}$Se (Se as SeIV or SeY), significant at $^{x}P<0.05$ and $^{X}P<0.01$, respectively

In contrast, the diet containing CLAmix or $_{H}$SeY increased the concentration of the above-mentioned individual polyunsaturated fatty acids as well as PUFA, LPUFAn-3, LPUFAn-6, and PUFAn-3 in plasma compared with the control group. Other experimental diets showed a small and inconsistent influence on the concentrations of these fatty acids in plasma. Supplementing $_{L}$SeY or $_{H}$SeY alone to diets or to the diet containing CLAmix decreased PUFAn-6/PUFAn-3 ratios in plasma.

The rat diet enriched in 1.5% CLAmix with or without Se (as SeIV or SeY) significantly increased Δ6-desaturase index values in the spleen and plasma (Table 5). Additionally, rats in the SeIV or SeY treatments presented a higher value of this index in plasma than the control group. Values of the Δ4-, Δ5-desaturase indexes and the elongase index were slightly higher in the spleen of rats fed the diet enriched in CLAmix; the addition of SeIV or SeY to the diet with CLAmix increased the values of these indexes in the spleen in comparison with the diet containing only CLAmix. Similarly, values of the plasma elongase index were higher rats fed the diets enriched in both CLAmix and Se (as SeIV or SeY) than in the control group or rats in the SeIV, SeY, or CLAmix treatments.

Unexpectedly, the diet enriched in CLAmix resulted in a decrease in the ratios of PUFA/SFA, MUFA/SFA, PUFA/ΣFA (Table 5) and UFA/ΣFA (Table 1) in the spleen and plasma. The addition of SeIV or SeY to the diet containing CLAmix usually slightly decreased the values of these ratios in the spleen and plasma in comparison with rats fed the diet with CLAmix. Similarly, the $_{H}$SeY treatment decreased the values of these ratios in both examined tissues. Considering the above, it appears that dietary CLAmix and/or Se (as SeIV or SeY) resulted in a different composition pattern of SFA, MUFA and PUFA in the spleen and blood plasma.

CONCLUSIONS

This study describes changes in fatty acid profiles in the spleen and plasma as a result of the addition of 1.5% CLAmix and various amounts of sodium selenite (SeIV), selenized yeast (SeY) to the rat diet. The results document that the CLAmix treatment more significantly stimulated the metabolic, especially catabolic, processes of poly- (PUFA) and monounsaturated fatty acids (MUFA) in the spleen than saturated fatty acids (SFA). The efficiency of PUFA and MUFA metabolism in the spleen was increased by dietary supplementation of SeIV or SeY to the diet containing CLAmix. These final conclusions are well confirmed by changes in the values of the elongase and Δ4-, Δ5-, and Δ6-desaturase indexes and the PUFA/SFA and MUFA/SFA ratios in the spleen. On the other hand, the

diet containing CLAmix, irrespective of the presence of SeIV or SeY, stimulated the accumulation of MUFA, PUFA, and SFA in blood plasma.

The diet enriched CLAmix and the higher amount of SeY most efficiently stimulated accumulation of CLA isomers, especially health-promoting *c9t11*CLA in the spleen and plasma. We demonstrated that *c9t11*CLA in the rat spleen is more efficiently metabolized than *t10c12*CLA, while in other internal organs, muscles, and adipose tissues, *t10c12*CLA is metabolized faster than the *c9t11* isomer.

Further research is needed to determine if dietary selenium compounds and individual CLA isomers also induce changes in fatty acid profiles and elongase and desaturase indexes in the spleen and blood plasma of rats.

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