

Comparison of the influence of different chemical forms of selenium and the profiles of CLA isomers in the diet on the fatty acid and amino acid contents in the liver and femoral muscles of rats*

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ABSTRACT

The influence of diets enriched in 2% CLA isomer mixture (as CLAmix_A or CLAmix_B) and/or Se as selenate (Se^{VI}) or selenized yeast (Se-Y) on the concentration of fatty acids (FA) and amino acids (AA) in the liver and femoral muscles was studied on 8 groups of 7-8 rats aged 8 weeks. Rats were fed a basal diet for 29 days or diets enriched with two combinations of 2% CLAmix_A with/without 2 ppm Se (as Se^{VI}) or 2% CLAmix_B with/without 1.2 ppm Se (as Se-Y). The dietary CLAmix_B containing the higher concentration of *trans10cis12CLA* (*t10c12CLA*) resulted in a higher decrease of body weight gain (BWG) ($P < 0.1$) than the dietary CLAmix_A with the lower concentration of *t10c12CLA*. The diet enriched in Se^{VI} reduced the BWG ($P < 0.05$) in rats. The dietary CLAmix_A lowered the concentration of saturated FA (SFA), atherogenic SFA and thrombogenic SFA in the liver and muscles. The value of the $\Delta 9$ -desaturase index decreased in the liver and muscles of rats fed the diet enriched in CLAmix_A or CLAmix_B compared with that in the control rats. There were significantly higher ($P < 0.01$) concentrations of *c9t11CLA*, *t10c12CLA*, *ccCLA* and *ttCLA* in the muscles compared with the concentrations of these isomers in the liver of rats fed the diets enriched in CLAmix_A or CLAmix_B, regardless the presence of extra Se as Se^{VI} or Se-Y. The ratio ($R_{t10c12/c9t11}$) of *t10c12CLA* and *c9t11CLA* in the liver and muscles of rats fed the diets enriched in CLA isomers was, regardless of the presence of Se, smaller compared with the values of $R_{t10c12/c9t11}$ in the dietary CLA isomers. The concentrations of CLA isomers tended to or significantly increased in the liver and muscles of rats fed diets containing Se and CLA isomers compared with rats fed the diets enriched in CLA isomers. The concentrations of PUFA_{n-3}, PUFA_{n-6}, PUFA and the ratio of PUFA/SFA decreased in the muscles of rats fed the diet with CLAmix_A. The results suggest that the dietary CLAmix_A containing the higher concentration of *ttCLA*, regardless of the presence of Se^{VI}, more efficiently reduced the magnitude of lipoprotein synthesis in muscles than the diet containing CLAmix_B with/without Se-Y,

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while dietary Se-Y most effectively reduced the anti-obesity properties of CLA isomers in rats. The diets enriched in CLA isomers (as CLAmix_A or CLAmix_B), regardless of the presence of Se^{VI}, led to a decrease in the concentration of methionine (Met) in the liver and the concentration of all amino acids in the muscles, while increasing the concentration of Met in muscles.

KEY WORDS: CLA isomers, fatty acids, selenate, high-selenized yeast, liver, femoral muscles, rats

INTRODUCTION

Analyses of protein sequences, on a genomic scale, revealed a significant number of proteins that bind micronutrients, including proteins that contain some trace elements (e.g., Se, I or B). Selenium, in particular, has been utilized by biological systems because it endows proteins with unique coordination, catalytic and electron transfer properties (Rowntree et al., 2004; Suzuki, 2005). These properties have been employed by organisms in key functions in a variety of pathways, resulting in dependence of organisms on the chemical form and level of Se in diets. In turn, Se and other nutrients important for health, e.g., conjugated linoleic acid (CLA) isomers, can govern an organism's nutritional strategies and its evolution (Combs, 2005; Rayman, 2005). Se occupies important sites in proteins, often being present as a catalytic species or serving key structural functions. Indeed, dietary Se is reduced to selenide for further excretion and/or utilization (Weiss and Hogan, 2005). Formed selenide is known to be utilized to synthesize proteins containing Se-cysteine (Se-Cys); these Se-Cys-proteins possess a specific codon. On the other hand, Se-methionine (Se-Met), originated from e.g., selenized yeast (Rayman, 2004), does not have a specific codon and is incorporated into protein by the same codon as methionine. Moreover, Se-Met can also be transformed to Se-cysteine.

Numerous beneficial regulatory effects of CLA isomers on immune functions, cytokines, immunoglobulin production, the ratio of protein to fat in mammal bodies (repartition), lipids and eicosanoid metabolism have been reported (Akahoshi et al., 2003, 2004; Turpeinen et al., 2006). Moreover, CLA isomers can modulate the expression of numerous genes, either directly or through specific transcription factors involved in the many metabolic processes that they affect (Raes et al., 2004; Wahle et al., 2004). Fortunately in mammals, Se-proteins have important roles in protecting unsaturated fatty acids, including CLA isomers, from oxidative damage (Crespo et al., 1995; Tapiero et al., 2003; Suzuki, 2005). Indeed, our studies documented that dietary selenate stimulated the accumulation of unsaturated fatty acids (UNFA) as well as affected the content of amino acids in rat bodies (Czauderna et al., 2004a,b; Niedźwiedzka et al., 2006a).

Therefore, the aim of the current study was to investigate the influence of various chemical forms of dietary Se on the magnitude of the accumulation of differ-

ent geometrical and positional isomers of CLA, especially on the composition of long-chain polyunsaturated fatty acids (PUFA) in the liver and femoral muscles in rats. We intended especially to compare the effectiveness of dietary selenate (Se^{VI}) and high-selenized yeast (Se-Y) (Rayman, 2004) on the magnitude of the accumulation of *cis9trans11*CLA (*c9t11*CLA), *trans10cis12*CLA (*t10c12*CLA), n-3 long-chain PUFA (LPUFAn-3) and the ratio of PUFA to saturated fatty acids (PUFA/SFA) in the liver and muscles.

MATERIAL AND METHODS

Animals, treatment and feeding

Sixty-one female rats (Wistar, Ifz: BOA), at 8 weeks of age, each weighing 201 ± 1 g at the beginning of the experiment, were housed individually in plastic cages at a temperature of $22 \pm 1^\circ\text{C}$ with a 12 h light-dark cycle and relative humidity ~60%. Each group comprised 7-8 rats (Table 1).

The experimental protocol was approved by the Local Animal Care and Use Committee (The Agriculture University, Warsaw, Poland). During a one-week preliminary period the rats were fed the Labofeed H diet produced by the Feeds and Concentrates Production Plant in Kcynia (Poland) (Pastuszewska et al., 2000) given at a submaintenance level (9 g of Labofeed H diet/day/rat) to reduce the body fat of rats (Table 1). During that time the rats decreased body weight by about 10% of their initial weight. Afterwards for 29 days the rats were fed the experimental diets enriched with 2% of a mixture of CLA isomers (as CLAmix_A or CLAmix_B), 2 ppm Se as Se^{VI} (Se^{VI} Experiment) or 1.2 ppm Se as the high-selenized yeast (Se-Y Experiment) (Table 1). The amount of the diet was adjusted each day to ensure an *ad libitum* feeding level. At the end of the experiment the rats were killed. The livers and femoral muscles were removed, weighed and frozen. Fatty acids (FA) in the livers and femoral muscles were analysed individually, while amino acids (AA) were analysed in pooled samples prepared by a combination of the livers or muscles from rats fed the same diet.

Reagents and chemicals

Sodium selenate and AA standards were provided by Sigma (USA), whereas absolute methanol, 99.9% acetonitrile and 95% heptane were HPLC grade and purchased from Lab-Scan (Ireland). The two CLA isomer mixtures (CLAmix_A and CLAmix_B) were supplied by Larodan Fine Chemicals AB (Sweden). Composition details (Table 1) and the purities of CLA isomer standards and the dietary CLA

isomer mixtures were examined using silver-liquid chromatography (Ag^+ -HPLC) with photodiode array detection (DAD) at 234 nm (Czauderna et al., 2003b) and 100 m capillary-column gas liquid-chromatography with flame-ionization detection (GLC-FID) (Czauderna et al., 2007).

All FA standards, 50% BF_3 in methanol and *o*-phthaldialdehyde (OPA) were provided by Sigma (USA) and Suppelco. Ethanethiol, tetrahydrofuran and sodium hypochlorite water solution (4% available Cl) were from Fluka. The high-selenized yeast (Se-Y) used was Sel-Plex (Alltech Inc., USA). Eighty-three per cent of the total Se content of Se-Y represent Se in the form of Se-methionine (Se-Met) incorporated into the proteins of *Saccharomyces cerevisiae* (Rayman, 2004). Other reagents, including dichloromethane (DCM), KOH, NaOH, Na_2SO_4 and conc. HCl, were analytical grade and were purchased from POCh (Gliwice, Poland).

Chromatographic equipment and methods

An alliance separation module (model 2690, Waters) with a Waters 996 photodiode array detector and Waters 474 fluorescence detectors were used for determination of the concentration of AA, CLA isomers and other fatty acids containing conjugated double bonds (CFA) in the livers and muscles of rats. The underivatized CLA isomers and CFA in the livers and muscles of rats were determined according to Czauderna et al., (2003b). Methylated non-CLA fatty acids in the livers and femoral muscles were determined using GLC-FID (Czauderna et al., 2007). Methylated nonadecanoic acid (C19:0) was used as the internal standard.

The methods of protein amino acid (AA) conversion to *o*-phthaldialdehyde (OPA)-derivatives (OPA-AA) followed by reversed-phase (RP) HPLC separations and quantifications of OPA-AA in the livers and muscles of rats were applied as previously described (Czauderna et al., 2002, 2003a; Niedźwiedzka et al., 2006b).

Statistical analysis

The results in Tables 1-5 are presented as means of 7-8 individually analysed concentrations of FA in the livers and femoral muscles of rats. Statistical analyses of the effects of the CLA isomer mixture (as CLAmix_A or CLAmix_B) and/or Se (as Se^{VI} or Se-Y) in the diets were conducted using the nonparametric Mann-Whitney U test (Statistica, 2002) for comparing pairs in an independent experimental group (one-factor analysis), while statistical analyses of the interaction between the CLA isomer mixture (as CLAmix_A or CLAmix_B) and Se (as Se^{VI} or Se-Y) were performed using two-factorial ANOVA analysis (CLA isomers \times Se). The statistical analyses were performed using the Statistica v. 6 package (Statistica,

Table 1. Dietary effects¹ of 2% CLA isomer mixtures (CLAmix_A and CLAmix_B), selenate (Se^{VI}) and high-selenized yeast (Se-Y) on the body weight gain (BWG)³ of rats after 29 days feeding with experimental diets

| Group | Se ^{VI} Experiment | | | | Se-Y Experiment | | | |
|----------------------------------|---|----------------------|---|----------------------|---|----------------------|------------------------------------|----------------------|
| | rats fed diets enriched in CLA isomers and Se ^{VI} | | rats fed diets enriched in CLA isomers and Se-Y | | additives | | content ⁷ | |
| | additives | content ⁷ | BW, g after 7 days ² | BWG ³ , g | additives | content ⁷ | BW, g after 7 days ² | BWG ³ , g |
| A Control ¹ | - | - | 184.9 (8) ⁸ | 59.4 ^a | - | - | 177.0 (8) ⁸ | 61.1 ^a |
| Se ^{VI} | selenate | 2 ppm | 185.3 (8) | 52.8 ^{ab} | Se-Y | 1.2 ppm | 179.0 (8) | 60.9 |
| A CLA ⁵ | CLAmix _A ⁵ | 2 ppm | 183.3 (7) | 56.8 | CLAmix _B ⁶ | 2% | 178.0 (7) | 56.4 ^a |
| A CLA _{Se^{VI}} | CLAmix _A ⁵ + selenate | 2 ppm | 182.8 (7) | 58.4 ^a | CLAmix _B ⁶ +Se-Y | 2% 1.2 ppm | 182.0 (8) | 60.4 |

¹ means in columns with the same letter are significantly different: ^a - P<0.05 and ^a - P<0.1. Statistical analyses of simultaneously the CLA isomer mixture (as CLAmix_A or CLAmix_B) and Se (as Se^{VI} or Se-Y) treatments were performed applying two-factorial ANOVA analysis

² the body weight (g) of individually adapted rats after 7 days of submaintenance feeding (9 g/the Labofeed H diet/a day/a rat). The initial body weights of rats and after 7 days of adaptation did not statistically differ among the groups at the P<0.05 level

³ BWG after feeding for 29 days with the experimental diets enriched in CLA isomer mixture and/or Se as: Se^{VI} (2 ppm Se) or Se-Y (1.2 ppm) concentrations of Se (as Na₂SeO₃), Zn, Fe, Mg and Ca in the standard Labofeed H diet found: 0.63, 137, 698, 1653 and 10683 µg/g, respectively

⁵ the dietary CLA isomers (CLAmix_A) contain: *t11t13* - 2.9%; *t10t12* - 5.1%; *t9t11* - 4.3%; *t8t10* - 2.9%; *c11t13* - 13.4%; *t10c12* - 28.0%; *c9t11* - 28.6%; *c8t10* - 9.6%; *c11c13* - 1.6%; *c10c12* - 1.5%; *c9c11* - 1.4%; *c8c10* - 0.7%. Linoleic acid <1%; no other fatty acids were detected. The concentration ratio of *c9t11*/CLA/*t10c12*CLA in the CLA isomer mixture: 1.0242; the sum of *t*CLA - 15.2%; the sum of *c*CLA 5.2%

⁶ the dietary CLA isomers (CLAmix_B) contains: *t1CLA* - 1.94%; *c9t11* - 47.3%; *t10c12* - 48.2%; *c1CLA* - 1.48%; *LA* - 1%. The concentration ratio of *c9t11*/CLA to *t10c12*CLA in the CLA isomer mixture: 0.9813; the percentage sum of *c9t11* and *t10c12* - 98.5%

⁷ the concentration of CLA isomer mixture (%) and Se (ppm) in diets of rats

⁸ in parenthesis - the number of rats in a group

Table 2. The concentration of saturated fatty acids (SFA), CLA isomers, oleic acid (*c9*C18:1), linoleic acid (LA), *c9c12c15*C18:3 (αLNA), *c5c8c11c14*C20:4 (ArA) and the concentration sum of all fatty acids (ΣFA) in the liver and femoral muscles of rats fed the control diet and the experimental diet enriched in the CLA isomer mixture (CLA_{mix}) and/or selenate (Se^{VI})

| Group | Fatty acids | | C18:0 mg/g | A-SFA mg/g ¹ | T-SFA mg/g ² | SFA ³ mg/g | <i>c9</i> C18:1 mg/g | <i>c9t11</i> CLA μg/g | <i>t10c12</i> CLA μg/g | <i>tt</i> CLA μg/g ⁴ | α CLA μg/g ⁴ | ΣCLA mg/g ⁴ | LA mg/g | αLNA mg/g | ArA mg/g | ΣFA mg/g |
|---------------------------------------|-------------|--------------------|--------------------|----------------------------|----------------------------|--------------------------|-------------------------|-----------------------------|------------------------------|---------------------------------------|-------------------------------|---------------------------|-------------------|-------------------|-------------------|--------------------|
| | Tissue | C18:0 mg/g | | | | | | | | | | | | | | |
| A Control ⁸ | Liver | 9.73 ^{ab} | 4.30 ^{ab} | 13.9 ^{ba} | 14.9 ^{ab} | 1.22 ^{ba} | 4.2 ^b | 26 ^b | 27 ^b | 62 ^b | - ^s | 68 ^b | 2.28 | 0.44 | 2.47 | 250 ^b |
| | Muscle | 3.09 | 11.9 ^b | 14.7 ^b | 15.1 ^b | 8.7 ^b | 61 ^b | 24 | 25 | 50 | 84 | - ^s | 0.15 ^b | 11.5 ^b | 4.35 ^b | 1.27 ^{ba} |
| Se ^{VI} ⁸ | Liver | 8.71 ^A | 3.70 ^c | 12.3 ^a | 13.1 ^A | 0.77 ^a | 24 | 25 | 50 | 84 | - ^s | 0.05 | 2.09 | 0.35 | 2.41 | 22.0 |
| | Muscle | 3.10 | 11.2 | 13.9 | 14.3 | 7.9 | 84 | 50 | 84 | 84 | - ^s | 0.22 | 11.9 | 4.39 | 1.39 ^a | 48.6 |
| A CLA ⁸ | Liver | 7.67 ^B | 3.25 ^A | 10.9 ^B | 11.2 ^B | 0.66 ^B | 459 ^B | 6315 ^B | 7374 ^X | 158 | 48 | 0.97 ^B | 2.96 | 0.46 | 3.23 | 238 ^B |
| | Muscle | 2.89 | 8.9 ^B | 11.6 ^B | 11.9 ^B | 4.2 ^B | 2664 ^B | 61779 ^B | 71971 | 790 | 249 | 5.40 ^B | 5.6 ^b | 2.89 ^b | 1.06 ^B | 400 |
| A CLA _{SeVI} ⁹ | Liver | 7.51 ^X | 3.34 | 10.8 ^X | 11.1 ^X | 0.67 ^W | 502 ^Y | 7374 ^X | 158 | 50 | 50 | 1.09 | 3.26 | 0.51 | 3.12 | 24.8 |
| | Muscle | 2.89 | 8.9 | 11.6 | 12.0 | 4.8 | 2870 | 71971 | 790 | 259 | 259 | 5.89 | 5.7 | 3.19 | 0.97 ^Y | 42.3 |

¹ A-SFA: the concentration sum of C12:0, C14:0 and C16:0

² T-SFA: the concentration sum of C14:0, C16:0 and C18:0

³ SFA - the concentration sum of saturated fatty acids (i.e.: C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C22:0 and C24:0)

⁴ *cc*CLA, *tt*CLA and ΣCLA: *cis,cis*CLA, *trans,trans*CLA and the concentration sum of CLA isomers, respectively

⁵ below the quantification limit

⁶ 0.6863 and 0.6678 - the concentration ratio ($R_{t10c12CLA/c9t11CLA}$) in the liver and muscles, respectively (CLA group)

⁷ 0.7450 and 0.6868 - the concentration ratio ($R_{t10c12CLA/c9t11CLA}$) in the liver and muscles, respectively (CLA_{SeVI} group)

⁸ means in a column with the same letter are significantly different: ^{A,B}- P <0.01, ^{ab}- P <0.05 and ^{α,β}- P <0.1. Superscripts: ^{A, a, α} and ^{B, b, β} - for Se^{VI} and ^A CLA group, respectively

⁹ statistical analyses of simultaneously the CLA isomers and Se^{VI} treatment were performed applying two-factorial ANOVA analysis: ^{X,Y}-P<0.01, ^{X,Y}-P<0.05 and ^W-P<0.1. Superscripts: ^{X, X, Y, Y, W}- for the liver and muscles, respectively

Table 3. The concentration of saturated fatty acids (SFA), CLA isomers, oleic acid (*c9c18:1*), linoleic acid (LA), *c9c12c15c18:3* (α LNA) *c5c8c11c14c20:4* (ArA) and the concentration sum of all fatty acids (Σ FA) in the liver and femoral muscles of rats fed the control diet and the experimental diet enriched in the CLA isomer mixture (CLAmix_B) and/or the high-selenized yeast (Se-Y)¹

| Group | Tissue | C18:0 | | A-SFA | | T-SFA | | SFA | | <i>c9c18:1</i> | | <i>c9H1 t10c12</i> | | μ | | <i>cc</i> | | Σ CLA | | LA | | α LNA | | ArA | | Σ FA | | | | |
|------------------------------------|--------|-------------------|--------------------|-------------------|--------------------|--------------------|-------------------|--------------------|------------------|------------------|-------------------|--------------------|-----------------|-------------------|-------------------|-----------|-------------------|-------------------|------|------|------|--------------|------|------|------|-------------|------|------|------|--|
| | | mg/g | mg/g | mg/g | mg/g | mg/g | mg/g | μ g/g | μ g/g | μ g/g | μ g/g | μ g/g | μ g/g | μ g/g | μ g/g | μ g/g | μ g/g | μ g/g | mg/g | mg/g | mg/g | mg/g | mg/g | mg/g | mg/g | mg/g | mg/g | mg/g | mg/g | |
| B Control ¹ | Liver | 7.3 ^B | 3.04 ^{AB} | 10.3 ^A | 10.5 ^{Ba} | 0.93 ^{AB} | 82 ^B | 24 ^{Ba} | - ² | 82 ^B | 111 ^B | 111 ^B | 82 ^b | - ² | 2.31 ^A | 0.44 | 5.33 ^b | 24.3 ^B | | | | | | | | | | | | |
| | Muscle | 3.44 ^a | 12.0 | 15.4 | 16.4 | 9.1 ^b | | | | | | | | | | | | | | | | | | | | | | | | |
| Se-Y ³ | Liver | 7.4 | 3.58 ^A | 11.0 ^B | 11.9 ^A | 1.13 ^A | - ² | 18 ^a | - ² | - ² | - ² | - ² | - ² | - ² | 3.62 ^A | 0.25 | 5.29 | 26.5 ^B | | | | | | | | | | | | |
| | Muscle | 2.93 ^a | 10.8 | 13.7 | 14.7 | 8.1 | 82 | 32 ^a | 119 | 55 | 0.29 | 12.6 | 3.68 | 1.58 ^A | 47.7 | | | | | | | | | | | | | | | |
| B CLA ³ | Liver | 8.1 ^B | 2.72 ^B | 10.8 | 12.1 ^B | 0.56 ^B | 420 | 5269 ^B | 57 | 153 | 0.90 | 2.58 | 0.26 | 2.89 ^b | 21.1 | | | | | | | | | | | | | | | |
| | Muscle | 3.50 | 9.9 | 13.4 | 14.2 | 6.4 ^b | 3640 ^B | 52274 ^B | 316 ^B | 129 ^b | 6.36 ^B | 11.0 | 3.33 | 1.39 ^B | 47.8 | | | | | | | | | | | | | | | |
| B CLA _{Se-Y} ⁴ | Liver | 9.0 | 3.24 | 12.3 | 14.1 | 0.68 | 492 | 6402 ^x | 77 | 38 | 1.01 | 2.00 | 0.26 | 4.82 | 26.3 | | | | | | | | | | | | | | | |
| | Muscle | 3.76 ^y | 10.9 | 14.7 | 15.5 | 6.9 | 3685 | 63106 | 288 | 120 | 7.20 | 8.7 | 3.01 | 1.38 | 51.9 | | | | | | | | | | | | | | | |

¹ all abbreviations as in the Tables 1-2

² below the quantification limit

³ means in a column with the same letter are significantly different: ^{A,B} - $P < 0.01$, ^{a,b} - $P < 0.05$ and ^{α,β} - $P < 0.1$. Superscripts: ^{A,a α} and ^{B,b β} - for Se-Y and ^B CLA groups, respectively

⁴ statistical analyses of simultaneously the CLA isomers and Se-Y treatment were performed applying two-factorial ANOVA analysis: ^{X,Y} - $P < 0.01$, and ^{Y,Y} - for Se-Y

^{X,Y} - $P < 0.05$ and ^{Y,Y} - $P < 0.1$. Superscripts: ^{X, X}, ^{Y, Y} and ^{Y, Y}, ^Y - for the liver and muscles, respectively

⁵ 0.6405 and 0.6247 - the concentration ratio ($R_{t10c12,c9H1}$): *t10c12CLA/c9H1* CLA in the liver and muscles, respectively (_B CLA group)

⁶ 0.8171 and 0.8429 - the concentration ratio ($R_{t10c12,c9H1}$): *t10c12CLA/c9H1* CLA in the liver and muscles, respectively (_B CLA_{Se-Y} group)

Table 4. The Δ9 desaturase index, the concentrations of unsaturated fatty acids and amino acids in the liver and femoral muscles of rats fed the control diet and the experimental diet enriched in the CLA isomer mixture (CLAmix_A) and/or selenate (Se^{VI})

| Group | Tissue | Δ9- index ¹ | | PUFA | PUFA | PUFA | PUFA | PUFA | C20:2 | C20:5 | C22:5 | C22:6 | AA ⁷ | Met ¹¹ | Cys ¹² |
|--|--------|------------------------|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|------------------|-------------------|-----------------|-------------------|-------------------|
| | | mg/g | mg/g ² | n-3 | n-6 | n-3 | n-6 | n-3 | n-6 | n-3 | n-3 | n-3 | n-3 | mg/g | mg/g |
| ^A Control ⁸ | Liver | 0.082 ^B | 1.88 ^B | 2.91 | 4.89 ^B | 7.8 ^B | 1.68 ^B | 0.52 ^B | 54 ^B | 1.25 | 260 ^B | 0.96 ^B | 464 | 29 | 11 |
| | Muscle | 0.434 ^B | 13.7 ^B | 7.5 ^B | 13.0 ^B | 20.6 ^B | 1.73 ^B | 1.34 ^A | 117 | 0.54 ^B | 541 ^B | 2.03 ^B | 412 | 22 | 8 |
| Se ^{VI} ⁸ | Liver | 0.070 | 1.31 | 2.67 | 4.58 | 7.3 | 1.72 | 0.55 | 18 | 1.16 | 238 | 0.93 | 443 | 14 | 10 |
| | Muscle | 0.422 | 12.6 | 7.8 | 13.5 | 21.4 | 1.73 | 1.47 ^A | 101 | 0.55 | 583 | 2.24 ^A | 312 | 22 | 8 |
| ^A CLA ⁸ | Liver | 0.065 ^B | 1.16 ^B | 4.16 | 6.34 ^B | 10.5 ^B | 1.57 ^B | 0.94 ^B | 110 ^B | 1.19 | 436 ^B | 2.03 ^B | 465 | 11 | 8 |
| | Muscle | 0.311 ^B | 7.2 ^B | 5.2 ^B | 10.0 ^B | 15.2 ^B | 1.88 ^B | 1.26 | 115 | 0.25 ^B | 334 ^B | 1.72 ^B | 323 | 25 | 9 |
| ^A CLA _{Se^{VI}} ⁹ | Liver | 0.065 ^w | 1.17 | 4.92 | 6.53 | 11.5 | 1.30 ^w | 1.03 | 107 | 1.48 | 434 | 2.18 | 460 | 15 | 11 |
| | Muscle | 0.336 | 7.4 | 5.4 | 11.6 | 17.0 | 2.14 | 1.40 | 95 | 0.25 | 341 | 1.65 ^y | 345 | 27 | 9 |

¹ Δ9 desaturase index (Δ9-index): (C14:1 + C16:1 + C18:1) / (C14:1 + C16:1 + C18:1 + C18:0 + C16:0 + C14:0)

² PUFAn-3, PUFAn-6 – polyunsaturated fatty acids n-3 and n-6, respectively

³ the concentration ratio of PUFAn-3/PUFAn-6

⁴ the concentration ratio of PUFA/SFA

⁵ *c11c14C20:2*

⁶ *c11c14c17C20:3*, *c5c8c11c14c17C20:5*, *c7c10c13c16c19C22:5* (DPA) and *c4c7c10c13c16c19C22:6* (DHA), respectively

⁷ the concentration sum of protein amino acids determined by RP-HPLC procedure (Czauerna et al., 2002). All amino acids were analysed in pooled samples prepared by combination of the liver or muscle samples from each rats fed the same diet

⁸ means in a column with the same letter are significantly different: ^{A,B} - P<0.01, ^{ab} - P<0.05 and ^{α,β} - P<0.1. Superscripts: ^{A,α,α} and ^{B,β,β} - for Se^{VI} and ^A CLA groups, respectively

⁹ statistical analyses of simultaneously the CLA isomers and Se^{VI} treatment were performed applying two-factorial ANOVA analysis:

^{x,y} - P<0.01, ^{x,y} - P<0.05 and ^y - P<0.1. Superscripts: ^{x,s,y} and ^{y,s,y} - for the liver and muscles, respectively

¹⁰ below of quantification detection

¹¹ the concentration of methionine (Met); ¹² the concentration of cysteine (Cys)

2002). Differences were considered significant at the a,b - $P < 0.05$ or A,B - $P < 0.01$ levels, while a tendency was concluded at the $^{\alpha,\beta}$ - $P < 0.1$ level. Statistical analyses of the interaction between the CLA isomers and Se were performed using two factorial ANOVA analysis (the CLA isomers \times Se). The interaction was considered statistically significant at x,y - $P < 0.05$, X,Y - $P < 0.01$ or $^{\psi}$ - $P < 0.1$ levels.

RESULTS AND DISCUSSION

Effects of the dietary CLA isomer mixtures, Se^{VI} and Se-Y on the body weight gain in rats

In the current study, no macroscopic lesions or pathological changes were found in the liver and femoral muscles or in any of the other organs of rats fed the diets enriched in the CLA isomer mixture (as CLAmix_A or CLAmix_B) and/or selenium (as Se^{VI} or Se-Y) (Czauderna et al., 2004a; Korniluk et al., 2006). There were no differences in the body weight gain (BWG) among the groups of rats fed the diets enriched in the CLA isomer mixture (as CLAmix_A or CLAmix_B) and Se (as Se^{VI} or Se-Y) compared with the appropriate control groups (Table 1). In contrast, BWG was numerically lower (-4.4%) and tended to be lower (-7.7%) in the group of rats fed the diet containing only CLAmix_A or CLAmix_B, respectively. Thus, in the current study the anti-obesity effect of CLA isomers, *t10c12*CLA in particular, was confirmed in all rats fed diets enriched in the CLA isomer mixture (as CLAmix_A or CLAmix_B), regardless of the presence of the higher concentration of *cc*CLA and *tt*CLA isomers in the dietary CLAmix_A than in the dietary CLAmix_B. The obtained results suggest that the concentration of *t10c12*CLA in the dietary CLA isomer mixture determines the size of the anti-obesity effect of CLA isomers in rats. Consequently, the dietary CLAmix_B containing the higher concentration of *t10c12*CLA (48.2%) resulted in a higher decrease of BWG than the dietary CLAmix_A with the significantly lower (28.0%) concentration of *t10c12*CLA. This conclusion is in agreement with results of our previous studies in which dietary *t10c12*CLA most efficiently decreased BWG in rats (Czauderna et al., 2004a, b). On the other hand, recent investigations that have not documented a considerable reduction in BWG are usually those in which low concentrations of CLA isomers were used in diets ($\leq 0.5\%$) or the dietary CLA isomer mixtures contained lower amounts of *t10c12*CLA (Sisk et al., 2001). Our results are also supported by several other investigations, which have indicated that the *t10c12* isomer, due to its positional and geometric property, is the most potent CLA isomer in terms of anti-obesity activity, thus the one which most efficiently reduces feed intake (Czauderna et al., 2004a), body weight gain (Akahoshi et al., 2003, 2004), concentration

of plasma leptin and the abundance of sterol regulatory element-binding protein mRNA (Wang et al., 2004).

Our investigation confirms the results of other studies in rodents documenting that CLA isomers lowered body fat content and energy retention, while increasing energy expenditure as well as faecal energy and fat excretion (Terpstra et al., 2002). Indeed, the sum of FA (Σ FA) concentrations numerically or statistically decreased in the liver and muscles of rats fed the diets enriched in the CLA isomer mixture (as CLAmix_A or CLAmix_B; Tables 2 and 3). In addition, the dietary CLAmix_A or CLAmix_B numerically decreased the sum of protein amino acids (AA) in femoral muscles of rats (Tables 4 and 5). Interestingly, the dietary CLAmix_B containing the higher *l10c12CLA* content also numerically lowered the concentration of AA in the liver of rats fed the diet enriched only in CLAmix_B (Table 5). Surprisingly, addition of Se (as Se^{VI} or Se-Y) to a diet enriched in a CLA isomer mixture (as CLAmix_A or CLAmix_B) offset the decreasing effect of CLA isomer mixtures on BWG in rats. Therefore, we suggest that interactions between metabolites of CLA isomers and Se neutralized the anti-obesity properties of CLA isomers in rats.

As shown in Table 1, there was an obvious difference in the influence of dietary Se^{VI} and Se-Y on BWG in rats. Se^{VI}, the inorganic selenium compound, reduced BWG ($P < 0.05$) in rats. In accordance with current observations, our previous studies also reported that dietary Se^{VI} was most efficient in decreasing the feed conversion efficiency (FCE) in rats (Czauderna et al., 2003c). On the other hand, dosed Se-Y containing mainly seleno-methionine (~85%) (Rayman, 2004) resulted in a negligible influence on BWG in rats, which occurred concomitantly with the insignificant effect of dietary Se-Y on FCE in rats (Korniluk et al., 2006).

The influence of the diets on the concentration of saturated and monounsaturated fatty acids and CLA isomers in the liver and muscles

On analysing the concentration of saturated fatty acids (SFA), differences were found in the liver and muscles of rats fed the experimental diets. The dietary CLAmix_A lowered the concentration of SFA, atherogenic SFA (A-SFA: the sum of C12:0, C14:0 and C16:0) and thrombogenic SFA (T-SFA: the sum of C14:0, C16:0 and C18:0) in the liver and muscles (Table 2). This additive in the diet also decreased the concentration of C18:0 in the liver, while only numerically lowering it in the muscles. In contrast, the diet enriched in CLAmix_B usually less effectively decreased the concentration of these fatty acids in the liver and muscles of rats (Table 3) compared with the dietary CLAmix_A. Indeed, CLAmix_B containing the higher concentration of *l10c12CLA* (48.2%) than CLAmix_A (i.e. *l10c12CLA* - 28.0%) caused a stronger reduction of the desaturation capacity through $\Delta 9$ -desaturase. So, the current results are consistent with other studies in which dietary

individual *t10c12CLA* or mixtures of *t10c12CLA* with other CLA isomers caused a reduction in $\Delta 9$ -desaturase capacity, inhibited stearyl-CoA desaturase mRNA expression and fatty acid synthesis (Czauderna et al., 2004a,b; Raes et al., 2004; Wang et al., 2004). Therefore, the values of the $\Delta 9$ -desaturase index ($\Delta 9$ -index) decreased in the liver and muscles of rats fed the diet enriched in CLAmix_A or CLAmix_B compared with that in the control rats (Tables 4 and 5). Moreover, the highest decrease of the $\Delta 9$ -index value in the liver of rats fed the diet enriched in CLAmix_B was accompanied by the highest increase of the liver concentration of C18:0 and SFA. Therefore, as shown in Tables 2 and 3, there was an obvious decrease in the concentration of oleic acid (*c9C18:1*) and monounsaturated fatty acids (MUFA) (Tables 4 and 5) in the liver and muscles of rats fed the diet containing CLAmix_A or CLAmix_B.

The diet enriched in CLAmix_A and Se^{VI} decreased the concentration of C18:0, T-SFA, SFA, *c9C18:1* and $\Delta 9$ -index in the liver (the interaction significances: $P < 0.05$ and $P < 0.1$), while numerically lowering the liver concentrations of A-SFA and MUFA. There was only a numerical decrease in the concentration of A-SFA, *c9C18:1*, MUFA and the $\Delta 9$ -index in the muscles (interaction $P > 0.05$) of rats fed this diet. Surprisingly, simultaneous addition of CLAmix_B and Se-Y to the diet caused a numerical increase of the concentration of C18:0, A-SFA, T-SFA and SFA in the liver, whereas in muscles no consistent differences were observed. There was a numerical decrease in the value of the $\Delta 9$ -index and the concentration of MUFA and *c9C18:1* in the liver and muscles of rats fed the diet enriched in CLAmix_B and Se-Y (Tables 3 and 5).

Fortunately, the diet enriched in Se^{VI} decreased the concentrations of C18:0, A-SFA, T-SFA and SFA in the liver, while there were no noticeable or consistent differences in the muscles. On the other hand, the dietary Se-Y increased the concentration of A-SFA ($P < 0.01$), T-SFA ($P < 0.05$), SFA ($P < 0.05$), *c9C18:1* ($P < 0.01$), MUFA ($P < 0.05$) and the value of the $\Delta 9$ -index ($P < 0.1$) in the liver, while only numerically decreasing the concentrations of these fatty acids and the value of the $\Delta 9$ -index in the muscles (Tables 3 and 5). A considerable influence of dietary Se^{VI} on the $\Delta 9$ -index values and the concentration of *c9C18:1* and MUFA in the liver and muscles was not found. However, this additive numerically decreased the concentration of MUFA and the $\Delta 9$ -index value in the liver and muscles, while a statistically significant decrease was observed only for the concentration of *c9C18:1* in the liver ($P < 0.05$).

The addition of CLAmix_A or CLAmix_B to the diet induced a significant increase in the concentrations of *c9t11CLA*, *t10c12CLA*, *ccCLA* and *ttCLA* in the liver and muscles of rats. As shown in Tables 2 and 3, there were significantly higher ($P < 0.01$) concentrations of CLA isomers in the muscles compared with the concentrations of CLA isomers in the liver of rats fed the diets enriched in CLAmix_A or

CLAmix_B, regardless of the presence of extra Se as Se^{VI} or Se-Y. The current study also investigated the relationship between the experimental diets and preferential accumulation of *t10c12*CLA and *c9t11*CLA in the liver and muscles (Tables 2 and 3). The present results clearly demonstrated that the concentration ratios ($R_{t10c12/c9t11}$) of *t10c12*CLA and *c9t11*CLA in the liver and muscles of rats fed the diets enriched in CLAmix_A or CLAmix_B were, regardless of the presence of Se (as Se^{VI} or Se-Y), smaller compared with the values of $R_{t10c12/c9t11}$ of these isomers in CLAmix_A ($R_{t10c12/c9t11} = 1.0242$) and CLAmix_B ($R_{t10c12/c9t11} = 0.9813$) added to the rat diets (i.e. in the liver, muscles: 0.6863, 0.6678 and 0.6405, 0.6247, respectively; Tables 2 and 3). Thus, our results are in agreement with the studies of Alasnier et al. (2002) in which *t10c12*CLA and *t10t12*CLA were also more efficiently driven through β -oxidation in the cells of the liver, muscles, kidneys or adipose tissue compared with their 9,11 homologues. Moreover, based on the concentration sums of all assayed fatty acids (Σ FA) in the liver and muscles (Tables 2 and 3) and other studies (Alasnier et al., 2002; Akahoshi et al., 2003), we suggest that CLAmix_A and CLAmix_B added to the rat diet stimulated FA oxidation in rats.

The results summarized in Tables 2 and 3 suggest that the addition of Se (as Se^{VI} or Se-Y) to the diet enriched in CLAmix_A or CLAmix_B affected the capacity of β -oxidation of CLA isomers. In our studies, the values of $R_{t10c12/c9t11}$ were higher in the liver and muscles of rats fed the diet containing Se (as Se^{VI} or Se-Y) and the CLA isomer mixture (as CLAmix_A or CLAmix_B) compared with the $R_{t10c12/c9t11}$ values in the liver and muscles of rats fed the diet enriched in only the CLA isomer mixture. Moreover, we suggest that adding Se-Y to the diet containing CLAmix_B more efficiently reduced the β -oxidation of *t10c12*CLA in the liver and muscles than the diet enriched in Se^{VI} and CLAmix_A. Therefore, in the liver and muscles, Se-Y in the diet with CLAmix_B gave a higher value of $R_{t10c12/c9t11}$ in comparison with the values of $R_{t10c12/c9t11}$ in the liver and muscles of rats fed the diet containing Se^{VI} and CLAmix_A (i.e. 0.8171 vs 0.7450 and 0.8429 vs 0.6868, respectively; see Tables 2 and 3). The present results on this subject also reinforce the hypothesis that the CLAmix_B \times Se-Y interaction more efficiently diminished the yield of FA oxidation than the dietary CLAmix_A and Se^{VI} (Tables 2 and 3). Therefore, BWG, concentrations of Σ FA and the percentage contributions of *t10c12*CLA in the liver and muscles of the CLA_{Se-YB} rats' group are more similar to the ones in the control rats. Concomitantly, stronger effects of the CLAmix_A \times Se^{VI} interaction on BWG, the sum of concentrations of all assayed fatty acids (Σ FA) and the abundance of the *t10c12* isomer were observed in rats fed the diet enriched in CLAmix_A and Se^{VI}.

The concentrations of *c9t11*CLA, *t10c12*CLA, *cc*CLA and *tt*CLA numerically or significantly ($P < 0.05$ or $P < 0.01$) increased in the liver and muscles of rats fed diets containing Se (as Se^{VI} or Se-Y) and the CLA isomer mixture (as CLAmix_A

or CLAmix_B) compared with rats fed the diets enriched in CLAmix_A or CLAmix_B. For the concentration of *t10c12CLA* in the liver, interactions of CLAmix_A x Se^{VI} and CLAmix_B x Se-Y interactions were found ($P < 0.05$), while for the concentration of *c9t11CLA* in the liver, there a CLAmix_A x Se^{VI} interaction was observed ($P < 0.1$). It is well established that dietary supplementation of both selenate and Se-Y results in increased activities of glutathione peroxidases and other specific selenoproteins. Thus, we hypothesized that both chemical forms of Se are effective in maintaining the antioxidant Se status in rats.

The influence of diets on the concentration of PUFA in the liver and muscles

The concentration of PUFAn-3 ($P < 0.01$), PUFAn-6 ($P < 0.1$), PUFA ($P < 0.1$) and the value of the concentration ratio of PUFA and SFA (PUFA/SFA) decreased in the muscles of rats fed the diet containing CLAmix_A (Table 4). Similar changes were observed in the muscles of rats fed the diet enriched in CLAmix_B, however, the decrease was not statistically significant ($0.10 < P < 0.17$; Table 5). Moreover, this diet resulted in a decrease in the concentrations of these fatty acids and the ratios of PUFAn-6/PUFAn-3 and PUFA/SFA in the liver. Surprisingly, the diet containing CLAmix_A increased the concentrations of PUFAn-3, PUFAn-6, PUFA and the value of PUFA/SFA in the liver.

No consistent differences in the concentrations of PUFAn-3, PUFAn-6, PUFA and the ratio value of PUFA/SFA, were observed in the liver and muscles of rats fed the diet enriched in Se^{VI} and Se-Y. On the other hand, as shown in Table 4, the diet enriched in CLAmix_A or Se^{VI} increased the concentration of PUFAn-3, PUFAn-6, PUFA and the ratio of PUFA/SFA in the liver, while there was decrease in the concentration of PUFAn-3, PUFAn-6 and PUFA in the muscles. For these fatty acids and the ratio of PUFA/SFA, there were no significant CLA isomer (as CLAmix_A and CLAmix_B) x Se (as Se^{VI} and Se-Y) interactions in the liver and muscles, with the exception of the ratio of PUFA/SFA ($P < 0.1$) in the muscles of rats fed the diet containing CLAmix_B and Se-Y.

The concentrations of linoleic (LA), linolenic (α LNA) and arachidonic (ArA) acids were statistically or numerically lower in the muscles of rats fed the diet containing CLAmix_A ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively) or CLAmix_B ($P = 0.22$, $P = 0.22$ and $P < 0.01$, respectively) (Tables 2 and 3). Unexpectedly, the concentrations of these fatty acids in the liver showed no significant changes ($P > 0.05$) with the exception of the level of ArA in the liver of rats fed the diet enriched in CLAmix_B ($P < 0.05$). So, the current study demonstrates that the dietary CLA isomer mixture containing higher concentrations of *c9t11CLA* (47.3%) and *t10c12CLA* (48.2%) (i.e. CLAmix_B) more efficiently decreased the accumulation of AAs than the dietary CLAmix_A possessing lower concentrations of *c9t11CLA* and *t10c12CLA* (i.e. 28.6

and 28.0%, respectively). Thus, the present results are consistent with other studies showing that dietary *c9t11CLA* and *t10c12CLA* decreased the concentration of LA (i.e. PUFAn-6 in particularly) and its metabolites (e.g., C18:3n-6, C20:3n-6 and ArA) in animals (Wang et al., 2004; Korniluk et al., 2006). The current results documented that dietary *c9t11CLA* and *t10c12CLA* resulted in dose-dependent interference in the conversion of LA to anabolites by competing for the same enzymes (the Δ 6-, Δ 5-, Δ 4-desaturases and elongase) (Alasnier et al., 2002; Korniluk et al., 2006). These CLA isomers also modified the metabolism of α LNA (PUFAn-3), however, the effects of CLA isomers are weaker and less consistent than on the metabolism of LA (Tables 2 and 3). On the other hand, no consistent differences were observed in the concentrations of LA, α LNA and AAs in the liver and muscles of rats fed the diet containing Se^{VI} or Se-Y. Similarly, for these fatty acids in the liver and muscles, there also were no significant CLA isomer mixture (as CLAmix_A or CLAmix_B) \times Se (as Se^{VI} or Se-Y) interactions, although the diet enriched in CLAmix_B and Se-Y resulted in a numerical decrease of the LA, α LNA and AAs in the liver and muscles, while CLAmix_A \times Se^{VI} treatment numerically lowered the levels of these fatty acids only in the muscles.

The concentration of *c11c14C20:2* in the liver and muscles usually numerically or significantly ($P < 0.01$ and $P < 0.05$) increased in rats fed the diet enriched in CLAmix_A or CLAmix_B, respectively (Tables 4 and 5). Therefore, we suggest that dietary *c9t11CLA* and *t10c12CLA* tended to increase elongase capacity, particularly in the liver. On the other hand, for the capacity of elongase, there were no significant CLA isomer (as CLAmix_A or CLAmix_B) \times Se (as Se^{VI} or Se-Y) interactions.

The stimulatory effect of *c9t11CLA* and *t10c12CLA* on elongase capacity as well as Δ 4-desaturase in the liver was confirmed by the increase of the *c7c10c13c16c19C22:5* (C22:5n-3) and *c4c7c10c13c16c19C22:6* (C22:6n-3) concentrations of in the liver of rats supplemented with CLAmix_A or CLAmix_B in the liver of rats (Tables 4 and 5). For these fatty acids, a similar effect was observed in the liver of rats fed the diet enriched in both CLAmix_A and Se^{VI}. Unexpectedly, the products of elongase and Δ 4-desaturase (i.e. C22:5n-3 and C22:6n-6) were less efficiently accumulated in the muscles of rats fed the diets containing CLAmix_A or CLAmix_B compared with the control rats. Moreover, no consistent effects of Se^{VI} and Se-Y on C22:5n-3 and C22:6n-6 in the liver and muscles were found.

Influence of the diets on the concentration of amino acids in the liver and muscles

It has been shown that the diet enriched in the CLA isomer mixture (as CLAmix_A or CLAmix_B), regardless of the presence of Se as Se^{VI} (Tables 4 and 5), leads to a decrease in the concentration of methionine (Met) in the liver and the sum of all amino acid (AA) concentrations in the muscles, while increasing the

concentration of Met in the muscles. The presented data suggested that dietary CLAmix_A containing the higher concentration of *tt*CLA, regardless of Se^{VI} supplementation, more efficiently reduced lipoprotein synthesis in the muscles of rats than the diet with CLAmix_B, regardless of the presence of Se-Y. Consequently, the sums of AA concentrations (Tables 4 and 5) and ΣFA (Tables 2 and 3) in the muscles decreased in rats fed the diet containing CLAmix_A or CLAmix_B, although the addition of Se^{VI} and especially Se-Y to the diet containing CLA isomers reduced the effect of CLAmix_A and CLAmix_B. We also hypothesize that dietary Se^{VI} and, particularly, Se-Y, reduced the metabolism of Met in the muscles, while stimulating the catabolism or/and transfer of liver Met to other organs and tissues of rats (e.g., into muscles). Indeed, the concentration of Met ($P < 0.05$) and the sum of AA ($P < 0.05$) increased in the blood plasma of rats fed the diet enriched in 2% CLAmix_A (Niedzwiedzka et al., 2006a). Interestingly, the diet containing only Se^{VI} resulted in a considerable decrease in the sum of AA concentrations in the liver and, especially, in the muscles, concomitantly with a lower BWG (Table 1). On the other hand, Se-Y added to the diet enriched in CLAmix_B most efficiently elevated the concentrations of AA, Met and cysteine in the muscles (Table 5). Moreover, addition of Se-Y to the diet with CLAmix_B most efficiently diminished the ability of CLAmix_B to decrease the value of BWG and the concentration of ΣFA in the muscles. Therefore, we suggest that Se-Y most effectively reduced the anti-obesity property of CLA isomers.

No consistent effects were observed on the content of cysteine in the liver and muscles of rats fed the diets enriched in CLA isomers (CLAmix_A or CLAmix_B) or/and Se (as Se^{VI} or Se-Y).

CONCLUSIONS

For monogastric animals both dietary CLA isomer mixtures, regardless of the different content of *t,t* and *c,c* isomers of CLA, seem to be an efficient way of increasing the deposition of *c9t11*CLA and *t10c12*CLA in the liver and muscles, as well as of DPA and DHA in the liver. The mixture of *c9t11*CLA *t10c12*CLA considerably decreased the abundance of A-SFA and T-SFA in the liver and muscles, so, we suggest that the diets enriched in these CLA isomers improve the nutritional properties of meat derived from monogastric animals. Selenate added to the diet with CLAmix_A more efficiently decreased the concentration of A-SFA and T-SFA as well as other SFA in the liver and muscles than the diet enriched in CLAmix_B and Se-Y.

In a final conclusion, dietary Se-Y and Se^{VI} were shown to produce generally different effects on body weight gain of rats and FA profiles in the animals' bodies, regardless of the presence of CLA isomers in the diet.

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