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The influence of dietary conjugated linoleic acid isomers on the essential amino acid profile in rats*

K.M. Niedźwiedzka, M. Czauderna[1] and J. Kowalczyk

The Kielanowski Institute of Animal Physiology and Nutrition,
Polish Academy of Sciences
05-110 Jabłonna, Poland

ABSTRACT

Feeding diets with 1% individual conjugated linoleic acid (CLA) isomers increased the concentration of essential and non-essential free amino acids (AAs) in blood plasma of rats. *Trans10cis12*CLA or 1% CLA isomer mixture added to diets had a negligible effect on the content of essential and non-essential protein primary AAs in femoral muscles. Diets with *cis9trans11*CLA or 2% CLA isomer mixture decreased the content of all protein primary AAs in muscles.

KEY WORDS: essential amino acids, conjugated linoleic acid, blood, liver, muscles, rats

INTRODUCTION

The major body constituents of domestic animals (lipids, proteins, carbohydrates and salts) and their concentrations can be affected by physiological, genetic, nutritional and environmental factors. Among these, two major factors determine body composition: genetics (species of the animal) and nutrition. Nutrition has a profound effect on lipid composition (Czauderna et al., 2004; Korniluk et al., 2006), while the composition of the protein fraction is generally little affected (Coulon et al., 2001). Amino acids (AAs) and fatty acids are important components determining the nutritional quality of products derived from domestic animals. In particular, essential AAs are often believed to exert a favourable effect on animal and human health. Conjugated linoleic acid (CLA) isomers were shown recently to have a variety of beneficial effects, such as anticarcinogenic action or immune system enhancement. Therefore, the aim of the present study was to investigate the influence of dietary CLA isomer(s) on the profile of protein primary AAs in meat, liver and free AAs in blood plasma of rats.

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[1] Corresponding author: e-mail: m.czauderna@ifzz.pan.pl

MATERIAL AND METHODS

The experiments were conducted on female rats (Wistar, Ifz:BOA). Five groups of 7 or 8 rats at 8 weeks of age and 200 g initial body weight were housed individually in metabolic cages for the entire experimental period as described previously (Czauderna et al., 2004). For four weeks the rats were fed *ad libitum* a diet enriched in the CLA isomer mixture (CLA mix) at a level of 1 or 2%, *cis9 trans11*CLA (*c9t11*) at a 1% level, or *trans10cis12*CLA (*t10c12*) at a 1% level (Table 1). At the end of the experimental period the rats were killed by CO_2; femoral muscles, livers, and heparinized blood samples were collected for analyses.

Blood samples were centrifuged at 2000 *g* for 15 min (at 1-2°C). The obtained plasma was deproteinized with trichloroacetic acid. Livers and muscles were frozen, lyophilized and the obtained residues were stored at -20°C. An Alliance separation module (model 2690, Waters) with a Waters 996 photodiode array detector and a Waters 474 fluorescence detector was used. All methods of hydrolysis and *o*-phthaldialdehyde (OPA)-derivatization, HPLC separation and quantification of OPA amino acid derivatives (OPA-AAs) were as previously described (Czauderna et al., 2002, 2003). All reagents were analytical grade, whereas methanol was HPLC grade. The CLA isomer(s) were supplied by Larodan Fine Chemicals AB (Sweden). Ethanethiol was obtained from Aldrich, while *o*-phthaldialdehyde (OPA) and amino acid (AA) standards were from Sigma. Statistical analyses of the effects of dietary CLA isomer(s) on the concentrations of free AAs in blood plasma were conducted using the non-parametric Mann-Whitney U test.

RESULTS AND DISCUSSION

No lesions or symptoms of CLA isomer intoxication were observed in rats fed diets enriched in the CLA isomer(s). The content of AAs in the blood plasma, femoral muscles and liver of rats fed experimental diets are shown in Table 1. As can be seen, all of the experimental diets showed a tendency towards lowering the body weight gain of rats. We found that the individual CLA isomer or 2% CLA mix tended to increase, or increased the contents of the sum of free essential AAs and the sum of assayed free AAs in plasma.

It was found that the diet enriched in *t10c12* particularly effectively elevated the content of the sum of non-essential and essential AAs and CLA isomers in plasma and liver, while *t10c12* or the 1% CLA mix had a negligible influence on the content of protein AAs in muscles. It was surprising that the diet with *c9t11* or 2% CLA mix in generally resulted in a decreased content of essential and non-essential protein AAs in muscles. So, we suggest that the presence of ~1% *c9t11* in the diet with the 2% CLA mix or *c9t11* reduced the beneficial influence of *t10c12* on the levels of essential

Table 1. Mean concentrations of free amino acids (AAs) and CLA isomers in plasma (µg/ml), the concentration of amino acids (AAs) and CLA isomers in liver[1] (mg/g) and femoral muscles[1] (mg/g) of rats fed diets with CLA isomer(s)

Group[3]	Amino acids		Essential amino acids								ΣEAA[2]	ΣNEAA[2]	ΣAA[2]	ΣCLA[2]
	BMG[4]	Sample	His	The	Met	Val	Phe	Ile	Leu	Lys				
		Plasma	45^{aA}	37^{A}	18^{a}	46^{a}	20^{a}	30^{a}	43^{a}	98^{a}	337^{A}	460^{a}	797^{aA}	-
Control	59.4	Liver	10.0	40.8	24.5	29.6	25.0	24.1	45.2	30.4	230	228	458	67
		Muscles	9.0	36.7	5.8	26.6	22.5	21.6	40.7	27.3	190	221	411	-
		Plasma	39^{abAB}	33^{AB}	22^{abc}	44^{a}	19^{ab}	29^{a}	42^{a}	95^{abc}	324^{ABC}	424^{abc}	748^{abAB}	45.6^{a}
1% CLA mix	54.8	Liver	9.7	42.8	14.8	31.7	26.5	25.5	47.6	34.2	233	238	471	2600^{a}
		Muscles	8.3	36.6	9.4	27.1	22.7	21.8	40.8	29.3	196	221	417	4920^{A}
		Plasma	61^{bAB}	45^{AB}	28^{bc}	55^{a}	25^{ab}	35^{a}	51^{a}	115^{bc}	415^{BC}	551^{bc}	966^{bAB}	42.3^{a}
1% *c9t11*	59.7	Liver	8.5	41.5	14.2	30.1	24.8	24.3	45.5	32.8	222	227	449	2603^{ab}
		Muscles	5.6	27.4	7.0	19.9	16.4	16.0	30.1	21.7	144	164	308	6190^{AB}
		Plasma	70^{bB}	45^{B}	21^{abc}	49^{a}	23^{b}	32^{a}	46^{a}	105^{abc}	392^{ABC}	532^{abc}	924^{abB}	43.6^{a}
1% *t10c12*	54.1	Liver	11.2	48.8	15.0	36.4	30.2	29.6	55.6	38.5	265	275	541	2904^{ab}
		Muscles	7.7	33.7	8.3	25.1	20.8	20.5	38.4	26.6	181	206	388	5800^{AB}
		Plasma	61^{bAB}	39^{AB}	21^{abc}	43^{a}	21^{ab}	28^{a}	41^{a}	96^{abc}	351^{ABC}	480^{abc}	831^{abAB}	67.3^{a}
2% CLA mix	56.8	Liver	9.3	42.7	10.9	31.4	26.1	25.2	47.4	32.6	226	237	463	9622^{b}
		Muscles	6.1	27.6	5.6	20.3	16.9	16.3	30.7	21.1	145	178	323	9310^{B}

[1] concentrations of AAs analyzed in pooled samples prepared by combination of all livers or muscles from rats fed the same diet

[2] the sum of essential (EAA), non-essential (NEAA), all assayed AAs (Czauderna et al., 2002), and the sum of CLA isomers (Czauderna et al., 2004)

[3] means in columns with different superscripts are significantly different at $^{a,b}P<0.05$ or at $^{A,B}P<0.01$

[4] the body weight gain (g) of rats after 4 weeks of feeding

[5] eight rats fed the diet without added CLA isomer(s) (control group)

and non-essential AAs in plasma, liver and meat. Our results are consistent with data concerning AA contents in plasma, liver and muscles. The added individual isomers or 1% CLA mix have antiobesity and repartitioning properties. Feeding these additives increased in the content of protein AAs in the liver, while decreasing the sum of fatty acids (Czauderna et al., 2004). Rats fed the diet with 1% CLA mix or *t10c12* showed minor changes in the sum of the assayed AAs, while the added *c9t11* or 2% CLA mix decreased the content of essential and non-essential protein AAs in muscles.

CONCLUSIONS

Considering the above results, we could hypothesize that a diet with the higher content of *c9t11* (~1%) decreased the formation yield of protein in muscles, while dietary *t10c12,* in particular, most efficiently stimulated liver protein synthesis (repartitioning of the body composition). Finding that *t10c12* fed to rats increased the content of non-essential and essential AAs in plasma and liver (i.e. proteins), as well as the content of CLA isomers in muscles, is valuable information for nutritionists carrying out research to improve the nutritive value of food for human and animal health.

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