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Influence of conjugated linoleic acid (CLA) on intramuscular fatty acid composition in rabbit

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RIASSUNTO – Effetto dei coniugati dell'acido linoleico (CLA) sulla composizione acidica del grasso intramusicolare del coniglio. Sono stati utilizzati 42 conigli, del peso vivo (p.v.) medio di circa 1,8 kg, suddivisi in 3 gruppi ed alimentati fino alla macellazione (circa 3,1 kg p.v.) con mangimi che differivano per i livelli d'integrazione di CLA (0; 0,25 o 0,50%). Sul grasso intramusicolare del *Longissimus dorsi* sono stati determinati il contenuto degli isomeri CLA *cis*-9, *trans*-11 e *trans*-10, *cis*-12 ed il profilo acidico. Il trattamento alimentare ha determinato un incremento ($P<0,01$) del tenore di entrambi gli isomeri CLA nel grasso intramusicolare e ne ha modificato il profilo acidico causando un aumento ($P<0,01$) degli acidi grassi saturi, una riduzione ($P<0,01$) degli acidi grassi monoinsaturi e variazioni modeste nel tenore in polinsaturi. Laggiunta di CLA alla dieta del coniglio risulta un mezzo efficace per aumentarne il contenuto nelle carni, anche se aumenta il rapporto saturi/insaturi e gli indici di aterogenicità e di trombogenicità.

Key words: nutrition, rabbit meat, conjugated linoleic acid, fatty acid.

INTRODUCTION – The impact of feeding CLA has been thoroughly investigated in pigs, and Thiel-Cooper *et al.* (2001), Ostrowska *et al.* (2003), Lo Fiego *et al.* (2004), found that CLA modifies lipid fatty acid profile, negatively affecting some nutritional lipid indexes. So far, much less attention has been paid to rabbits. Recently, Corino *et al.* (2003) have shown that supplementing rabbit diets with CLA has limited effect on the chemical composition of meat and at a high slaughter weight reduces intramuscular fat content. The present research has been carried out to evaluate the effect of dietary CLA supplementation on *cis*-9, *trans*-11 and *trans*-10, *cis*-12- C18:2 isomers content, and on fatty acid composition of rabbit intramuscular lipids.

MATERIAL AND METHODS – Forty-two New Zealand White rabbits, half males and half females, 55 days old, 1.8 kg mean live weight (l.w.), were randomly assigned to three weight - and sex - balanced groups, fed *ad libitum* conventional diets (crude protein 16%, crude fiber 14%, ether extract 3%, and ash 8% as fed) supplemented with 0, 0.25 or 0.50% of a CLA preparation containing 65% CLA isomers, half *cis*-9, *trans*-11 and half *trans*-10, *cis*-12, in free fatty acid form (Colinco, Inc. Minnesota, USA). The rabbits were slaughtered at 104 days, at about 3.1 kg of l.w. *Longissimus dorsi* muscle was removed from each carcass after 24 h of storage at $1\pm0.5^\circ\text{C}$ and submitted to lipid extraction (Folch *et al.*, 1957). Fatty acid methyl esters were prepared using sodium methoxide 0.5 M in methanol for 15 min. at 50 – C and analyzed for fatty acid composition by GLC and CLA isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) by HPLC (Kramer *et al.*, 1998). Besides, atherogenic and thrombogenic indexes were calculated according to Ulbricht and Southgate (1991) and Δ^9 desaturase

index according to Smith *et al.* (2002). Data were processed by analysis of variance using the GLM procedure of SAS (SAS Institute, 1996) with dietary treatment as independent variable.

RESULTS AND CONCLUSIONS – Dietary CLA supplementation (table 1) led to a significant increase ($P<0.01$) of both CLA isomers in intramuscular fat. In agreement with findings of Chin *et al.* (1992) in foods of animal origin and Lo Fiego *et al.* (2004) in subcutaneous adipose tissue of pigs, the *cis*-9, *trans*-11 was the more represented isomer. This could be caused by the more efficient mechanism of incorporation of this isomer into tissues respect to the *trans*-10, *cis*-12 (Ostrowska *et al.*, 2003). The presence of the *cis*-9, *trans*-11 isomer in the control group tissues is likely to be due to endogenous desaturation of *trans*-11-C18:1 (Gläser *et al.*, 2002) or to biohydrogenation reactions brought about by the microbial flora populating the large intestine of rodents, which is able to convert linoleic acid to *cis*-9, *trans*-11 isomer (Chin *et al.*, 1994).

Table 1. Effect of dietary CLA supplementation on CLA isomers content (mg/g lipids) and fatty acid composition (%) of intramuscular lipids of rabbits *longissimus dorsi*.

	Dietary CLA supplementation %			P	SEM
	0	0.25	0.50		
<i>cis</i> -9, <i>trans</i> -11	0.40	2.54	7.59	**	2.257
<i>trans</i> -10, <i>cis</i> -12	trace	1.74	6.66	**	2.859
C14:0	1.90	2.13	2.13	ns	0.119
C15:0	0.45	0.49	0.50	*	0.003
C16:0	25.73	26.59	27.65	**	1.787
C17:0	0.50	0.58	0.65	**	0.005
C18:0	7.32	7.46	8.80	**	0.440
Total SFA	36.09	37.46	39.97	**	2.325
C16:1	3.11	2.48	2.02	*	0.760
C17:1	0.20	0.17	0.10	**	0.002
C18:1	24.67	21.91	21.19	**	2.050
C20:1	0.16	0.18	0.14	ns	0.003
Total MUFA	28.38	24.98	23.66	**	4.583
C18:2-n6	26.94	27.19	26.29	ns	2.803
C18:3-n3	1.87	2.14	2.10	ns	0.090
C20:2-n6	0.15	0.20	0.17	ns	0.003
C20:3-n6	0.33	0.39	0.27	**	0.009
C20:4-n6	4.15	4.68	4.01	ns	1.605
C22:4-n6	1.10	1.27	1.01	ns	0.124
C22:5-n6	0.29	0.38	0.26	*	0.018
Total PUFA	35.23	37.27	36.10	ns	6.954
Total UFA	63.61	62.24	59.76	**	2.344
SFA:UFA Ratio	0.57	0.60	0.67	**	0.002
Atherogenic Index	0.53	0.57	0.61	**	0.003
Thrombogenic Index	0.94	0.97	1.09	**	0.005
Δ^9 desaturase Index	0.44	0.40	0.37	**	0.001

**: $P<0.01$; *: $P<0.05$; ns: not significant.

SFA, MUFA, PUFA, UFA = saturated, monounsaturated, polyunsaturated and unsaturated fatty acids.

CLA dietary supplementation led to an increase of C15:0, C16:0, C17:0 and C18:0 fatty acids and, thus, of total saturated fatty acids (SFA) and to a decrease of monounsaturated fatty acids (MUFA), mainly C16:1 and C18:1. This effect, which involves both SFA and MUFA to a considerable degree, is probably linked to a modification that CLA exerts on desaturation mechanisms, i.e. a reduction in the Δ^9 desaturase index (table 1) and, hence, in the activity of Stearoyl-CoA desaturase (Lee *et al.*, 1998; Smith *et al.*, 2002), and, eventual-

ly, in a reduced conversion of stearic into oleic acid by desaturation within lipids. As regards polyunsaturated fatty acids (PUFA), the feeding of CLA caused only minor variations in some single components, but the total PUFA content was unaffected. Overall, the CLA dietary supplementation led to a significant reduction ($P<0.01$) of total unsaturated fatty acids (UFA) and an increase ($P<0.01$) of SFA:UFA ratio, which makes the fat less suitable for human nutrition. Further, dietary CLA supplementation negatively affected the atherogenic and thrombogenic indexes of intramuscular fat, with an increase of both parameters in supplemented groups (table 1), though CLA fed rabbits produced a leaner meat (Corino *et al.*, 2003). Indeed, the increase of CLA content in meat may offer some interesting perspectives, given the numerous positive effects that CLA is believed to have on human health.

In conclusion, dietary CLA supplementation is an effective tool for increasing, in a dose-dependent manner, the amount of CLA in intramuscular lipids of rabbits. Moreover, the data show that the *cis*-9,*trans*-11 is the predominant isomer in intramuscular lipids of rabbits. The dietary supplementation significantly influences lipid fatty acid composition causing an increase in SFA and a reduction in MUFA. These variations lead to a higher SFA:UFA ratio and a worsening of some lipid nutritional indexes, although other researches show that CLA dietary supplementation leads to a reduction of meat lipids content in rabbit. However, the possibility of increasing the CLA content in rabbit meat represents a highly interesting opportunity to provide value-added healthful meat product for human consumption. In fact, CLA is recognized to exert several beneficial effects on human health. In order to prevent an excessive reduction in the unsaturated fatty acid content of meat, appropriate feeding strategies, e.g. supplementing rabbit diets with UFA along with appropriate levels of antioxidants, must be taken into account.

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