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CONJUGATED LINOLEIC ACID CONTENT IN CECOTROPHES, SUPRARENAL AND INTRAMUSCULAR FAT IN RABBITS FED COMMERCIAL DIETS

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Abstract: An experiment was conducted to measure recycling through cecotrophes and retention of conjugated linoleic acid (CLA) in the body fat of rabbits. A commercial diet was formulated and given *ad libitum* to six New Zealand × Californian rabbits. Animals were weaned at 25 days of age and were reared in individual cages for five weeks, reaching an average body weight of 2.5 kg. Excretion of soft faeces was individually determined for 24 h after putting a wooden collar around the animals' neck. Ether extract content and fatty acid profile were determined in both soft and hard faeces, and also in the suprarenal and intramuscular fat. CLA cis-9, trans-11 isomer was detected in faeces and tissues. The CLA concentration was higher in soft than in the hard faeces (6.4 *vs.* 3.6 g/kg total fatty acids). No difference in CLA concentration was found between tissues (muscle vs. adipose). However, CLA incorporation in muscle was only detected in the neutral lipid fraction. It was concluded that the amount of CLA cis-9, trans-11 isomer recycled through cecotrophy in rabbits fed a commercial diet is retained in similar proportions in the suprarenal and intramuscular fat, but differs in the lipid classes.

Key words: cecotrophes, fat, dietary fibre, conjugated linoleic acid

INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of linoleic acid isomers that is produced in biohydrogenation processes, such as those occurring in the rumen. CLA is considered to have health benefits (anticarcinogenic, antiatherogenic, antioxidant and antidiabetic) at relatively low doses in laboratory animals and *in vitro* human models (Parodi, 1999; Williams, 2000; Pariza *et al.*, 2001; Azain, 2003). However, average CLA consumption in human diets (Ritzenthaler *et al.*, 2001) is below the recommended intake (Williams, 2000). As a result, there has been considerable interest in increasing CLA concentrations in animal feeds in order to provide healthful products for human consumption. Previous works (Corino *et al.*, 2002 and 2003) have shown that CLA supplementation of rabbit diets led to significant effects on lipid metabolism that improved carcass quality.

Concentrations of CLA in ruminant milk and meat fats is appreciable (from 3 to 6 g/kg; Chin *et al.*, 1992) and can be increased (up to 22 g/kg) through the use of appropriate dietary supplementation (Dhiman *et al.*, 1999). In contrast, CLA levels in products obtained from single-stomached animals such as pigs and poultry are much lower (Azain, 2003; Alvarez *et al.*, 2004). This difference is related to the lower amount of CLA precursors reaching the fermentative area and to the low capacity for long-chain fatty acids absorption in the hindgut. However, rabbits are able to recycle part of the end

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microbial fermentation products through cecotrophy, so that the CLA retained in their meat might be higher than in other non ruminant species.

The objective of this study was to measure the amount of CLA recycled through the soft faeces, and also the concentration of CLA in hard faeces, suprarenal and intramuscular fat of rabbits fed a commercial diet.

MATERIAL AND METHODS

A diet was formulated to meet nutrient recommendations from De Blas and Mateos (1998) and De Blas *et al.* (1999) by using typical ingredients from rabbit diets in Spain. The ingredients and chemical composition are shown in Table 1. Six New Zealand × Californian rabbits were weaned at 25 days of age and fed *ad libitum* the experimental diet in individual cages. Five weeks later, soft faeces excretion was individually determined throughout a 24 h period, after fitting the animals with a wooden collar (25-cm diameter) on the neck to prevent cecotrophy. The collar was put on at 8 am, before the usual

Table 1: Ingredient and chemical composition of the diet (g/kg as fed basis).

Ingredients	Barley grain	323
	Wheat bran	84
	Sunflower meal, 36%	71
	Soybean meal, 47%	111
	Alfalfa hay	288
	Sunflower hulls	60
	Beet pulp	23
	Lard	23
	L-Lysine HCl, 78% pure	4
	DL-methionine, 99% pure	1
	L-threonine	1
	Sodium chloride	6
	Vitamin-mineral premix[1]	5
Chemical analysis	Digestible Energy (MJ/kg)[2]	11.3
	Crude protein	180
	Lysine[2]	10.6
	Methionine[2]	3.7
	Threonine[2]	7.1
	Ether extract	50
	Starch	190
	Neutral detergent fibre	301
	Acid detergent fibre	164
	Acid detergent lignin	42
	Calcium[2]	6.2
	Phosphorus[2]	4.3

[1] Provided by Trouw Nutrition España, S.A. (Madrid, Spain). Mineral and vitamin composition (per kg of food): vitamin A, 8,375 IU/kg; vitamin D3, 750 IU/kg; vitamin E, 20 IU/kg; vitamin K3, 1 mg/kg; vitamin B2, 2 mg/kg; vitamin B6, 1 mg/kg; vitamin B1, 1 mg/kg; vitamin B3, 20 mg/kg; Fe: ($FeSO_4$), 76 mg/kg; Zn: (ZnO), 59 mg/kg; Mn: (MnO), 20 mg/kg; Cu: ($CuSO_4$), 10 mg/kg; Co: ($CoCO_3$), 0.7 mg/kg; I: (KI), 1.3 mg/kg; Choline, 250 mg/kg; S, 275 mg/kg; BHA-etoxiquin, 54 mg/kg; Flavofosfolipol, 2.5 mg/kg; Robenidine, 60 mg/kg.
[2] Calculated according to FEDNA (2003).

time rabbits begin to practise cecotrophy (Carabaño *et al.*, 1998). One day after finishing the cecotrophy trial, animals (weighing 2.44 ± 0.03 kg, SE) were slaughtered by cervical dislocation at 12 am. Samples of suprarenal fat and loin were taken for analysis.

Procedures of the AOAC (1995) were used to determine dietary contents of dry matter (930.15), N (954.01), ether extract, and starch according to the alpha-amyloglucosidase method (996.11). Neutral-detergent fibre, acid-detergent fibre and acid-detergent lignin were determined according to the sequential method of Van Soest *et al.* (1991). Fatty acids of diets were extracted and quantified by the one-step procedure described by Sukhija and Palmquist (1988) in freeze-dried samples with pentadecanoic acid (C15:0) (Sigma, Alcobendas, Madrid) as internal standard. Ether extract content in faeces was determined using Soxhlet extraction with diethyl ether. Intramuscular neutral and polar lipids were extracted by consecutive solvent elution with dichloromethane/methanol (90/10 v/v) according to the method developed by Marmer and Maxwell (1981). Fatty acid methyl esters (FAMEs) from muscle and faeces total lipids were prepared by acidic-trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) as described elsewhere (López-Bote *et al.*, 1997). All FAMEs were then analysed using a Hewlett Packard HP-6890 gas chromatograph equipped with flame ionisation detection and a 30 m × 0.32 mm × 0.25 mm cross linked polyethylene glycol capillary column (HP-Innowax). Analyses were performed with a temperature program from 170 to 240ºC at a rate of 1ºC/min. Injector and FID detector were maintained at 250ºC. Carrier gas was helium at a flow rate of 3 ml/min. Individual methyl esters were identified by comparison with retention times of the corresponding standards (Qualimix Fich S. Ref: 89-5550, LARODAN, Malmö, Sweden).

General linear model procedures of SAS version 6 (Statistical Analysis System Inst., 1990) were used to test, by non-orthogonal contrasts, differences between fatty acid profile of fat extracted from i) hard *vs.* soft feces, ii) suprarenal *vs.* intramuscular fat, and iii) polar *vs.* neutral intramuscular lipids.

RESULTS AND DISCUSSION

Feed intake during the three days before cecotrophy control averaged 149 (± 3.0, SE) g dry matter per day. Daily dry matter and ether extract recycling with cecotrophes were 17.7 (± 0.39) and 0.441 (± 0.101) g, which accounted for 10.7 and 5.0% of total intake (feed + cecotrophes), respectively.

Cis-9, trans-11 CLA isomer was detected in both soft and hard faeces (Table 2), the concentration being significantly higher in the former (6.4 *vs.* 3.6 g/kg total fatty acids). Previous works (García *et al.*, 1995, 2000) have shown that microbial nitrogen content in hard faeces was about half that in soft faeces, which indicates that a significant proportion of microorganisms are not recycled with cecotrophes. Also, ether extract concentration of total odd-numbered (C15:0 and C17:0) fatty acids, which are characteristics of microbial fat (Bauchart *et al.*, 1990) were higher (more than twice) in soft than in hard faeces (Table 2).

Ether extract recycled with cecotrophes might be higher when increasing the amount of energy substrate reaching the fermentative area, as observed by Gómez-Conde *et al*. (2004). Accordingly, CLA recycling could hypothetically be increased by using diets supplemented with highly digestible fibre.

CLA recycled with soft faeces was retained in a similar proportion (around 0.5 g/kg total fatty acids) both in suprarenal and total intramuscular fat. These values were close to those reported recently (Lo Fiego *et al.*, 2005) for cis-9, trans-11 concentration (0.4 g/kg) in *longissimus dorsi* samples from rabbits fed control diets with no CLA added. They were higher than those observed in meat from pigs (Azain, 2003) or eggs from poultry (Alvarez *et al.*, 2004), but lower than those found in meat or milk from ruminants (Chin *et al.*, 1992).

Table 2: Fatty acid profile (g/100 g fatty acids) of the diet (mean), soft and hard faeces, suprarenal and intramuscular fats (mean ± SE).

					Intramuscular fatty acids		*P*-value Contrasts[a]		
Fatty acids	Diet	Hard faeces	Soft faeces	Suprarenal fatty acids	Neutral	Polar	1	2	3
$C_{14:0}$	0.80	1.21 ± 0.06	1.85 ± 0.13	2.60 ± 0.06	2.84 ± 0.09	0.90 ± 0.09	0.001	0.02	0.001
$C_{16:0}$	20.9	25.7 ± 0.18	23.5 ± 0.38	27.8 ± 0.14	28.2 ± 0.51	25.2 ± 0.22	0.001	0.29	0.001
$C_{16:1\ n-7}$	1.40	1.12 ± 0.03	0.86 ± 0.04	5.03 ± 0.31	8.47 ± 0.72	3.10 ± 0.27	0.66	0.02	0.001
$C_{18:0}$	8.20	16.1 ± 1.57	17.9 ± 0.78	6.71 ± 0.20	6.01 ± 0.27	6.32 ± 0.09	0.08	0.60	0.75
$C_{18:1\ n-7}$	2.01	5.01 ± 1.16	4.88 ± 0.58	1.93 ± 0.04	2.02 ± 0.07	2.57 ± 0.10	0.85	0.71	0.43
$C_{18:1\ n-9}$	28.9	24.4 ± 0.38	17.6 ± 0.50	32.9 ± 0.41	33.5 ± 0.67	23.9 ± 0.71	0.001	0.013	0.001
$C_{18:2\ n-6}$	31.9	14.8 ± 1.78	12.5 ± 0.60	18.4 ± 0.59	15.3 ± 0.62	20.8 ± 0.79	0.06	0.29	0.001
$C_{18:3\ n-3}$	3.50	1.61 ± 0.23	1.06 ± 0.09	1.72 ± 0.05	1.40 ± 0.05	0.56 ± 0.04	0.001	0.001	0.001
$C_{20:1\ n-9}$	ND[b]	ND	ND	ND	0.47 ± 0.011	0.26 ± 0.016	-	-	0.001
$C_{20:3\ n-9}$	ND	ND	ND	ND	0.28 ± 0.009	0.31 ± 0.014	-	-	0.06
$C_{20:4\ n-6}$	ND	ND	ND	ND	0.30 ± 0.030	6.39 ± 0.36	-	-	0.001
$C_{22:6\ n-3}$	ND	ND	ND	ND	ND	0.82 ± 0.09	-	-	0.001
Total odd-numbered	ND	1.41 ± 0.04	3.31 ± 0.20	0.98 ± 0.005	0.87 ±0.04	0.69 ± 0.02	0.001	0.47	0.37
cis-9,trans-11 CLA[c]	ND	0.36 ± 0.07	0.64 ± 0.05	0.053 ± 0.003	0.082 ± 0.006	ND	0.001	0.98	-
Total NSFA[d]	30.5	44.4 ± 1.79	46.6 ± 0.98	38.1 ± 0.15	37.9 ± 0.61	33.1 ± 0.28	0.12	0.20	0.001
Total BSFA[e]	ND	3.14 ± 0.02	8.72 ± 0.88	0.39 ± 0.03	ND	ND	0.002	-	-
Total MUFA[f]	34.1	31.4 ± 1.15	24.0 ± 0.92	40.8 ± 0.71	44.4 ± 1.04	29.8 ± 0.85	0.001	0.40	0.001
Total PUFA[g]	35.4	16.6 ± 2.01	14.4 ± 0.67	20.5 ± 0.64	17.3 ± 0.64	28.8 ± 0.82	0.10	0.56	0.001

[a]Contrasts: 1 = hard *vs.* soft faeces; 2 = suprarenal *vs.* weighed average intramuscular fat; 3 = polar *vs.* neutral intramuscular fat . [b]ND = not detected. [c]Conjugated linoleic acid. [d]Normal saturated fatty acids. [e]Branched saturated fatty acids. [f]Monounsaturated fatty acids. [g]Polyunsaturated fatty acids.

Incorporation of CLA in muscle was only detected in the neutral lipid fraction. This may suggest that the amount of CLA cis-9, trans-11 concentration found in the soft faeces is not high enough to be detected in the intramuscular polar lipid fraction. Demaree *et al.* (2002) were unable to find CLA in the muscle polar lipid fraction of pigs fed corn oil or tallow, although feeding the same diets supplemented with a mixture of CLA isomers resulted in higher amounts of CLA in the polar than in the neutral lipid fraction of longissimus muscle.

From the results of this study it can be concluded that soft faeces, and hence meat from rabbits fed control diets with no CLA added, contain detectable amounts of cis-9 trans-11 CLA. Further work is necessary to establish the possibility of improving CLA concentration in rabbit meat through increasing caecal microbial growth.

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