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Effect of dietary condensed tannins on lamb intramuscular fatty acids

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SUMMARY – Carob pulp is a source of condensed tannins (CT), molecules able to form complexes with proteins and to inhibit ruminal microorganisms, responsible for dietary fatty acid bio-hydrogenation. The purpose of the present study was to investigate how condensed tannins from carob pulp affect intramuscular fatty acid composition in lamb *longissimus dorsi* muscle. Eighteen Comisana male lambs, weaned at the age of 60 days, were randomly assigned to one of two treatments: "carob" lambs (nine animals) were fed a diet based on carob pulp (45% as fed basis), while the remaining nine animals (PEG group) received the same diet as the carob lambs supplemented with polyethylene glycol (PEG, 42 g/kg of diet). Lambs were slaughtered at the age of 105 days. Lower amounts of conjugated linoleic acid (CLA), trans vaccenic acid (t11C18:1) ($P<0.0005$) and linolenic acid (C18:3n-3) ($P<0.05$) were found in animals fed the "carob" diet than in lambs fed the same diet supplemented with PEG, while arachidonic acid was found at a higher level in the "carob" group compared to PEG lambs ($P<0.05$). The CLA/t11C18:1 ratio was lower in intramuscular fat of PEG lambs ($P<0.05$) compared to the "carob" group, while no significant differences were found in total saturated-, mono- and poly-unsaturated fatty acids between treatments. Results suggest that the presence of condensed tannins in carob diet reduces ruminal bio-hydrogenation.

Keywords: Carob, fatty acids, lamb, meat, tannins.

RESUME – *"Effet des tannins condensés sur la composition intramusculaire en acides gras d'agneaux". La pulpe de caroube contient des tannins condensés (TC), molécules capables de former des complexes avec les protéines et qu'inhibent les microorganismes du rumen responsables de la bio-hydrogénation des acides gras. L'objectif de cette étude est de vérifier si les tannins condensés de la caroube affectent la composition en acides gras du muscle* longissimus dorsi *d'agneaux. Dix-huit agneaux mâles de race Comisana, sevrés à l'âge de 60 jours, ont étés divisés en deux lots : les agneaux du groupe "caroube" (neuf animaux) ont reçu un régime à base de pulpe de caroube (45% de la ration). Les neuf autres animaux (groupe PEG) ont reçu le même régime que le premier groupe supplémenté avec du polyéthylène glycol (PEG, 42 g/kg d'aliment). Les animaux ont été abattus à l'âge de 105 jours. L'acide linoléique conjugué (CLA, c9,t11C18 :2), l'acide trans-vaccénique (t11C18 :1) ($P < 0,0005$) et l'acide linolénique (C18:3n-3) ($P < 0,05$) ont étés détectés en quantités plus faibles dans le gras intramusculaire des animaux du groupe "caroube" que dans celui des agneaux du groupe PEG. Par contre, l'acide arachidonique était plus présent ($P < 0,05$) dans la viande du groupe caroube que dans celle du groupe PEG. Le rapport CLA/t11C18:1 était plus bas dans le gras intramusculaire des agneaux supplémentés avec le PEG ($P < 0,05$) que dans celui des animaux du groupe caroube. Les acides gras saturés, mono- et poly-insaturés ne différeraient pas dans les deux groupes. Ces résultats suggèrent que la présence de tannins condensés dans le régime caroube a réduit la bio-hydrogénation dans le rumen.*

Mots-clés : *Caroube, acides grasses, agneaux, viande, tannins.*

Introduction

Tannins are phenolic compounds widespread in the plant kingdom. Condensed tannins (CT) are polymers of flavonoid units with high molecular weight, able to bind with and precipitate proteins (Hagerman, 1992), interfering with several aspects of small ruminants nutrition (for a review see Min *et al.*, 2003).

Polyethylene glycol (PEG), a compound with high affinity for CT, is able to prevent the formation of tannin-protein complexes (Jones and Mangan, 1977). PEG can be used to eliminate the deleterious effects of condensed tannins on small ruminant performance (Silanikove *et al.*, 1996; Ben Salem *et al.*, 1997) and meat quality (Priolo *et al.*, 2000).

It has been reported that CT reduce microorganisms cells growth and division (Jones *et al*., 1994; Molan *et al.*, 2001). According to Kepler and Tove (1967) ruminal bacteria, and particularly *Butyrivibrio fibrisolvens*, are responsible for ruminal fatty acid bio-hydrogenation. The bio-hydrogenation pathway involves the isomerization of dietary linoleic acid (c9c12C18:2*n*-6) to a *cis*-9, *trans*-11 octadienoic acid (conjugated linoleic acid, or CLA) and its subsequent hydrogenation to *trans*-vaccenic acid (t11C18:1). The last compound obtained by biohydrogenation is stearic acid (C18:0) a saturated fatty acid (SFA) (Bauman *et al.*, 2000).

Carob (*Ceratonia siliqua* L.) is a legume tree widespread in the Mediterranean area (Rizzo *et al.*, 2004) containing condensed tannins in both pods and leaves (Silanikove *et al.*, 1994). Carob pulp – the pods after seed removal – is used in animal diets. In the present trial, we have chosen to feed lambs a diet based on carob pulp (with or without PEG), to test if high percentages of dietary condensed tannins affect meat fatty acids composition, and particularly CLA through a reduced ruminal bio-hydrogenation.

Materials and methods

Eighteen male Comisana lambs, born in the same farm within a 7 days period, were given free access to commercial starter concentrate and alfalfa hay from 15 d of age. At age 51 days, the animals were weaned and, after 9 days of adaptation to the experimental conditions, randomly assigned to one of two treatments (tannin, or PEG). The tannin diet contained carob pulp and barley (45% and 11% respectively, on an as fed basis). The PEG lambs received the same diet as the tannin lambs with 42 g/kg of diet of PG (molecular weight 4,000) mixed in the diet. At the age of 105 days the animals were slaughtered.

Feeds were analyzed for neutral detergent fiber (NDF) (van Soest *et al.*, 1991) and crude protein (CP) (AOAC, 1995) (AOAC method 984.13). CT were measured according to Porter *et al.* (1986) and total phenols (TP) were determined by Folin-Ciocalteu reagent using tannic acid as a standard (Makkar *et al.*, 1993; Table 1). Fatty acids content in the feed was determined according to Sukhija and Palmquist (1988). Fatty acids from muscle *longissimus dorsi* were extracted according to Folch *et al.* (1957) and were quantified as fatty acid methyl esters (French *et al.*, 2000). The gas-chromatograph used was a Thermo Finnigan, TRACE (Thermo Finnigan, San Jose, CA, USA), interfaced to a flame ionization detector (FID). Data were analyzed as a completely randomized design. Each animal was considered as an experimental unit. Data were analyzed by ANOVA using treatment as main effect and including experimental error.

Table 1. Chemical composition of the carob diet† (% of dry matter)

Dry matter	91.70
Crude protein	18.72
Ether extract	1.25
Neutral detergent fiber	37.49
Total phenols††	11.20
Condensed tannins†††	2.70

†PEG treatment incorporated 42 g/kg of diet of polyethylene glycol to tannin diet.
††Expressed as gram tannic acid equivalent per 100 g DM.
†††Expressed as gram leucocyanidin equivalent per 100 g DM.

Results and discussion

Diet

The carob diet contained 2.7% CT (equivalent leucocyanidin, on dry matter basis) (Table 1). This result is in agreement with previous data on the CT content of tannin-rich feedstuffs (carob pulp:

Priolo *et al.*, 2000; fresh sulla: Priolo *et al.*, 2005). The amounts of C18:3*n*-3 and c9c12C18:2*n*-6 in diet were similar (31.49 and 35.02 g/100 g of fatty acids respectively). The total concentration of unsaturated fatty acids was 80% of total fatty acids.

Intramuscular fatty acid composition

The intramuscular fat of the tannin-fed lambs contained lower amounts of *trans*-vaccenic acid (t11C18:1), CLA (c9t11C18:2) ($P < 0.000$) and of linolenic acid (C18:3*n*-3) ($P < 0.05$) compared to the PEG lambs (Table 2). The CLA/t11C18:1 ratio was higher for the animals fed the tannin diet ($P < 0.05$). Meat from lambs fed the tannin diet contained more arachidonic acid (C20:4*n*-6; $P < 0.05$) than PEG group. The total amount of saturated-, mono- and polyunsaturated fatty acids was not affected by the presence of PEG in the carob diet. No significant difference was found for *n*-6/*n*-3 ratio between treatments.

Table 2. Effect of the diets on intramuscular fatty acid composition (g/100 g identified fatty acid methyl esters)

	Treatment		SEM	*P*-Value
	Tannin	PEG		
No. of lambs	9	9	–	–
t11C18:1	0.95	1.68	0.114	0.001
c9t11C18:2 (CLA)	0.48	0.63	0.024	0.001
C18:3*n*-3	4.78	5.51	0.167	0.025
C20:4*n*-6	9.55	7.93	0.385	0.031
C22:6*n*-3	2.07	3.24	0.205	0.001
CLA/t11C18:1 ratio	0.55	0.39	0.036	0.023
Other fatty acids	81.62	80.62	–	–

While planning this trial, we formulated the hypothesis that condensed tannins from carob pulp could reduce ruminal bio-hydrogenation resulting in a lower content of those fatty acids produced by microorganisms in the rumen. The higher amount of t11C18:1 and CLA in the fat of lambs fed the PEG diet compared to animals fed the same diet without PEG supplementation seems to confirm this hypothesis. The most likely explanation of this result is that the presence of CT in carob pulp reduced ruminal bio-hydrogenation. In particular, *trans*-vaccenic acid resulted more affected than CLA by the presence of PEG in the diet. This was expected because all the *trans*-vaccenic acid originates in the rumen during biohydrogenation, while CLA is partially synthesized in tissues by the action of Δ^9-desaturase on *trans*-vaccenic acid (Bauman *et al.*, 2000). A further confirmation of our hypothesis is given by the higher CLA/t11C18:1 ratio for lambs fed the tannin diet, compared to control and PEG animals. Endogenous biosynthesis of CLA from *trans*-vaccenic acid was probably more important than its ruminal production, confirming results found in beef studies (Griinari *et al.*, 2000; Raes *et al.*, 2004).

The higher amount of C20:4*n*-6 in intramuscular fat from lambs fed the tannin diet, compared to PEG animals, could result from increased endogenous biosynthesis of this fatty acid in muscle from linoleic acid (Zhou and Nilsson, 2001). The inhibition of ruminal microorganisms by the action of CT in tannin diet could have produced higher escape of c9c12C18:2*n*-6 from rumen to tissues, which is the precursor of arachidonic acid in muscle.

Conclusions

The present trial has shown that the intramuscular fat of lambs fed a diet containing condensed tannins from carob pulp has a lower concentration of CLA and *trans*-vaccenic acid when compared to lambs fed the same diet supplemented with PEG. In this experiment CLA resulted to be less affected

by dietary CT than *trans*-vaccenic acid. This can be explained by the fact that CLA is also synthesized endogenously in muscles. It has also been shown that arachidonic acid, which is originated by linoleic acid, was present at lower amounts in lambs fed the carob diet supplemented with polyethylene glycol, indicating a partial inhibition of ruminal bio-hydrogenation of linoleic acid when condensed tannins are not deactivated by PEG. These findings suggest that dietary condensed tannins from carob pulp affect microorganisms activity in rumen, resulting in a reduced ruminal bio-hydrogenation.

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