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Original article

Reducing the impact of condensed tannins in a diet based on carob pulp using two levels of polyethylene glycol: lamb growth, digestion and meat quality

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Abstract — Fourteen male Comisana lambs were divided into two groups of seven at age 45 days and were fed a diet containing 56% of dried carob pulp (as fed basis). One group (P10) received a supplement of 10 g of polyethylene glycol (PEG) for each kg of diet, while the second group (P40) received a supplement of 40 g of PEG for each kg of diet. Both diets were ground and supplied ad libitum. The experimental diet contained 2.1% of condensed tannins (CT) on a dry matter basis. The lambs fed the P40 diet had higher average daily gain than the P10 lambs ($P < 0.05$) and obtained higher live weights at slaughter ($P = 0.08$) although the dry matter intake was not different between the treatments. The increased level of PEG (P40 vs. P10 lambs) increased dry matter digestibility ($P = 0.07$) and nitrogen (N) and neutral detergent fibre (NDF) digestibility ($P < 0.01$). The carcasses from the P40 lambs were heavier and fattier than those from the P10 lambs ($P < 0.05$). Meat (*l. dorsi* muscle) from the P10 lambs showed higher ($P < 0.05$) values of lightness (L*) compared to the P40 lambs. No differences in eating quality were registered among the groups. These results indicate that in a diet based on carob pulp (56%), 10 g of PEG per kg of diet are not sufficient to eliminate the effects of CT.

carob pulp / meat quality / polyethylene glycol / sheep / tannins

Résumé — Réduction de l'impact des tannins condensés dans un régime à base de pulpe de caroube en utilisant deux niveaux de polyéthylène glycol : croissance des agneaux, digestion et qualité de la viande. Quatorze agneaux mâles de race Comisana ont été séparés à l'âge de 45 jours en deux groupes de sept individus, et ont reçu un régime contenant 56 % de pulpe de caroube. Un groupe (P10) a reçu un supplément de 10 g de polyéthylène glycol (PEG) par kg de ce régime, tandis que le

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second groupe (P40) en a reçu 40 g·kg^{-1}. Dans les deux cas, le régime expérimental était broyé et distribué ad libitum aux animaux. Il contenait 2,1 % (de la matière sèche) de tannins condensés (CT). Les agneaux P40 ont eu un gain moyen quotidien plus important que les agneaux P10 ($P < 0,05$) et un poids vif à l'abattage également supérieur ($P = 0,08$), malgré une ingestion en matière sèche similaire entre traitements. Avec l'augmentation du niveau de PEG (agneaux P40 vs. P10), la digestibilité de la matière sèche a légèrement augmenté ($P = 0,07$), tandis que celles de l'azote et du NDF se sont accrues de façon plus marquée ($P < 0,01$). Les carcasses des agneaux P40 étaient plus lourdes et plus grasses que celles des agneaux P10 ($P < 0,05$). La viande de ces derniers (muscle *l. dorsi*) montrait des valeurs de luminosité supérieures à celle des agneaux P40 ($P < 0,05$). En revanche, leur qualité sensorielle ne semblait pas différente. Ces résultats indiquent qu'avec un régime à base de pulpe de caroube (56 %), un supplément de 10 g PEG · kg^{-1} n'est pas suffisant pour éliminer les effets des tannins condensés.

pulpe de caroube / qualité de la viande / polyéthylène glycol / mouton / tannins

1. INTRODUCTION

One of the main problems of the use of carob pulp for ruminant feeding is the presence of condensed tannins (CT) [14] that are phenolic compounds present in several legume forages and tree leaves and pods [3, 18]. Although several nutritional advantages were described when CT from some plant species were present up to 4% of the dry matter in sheep diets [2, 17], in a preceding paper [11] we found that 2.5% of CT in a diet containing carob pulp had deleterious effects on lamb growth and meat quality. This happened because CT link with and precipitate proteins in the rumen [7], reducing protein availability for ruminal micro-organisms. CT may also inhibit absorption from the intestine [18].

Polyethylene glycol (PEG) reacts preferentially with CT and prevents the formation of tannin-protein complexes [7] and therefore it has been used to eliminate CT effects for both experimental or production practices [15, 18]. In the preceding trial [11], PEG was used to eliminate the impact of CT from carob pulp. The quantity of PEG given corresponded to 1.8 times the CT content of the diet. Considering the high cost of PEG, the objective of this trial was to study the possibility of reducing the PEG given to sheep fed a diet similar to that used by Priolo et al. [11] and to evaluate the effects on lamb growth, digestion and carcass and meat quality.

2. MATERIALS AND METHODS

2.1. Animals and diets

Fourteen male Comisana lambs born on the same farm were weaned and randomly divided on the basis of live weight into two groups of seven at age 45 days and allotted into individual pens. The animals were adapted to the experimental diets during a 5-d period. From age 50 days (experimental day 1), both groups of lambs received the same diet containing 56% of dried carob pulp, 26.1% of alfalfa hay, 15% of soybean meal, 1% of urea, and a mineral/vitamin mixture (Tab. I). One group (P10) received 10 g of PEG (molecular weight 4000) added for each kg of the diet, in an as fed basis, while the second group (P40) received 40 g of PEG for each kg of the diet. The diets were ground and passed through a 5-mm screen. Lambs had ad libitum access to the diets (fresh feed was supplied every day at 09.00) and to water. Refusal was removed and weighed daily to calculate voluntary feed intake.

Table I. Ingredients and chemical composition of the diets[1].

Ingredient (% as fed)	
Alfalfa hay	26.1
Soybean meal	15.0
Carob pulp	56.0
Urea	1.0
NaCl	0.5
Vitamin/mineral premix	0.3
$CaCO_3$	1.1
Chemical Composition (% of DM)	
DM	88.9
CP	17.5
NDF	35.3
ADF	23.6
Hemicellulose	11.7
Cellulose	15.7
ADL	7.9
Acid insoluble ash	1.0
Protein-bound CT	2.0
Fibre-bound CT	0.1
Total CT	2.1

[1]The P10 group received the diet with 10 g of polyethylene glycol added for each kg of the diet; the P40 group received the diet with 40 g of polyethylene glycol added for each kg of the diet.
DM: dry matter, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, CT: condensed tannins.

2.2. Animal measurements

All lambs were weighed at 09.00 once a week, before removing refusals and supplying fresh feed. Harnesses for faecal collection were placed on the lambs on experimental day 28. The lambs were adapted to the harnesses for 2 days; from experimental day 30 to day 37 of the experimental period, the faeces were collected into bags and removed once daily, weighed and a subsample of 10% was composited per animal to determine the digestibility of dry matter (DM), nitrogen (N) and neutral detergent fibre (NDF). Calculation of dry matter digestibility was done after subtracting the daily intake of PEG from faecal DM [11].

2.3. Slaughter procedures and carcass classification

The animals were slaughtered at age 100 d. Slaughtering took place after electrical stunning, by exsanguination. The digestive tract content was removed and weighed to calculate the empty live weight. Immediately after slaughter the carcasses where weighed and classified for fatness using a 15-point scale [5]. Six hours after slaughter the carcasses were placed in a refrigerated room set to 4 °C.

2.4. Feed and faeces analyses

Offered and refused feed and faeces were analysed for fibre fractions [6] and for crude protein [1]. Condensed tannins were determined by a butanol/HCl method as described by Terrill et al. [16] to indicate total, free and bound CT. The standard was obtained from *Lotus pedunculatus*, extracted and purified by passing through a Sephadex LH-20 column.

2.5. Meat instrumental quality

The right *longissimus thoracis et lumborum* muscle was excised 24 h post mortem and the ultimate pH was determined by an Orion 210A pH-meter equipped with an Orion 9106 penetrating glass probe. Three-centimetre thick slices were then placed on polystyrene trays and were wrapped with a polyethylene film. The samples were allowed to bloom for 2 h and then colour (L*, a*, b*, Chroma and Hue angle) was measured by a Minolta CR300 colorimeter (Illuminant D_{65}). To calculate cooking loss, weighed muscle samples were held in plastic bags and immersed in water at 75 °C until the internal temperature of the meat reached 75 °C, as monitored by a probe. The bags were then placed under running cold water for 30 min. The meat was then patted dry with paper towels and reweighed. Shear force was calculated on meat samples cooked as above. Three strips (1 × 1 cm) were removed from each sample parallel to the muscle fibres and sheared perpendicularly to the direction of the fibres using an Instron 4411 apparatus equipped with a Warner-Bratzler shearing device. The speed was 100 $mm \cdot min^{-1}$. The right hind leg was also excised and dissected in order to determine the percentage of lean, bone and fat. For chemical analysis, raw meat was minced in a blender and AOAC [1] methods were used to measure moisture (39.1.02), fat (39.1.05) and ash (39.1.09). Protein was estimated by the difference.

2.6. Meat sensory evaluation

The sensory evaluation was conducted as described by Lanza et al. [9] and the same 10 panellists were involved in this trial as in [9]. Briefly, longissimus lumborum samples were cooked in a boiling water bath until the internal temperature reached 75 °C. The samples were served warm to the panellists that scored using a 9-point intensity scale for flavour, tenderness, juiciness and overall acceptability.

2.7. Statistical analysis

A first analysis of all data included the covariance for the body weight at 50 days. For the variables in which the covariance was not significant ($P > 0.05$), this was removed from the model and a t test was used to compare the two treatments. For the sensory evaluation, we used a two way ANOVA with diet and panellist as factors.

3. RESULTS

The experimental diet contained 17.5% crude protein, 35.3% NDF and 2.1% CT (Tab. I).

The lambs fed the P40 diet performed better than those fed the P10 diet with an average daily gain of 172 vs. 116 $g \cdot d^{-1}$ ($P < 0.05$) and obtained higher live weights at slaughter ($P = 0.08$) although the dry matter intake was not different between the treatments (Tab. II). The increased level of PEG from 10 to 40 $g \cdot kg^{-1}$, allowed a higher dry matter digestibility ($P = 0.07$) and a N and NDF digestibility decidedly higher

Table II. Lamb growth performance and digestibility.

Item	P10	P40	SEM	*P*-value
No. of lambs	7	7	-	-
BW at 50 d (kg)	13.1	13.1	0.49	0.989
BW at slaughter[1] (kg)	19.4	21.7	0.10	0.079
ADG (50–100 d) (g)	116	172	14.70	0.049
DMI[1] ($g \cdot d^{-1}$)	774	863	61.20	0.319
DM digestibility (%)	62.6	66.3	1.04	0.073
N digestibility (%)	63.7	71.2	1.48	0.004
NDF digestibility[1] (%)	31.6	40.8	2.04	0.009
DMI/live weight gain ($kg \cdot kg^{-1}$)	7.5	5.1	0.66	0.088

[1]For these items, analysis of covariance for BW at 50 days was significant ($P < 0.05$) and was included in the model. Means are intended as adjusted means.
P10 and P40: see Table I.
BW: body weight, ADG: average daily gain, DMI: dry matter intake, N: nitrogen, NDF: neutral detergent fibre, SEM: standard error of the mean.

($P < 0.01$) in the P40 lambs compared to the P10 group. The P10 lambs required an average dry matter intake of 7.5 kg to attain 1 kg of body weight gain, while the P40 lambs consumed 5.1 kg of dry matter to attain the same gain ($P = 0.09$).

Data at slaughter are presented in Table III. Carcasses from the P40 lambs were heavier than those from the P10 lambs ($P < 0.05$). The carcass yield was not significantly different between the groups. Carcasses from the P40 lambs showed a higher fatness score as judged by the visual classification ($P < 0.05$). However dissection of the hind leg did not indicate differences for the percentage of lean, fat and bone between the two treatments.

Meat ultimate pH was not different between the treatments. Meat colour was affected by the level of PEG in the diet since the P10 lambs showed higher ($P < 0.05$) values of lightness (L*) compared to the P40 lambs. Meat from the P10 lambs tended to have higher moisture percentages ($P = 0.06$) and lower crude fat contents ($P = 0.11$) (Tab. III).

Sensory evaluation (Tab. IV) did not show any significant difference of flavour, tenderness and juiciness of the samples from the two treatments; the meat from the P10 lambs was considered as acceptable as the meat from the P40 lambs.

4. DISCUSSION

Considering a feed dry matter of 88.9% containing 2.1% of CT, with the P10 diet, PEG supplied 0.54 times the CT content, while in the P40 diet it was 2.14 times the CT content. In the preceding trial [11], to eliminate the effect of CT in a diet containing the same ingredients, we supplied a quantity of PEG corresponding to 1.79 times the amount of CT as measured by the same method of Terrill et al. [16] applied in this trial. Silanikove et al. [14] and Ben Salem et al. [4] found that sheep given respectively carob leaves or *Acacia cyanophylla* leaves, improved their performance favourably when about 25 g PEG were supplied daily.

Table III. Lamb carcass and meat instrumental quality.

Item	P10	P40	SEM	*P*-value
No. of lambs	7	7	-	-
Carcass weight[1] (kg)	7.98	9.20	0.52	0.049
Carcass yield[1] (% empty live weight)	47.91	49.38	0.70	0.212
Carcass fatness[1] (score)	6.42	8.29	0.50	0.023
Hind leg lean (%)	57.46	59.89	0.95	0.212
Hind leg fat (%)	10.63	12.19	0.69	0.273
Hind leg bone (%)	31.93	27.91	1.42	0.165
Meat instrumental quality				
Ultimate pH	5.96	5.79	0.06	0.136
Lightness[1] (L*)	49.16	46.14	0.90	0.037
Redness (a*)	15.90	14.51	0.50	0.176
Yellowness (b*)	7.69	6.40	0.50	0.206
Chroma	17.72	15.89	0.63	0.152
Hue angle	25.48	23.69	1.15	0.460
Cooking loss (%)	18.50	18.81	0.77	0.846
Warner-Bratzler shear force (kgF·cm^{-2})	3.32	4.29	0.31	0.116
Meat chemical composition				
Moisture[1] (%)	78.06	76.63	0.45	0.062
Crude fat[1] (% wet weight)	0.94	1.18	0.09	0.108
Ash (% wet weight)	1.12	1.12	0.01	1.000
Protein (% wet weight)	19.88	21.07	0.39	0.395

[1]For these items analysis of covariance for BW at 50 days was significant ($P < 0.05$) and was included in the model. Means are intended as adjusted means.
P10 and P40: see Table I, SEM: standard error of the mean.

The Comisana is a dairy sheep breed and the growth rate of the lambs fed the P40 diet was similar to that attained by the lambs of this breed [8] fed a pelleted diet based on maize, barley and soybean meal and wheat straw. The P10 group, however, showed a worse performance, indicating a negative effect of carob CT. Evidently a PEG/CT ratio of 0.54 was not sufficient to eliminate all the negative effects of the carob tannins. However, 116 g·d^{-1} of daily gain obtained by the P10 lambs in this trial, was decidedly better than the 48 g·d^{-1} obtained in the preceding trial [11] by lambs fed a diet with the same ingredients at the same proportions but with no PEG supply.

One of the typical negative effects of dietary CT is the reduction of voluntary feed intake [12]. Increasing of voluntary feed intake when PEG is supplied in diets containing CT from carob leaves has been reported

Table IV. Sensory analysis results (1- to 9-point scale).

Item	P10	P40	SEM	*P*-value
No. of lambs	7	7	-	-
Flavour	5.97	6.27	0.17	0.243
Tenderness	6.90	6.64	0.15	0.346
Juiciness	6.36	6.64	0.17	0.320
Overall acceptability	6.26	6.30	0.16	0.878

P10 and P40: see Table I, SEM: standard error of the mean.

in both goats and sheep [13, 14]. In the preceding trial [11], the supply of PEG increased intake by 60% compared to lambs not receiving the PEG supplementation. In the current trial the lower percentage of PEG enabled the P10 lambs to have similar intakes as the P40 lambs.

CT from carob pods have been reported to depress the intestinal activity of digestive enzymes [15]. The higher N digestibility ($P < 0.01$) of the P40 lambs demonstrates the effect of CT on digestion, compared to lambs supplemented with a lower level of PEG (P10). In this trial the improvement of N digestibility between P10 and P40 lambs was the 11.8%. Addition of 40 g PEG per kg feed in our previous trial involving similar diets [11] increased N digestibility by 29.4% as compared with lambs given the same diet without PEG. In this trial, as well as in previous work with carob pulp for sheep [11], the CT depressed NDF digestibility to very low values. The low level of PEG supplementation resulted in a similar low NDF digestibility as previously fed unsupplemented diets [11]. There were differences between the trials in NDF digestibility with 40 g PEG per kg feed: 40.8% and 61.6% respectively in this and the previous trial [11].

The carcasses from the P40 lambs were heavier than those from the P10 animals reflecting the differences in growth rate and body weight at slaughter. Reduced carcass fatness in the P10 lambs reflected the results obtained in animals fed a CT-containing diet without PEG as reported in the previous trial [11]. However in this experiment, the small amount of PEG supplied (P10) resulted in a similar tissue composition of the hind legs for both levels of PEG supplementation. In contrast, lambs not receiving PEG in the previous trial [11] had higher bone and lower fat percentages compared to the animals receiving 40 g of PEG per kg of the diet.

Reduced meat lightness has been reported when the effects of CT, from carob pulp or *Acacia cyanophylla* leaves, have been reduced by the addition of PEG. The removal of the CT effects seem to be correlated with an increased utilisation of the absorbed iron for synthesis of myoglobin [10, 11].

In the preceding trial [11], the animals not receiving PEG supplementation gave a meat with a higher ultimate pH and a reduced flavour and overall acceptability as judged by the panellists. In the present trial the even low supplementation of PEG was sufficient to obtain samples with similar values of pH and comparable meat sensory attributes.

5. CONCLUSIONS

This trial was designed to verify the possibility that reducing PEG supplementation in a diet containing CT fed to lambs would achieve adequate lamb growth, and carcass and meat quality. In a preceding trial [11], we found a diet based on carob pulp (56% as fed) and supplemented with 40 g of PEG per kg of feed resulted in significant improvements of lamb growth and carcass and meat quality, compared to the animals fed the same diet without the addition of PEG. In this trial we demonstrated that a reduction in PEG supplementation from 40 to 10 $g \cdot kg^{-1}$ of feed, enabled good growth and digestion. Performance was lower than that of lambs supplemented with 40 g PEG per kg feed, but meat quality was similar for both treatments.

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