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Supplementation of *Acacia cyanophylla* Lindl. foliage-based diets with feed blocks and PEG 4000 and its effects on *in vitro* fermentation and performance in sheep

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SUMMARY – In trial I, the effect of urea-molasses blocks, without (B1) or with polyethylene glycol (PEG) 4000 (B2), on *in vitro* fermentation (batch system over 6 hours of incubation) of *Acacia cyanophylla* (4.1 g/100 g DM of condensed tannins) based diet was studied. Diets were composed of mixtures of hay and acacia (D1: 39 and 61 % respectively) and B1 (D2) or B2 (D3) (26, 55 and 19% respectively). Gas and volatile fatty acid (VFA) productions, fermented organic matter (FOM), nitrogen degradability (ND) and ammonia nitrogen uptake (ANU) were determined. In trial II, two groups of 4 month-old Noire de Thibar lambs were used to compare D2 and D3 for growth over 84 days. Adding blocks in D2, resulted in increased gas production ($P<0.01$) and an increased, though not significant, VFA production and FOM when compared to D1. PEG in D3 increased gas ($P<0.01$) and total VFA ($P<0.05$) productions and FOM ($P<0.05$) when compared to D1. Feed blocks in D2 resulted in increased ND and ANU comparatively with D1 ($P<0.01$). PEG (D3) increased ND and ANU when compared to both D1 and D2 ($P<0.01$). Consuming PEG (D3) resulted in greater intake of acacia ($P<0.05$) and higher growth of lambs ($P<0.01$) when compared to D2.

Keywords: *Acacia cyanophylla*, feed blocks, *in vitro*, fermentation, performance, sheep.

RESUME – *"Effets des blocs multinutritionnels et du PEG 4000 sur les fermentations* in vitro *et les performances d'ovins nourris à base d'*Acacia cyanophylla *Lindl". Dans un premier essai, nous avons étudié l'effet des blocs mélasse-urée, sans (B1) ou avec polyéthylène gycol (PEG) 4000 (B2) sur les fermentations in vitro (système batch pendant 6 h d'incubation) pour un régime à base de feuilles d'*Acacia cyanophylla*. Les régimes sont composés de mélange de foin et d'acacia (R1 : 39 et 61% respectivement) et de B1 (R2) ou B2 (R3) (26, 55 et 19% respectivement). La production de gaz et d'acides gras volatiles (AGV), la quantité de matière organique fermentée (MOF) ainsi que la dégradabilité de l'azote (DN) et l'azote ammoniacal fixé par les micro-organismes (NAF) ont été déterminés. Dans un second essai, nous avons comparé les deux régimes complémentés (R2 et R3) pour la croissance sur deux lots d'agneaux de la race Noire de Thibart âgés de 4 mois pendant 84 jours. L'apport des blocs dans R2 a entraîné une augmentation de la production de gaz ($P<0.01$) et une tendance non significative d'augmentation de la production d'acides gras volatils (AGV) et de la MOF par rapport à D1. L'introduction du PEG (D3) a augmenté la production de gaz ($P<0.05$) et d'AGV ($P<0.05$) et la MOF ($P<0.05$) en comparaison avec D1. Les blocs introduits dans D2 ont amélioré la DN ainsi que la quantité de NAF ($P<0.01$) par rapport à R1. Le PEG (R3) a engendré une augmentation spécifique de la DN et de la NAF en comparaison avec R1 et R2. La consommation du PEG par les jeunes ovins a permis une amélioration de l'ingestion de l'acacia ainsi qu'une meilleure croissance ($P<0,05$) comparé à R2.*

Mots-clés : Acacia cyanophylla*, blocs*, in vitro, *fermentation, performances, ovins.*

Introduction

Acacia cyanophylla consumption causes decrease in feed intake and digestion (Reed *et al*., 1990; Ben Salem *et al*., 1997) and depresses rumen fermentation of the diet (Moujahed *et al*., 2000), thus affects animal growth (Reed *et al.,* 1990). These effects are attributed to condensed tannins. The use of tannin-complexing agents, such as polyethylene glycol (PEG) 4000 appears to deactivate tannins in acacia leaves and to improve their nutritive value (Ben Salem *et al*., 1997). Indeed, PEG forms stronger complexes with tannins than tannin-protein complexes and releases protein from the complexes (Jones and Mangan, 1977). In a previous study (Moujahed *et al*., 2000), we found that supplementing acacia-based diets with feed blocks (urea, molasses and minerals) improved the feeding value of acacia-based diets by increasing intake and enhancing rumen fermentation. In the same study, including PEG in blocks seemed to reduce the inhibitory effects of acacia tannins in the rumen and probably in the gut. The present work aimed to investigate the effects of urea-molasses

blocks and PEG included in blocks on *in vitro* carbohydrate fermentation and nitrogen metabolism and to evaluate the effect of PEG on lambs growth for *A. cyanophylla* Lindl. foliage-based diet.

Material and methods

Plant material and feed blocks

Acacia cyanophylla Lindl. (acacia) leaves (Central Tunisia, semi-arid) were air-dried in the shade (about 85% of DM) and then homogenized and stored. Oat-vetch hay was produced at the INAT-Farm. The two types of blocks (B1 and B2) used in this study were made as described by Moujahed *et al*. (2000). Their composition differs mainly in PEG component in B2 (Table 1).

Table 1. Ingredient composition of blocks (% on a DM basis)

Ingredients	Block 1	Block 2
Urea	11.4	11.07
Molasses	9.54	9.26
PEG-4000	-	11.24
Dicalcium phosphate	5.7	5.53
Salt	5.76	5.6
Mineral and vitamin supplement	5.67	5.51
Cement	11.57	11.24
Olive cake	13.87	10.1
Wheat bran	36.45	30.34

Animals, diets, experimental designs and measurements

In trial I, three previously studied diets composed of mixtures of hay and acacia (D1: 39 and 61% respectively) and B1 or B2 (D2 and D3: 26, 55 and 19% respectively) were incubated *in vitro* using the closed anaerobic system described by Jouany and Thivend (1986). The fermenter is a 1 litre flask shaken in water-bath at 39°C during 6 h. Inocula were taken from four Noire de Thibar sheep fed hay and concentrate in two equal meals (8 and 16 h). The fermenter medium was composed of 200 ml of artificial saliva saturated with CO_2, 100 g of solid phase rumen content and 100 ml of rumen liquid. Eight fermenters were used simultaneously in the same water-bath. Each diet was studied as follows: (i) two fermenters with inocula + $(NH_4)_2SO_4$ (187 mg N) + 13 g of starch (control); and (ii) two fermeters with inocula + $(NH_4)_2SO_4$ (187 mg N) + x g of DM of substrate providing 125 mg of N + y g of starch (x + y = 13 g).

From the incubations, a sample was constituted with all products except diets and starch (T0). In the fermenters, the liquid was sampled (9 ml) after 1 and 6 hours of incubation. Samples were conserved for NH_3-N (Conway, 1962) and volatile fatty acids (VFA) analysis (Jouany, 1982). Gas volume was read each hour in the test tubes related to fermenters. The three diets were incubated simultaneously in 2 replications over 4 successive periods (Table 2).

Table 2. Experimental design of the *in vitro* trial

	Control	D1	D2	D3
Period 1	F1 and F2	F3 and F4	F5 and F6	F7 and F8
Period 2	F1 and F2	F3 and F4	F5 and F6	F7 and F8
Period 3	F1 and F2	F3 and F4	F5 and F6	F7 and F8
Period 4	F1 and F2	F3 and F4	F5 and F6	F7 and F8

D1: hay + acacia; D2: hay + acacia + B1; D3: hay + acacia + B2 (PEG); F: fermenter.

Fermented organic matter (FOM: g/fermenter) was calculated by the stoechiometric equation of Demeyer (1991) on the basis of the difference between registered VFA quantities at T0 and the quantity measured after 6 h of fermentation: FOM = 162 (0.5 acetate + 0.5 propionate + butyrate), in which 162 represent the molar weight of a hexose unit coming from polymeric carbohydrate.

Nitrogen degradability was estimated on the basis of the amount of protein N introduced into the experimental fermenter and the NH_3-N liberated by the added substrate. Microbial ammonia N uptake (ANU) was estimated by the difference between NH_3-N concentration at 1 and 6 h of incubation. Efficiency of microbial synthesis (EMS) was calculated as the ratio between ANU (mg) and FOM (g).

In trial II, twenty Noire de Thibar lambs (10 males and 10 females) aged 4 months (initial mean live weight: 19.3 kg) were used in a 84-day growth trial to test D2 and D3 (PEG). They were divided into two homogeneous groups (5 males and 5 females in each one) and housed in individual pens. Hay and acacia were offered on the cited proportions with 15% of refusal for acacia. During the 84-day period of the trial, offered and refused forages and blocks were daily weighed for feeds intake determination. Animals were weighed at the beginning and at the end of the trial for estimating daily weight gain.

For both experiments 1 and 2, the General Linear Model procedure (GLM) of SAS (1985) was used to analyse data. Duncan multiple range test was used to compare treatment means.

Laboratory analysis

Feeds and refusals were analysed for ash (550°C, 8 h) and crude protein (CP) contents (AOAC, 1984). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed in feeds as described by Goering and Van Soest (1970). The content of acacia condensed tannins (CT) was analysed (vanillin-HCl procedure: Broadhurst and Jones, 1978). Chemical composition of feeds is presented in Table 3.

Table 3. Chemical composition of feeds and *in vitro* incubated diets

	% DM					
	Ash	CP	NDF	ADF	ADL	CT
Acacia leaves	11	12.1	42.9	31.3	16.9	4.2
Oat-vetch hay	6.3	5.7	59.5	33.4	9.4	
Block 1	29.3	38.7	25.7	11.8	5.3	
Block 2 (PEG)	29.5	37.1	20.1	9.1	3.8	

Results and discussion

Effects of blocks and PEG on carbohydrate fermentation

The effects of blocks and PEG on carbohydrate fermentation are presented in Table 4. Adding urea-molasses blocks in D2 resulted in increased ($P<0.01$) gas production (+132 ml) and increased, though not significant, total VFA (+5 mmol) and FOM (+0.5 g) productions, compared with D1. This trend may indicate a tendency of enhanced microbial degradation due to more balanced supply of energy, nitrogen and minerals from B1. The slight, but significant increase of butyrate production in D2 may be due to molasses (∅rskov and Ryle, 1990). PEG in D3 increased gas (+204 ml, $P<0.01$) and total VFA (+10 mmol, $P<0.05$) productions and FOM (+0.9 g, $P<0.05$) when compared to D1. These results indicate that PEG (D3) had improved OM fermentation, which was constrained by the inhibitory effect of CT in D1. The negative effect of tannin was proved *in vitro* by Mathieu and Jouany (1993) who found that adding tannin from chestnut to soybean meal (5.3% DM) reduced significantly gas and VFA production. Additionally, Rosales *et al.* (1997) found negative interactions in some incubated associations of shrubs. This finding concerned mainly associations including tannin-

containing shrubs. Additionally, Osuji and Odenyo (1997) and El Hassan (1994) showed that *in vitro* gas and VFA production is reduced in presence of *Acacia angustissima*. The inhibitory effect of tannins on microbial fermentation is exerted mainly through enzyme inhibition, substrate deprivation and action on membrane (Scalbert, 1991). The positive effect of PEG on gas and VFA production in tannin-containing species may be explained mainly by increased OM fermentation. It has been observed in several studies dealing with tannin-rich species (Salawu *et al*., 1997; Hervás *et al*., 2001). These authors suggested that this effect is mainly related to a probable deactivation of tannin adverse effects on micro-organisms and enzymes.

Table 4. *In vitro* gas and VFA production, FOM, ANU and EMS in fermenter

	Diets			SEM
	D1	D2	D3	
Gas production (6 h, ml)**	1437.5^{c}	1568.7^{b}	1641.2^{a}	18.05
VFA production				
*Acetate (mmol)**	21.17^{b}	23.67^{ab}	27.48^{a}	1.3
*Propionate (mmol)**	9.87^{b}	11.38^{ab}	12.49^{a}	0.38
*Butyrate (mmol)***	2.93^{b}	3.81^{a}	4.15^{a}	0.2
*Total production (mmol)**	34.21^{b}	39.14^{ab}	44.45^{a}	1.93
Fermented organic matter (g)*	3.04^{b}	3.51^{ab}	3.96^{a}	0.17
Nitrogen degradability (%)**	15.4^{c}	29.93^{b}	38.15^{a}	0.87
Ammonia nitrogen uptake (mg)**	71.2^{c}	78.76^{b}	83.08^{a}	0.82
Efficiency of microbial synthesis (mg/g)	25.01	22.63	21.06	1.75

D1: hay + acacia; D2: hay + acacia + B1; D3: hay + acacia + B2 (PEG).
$^{a, b, c}$ For the same line values with the same letter do not differ significantly, * P<0.05, **P<0.01.

Nitrogen metabolism

The effects of blocks and PEG on N metabolism are presented in Table 4. Including B1 in D2 increased (P<0.01) ND (+14 percentage points) comparatively to D1. This result could be explained both by the supplied urea-N in blocks and the lower amount of acacia, thus tannin, comparatively with D1. PEG in D3 resulted in an increased (P<0.01) ND comparatively with D1 (+23 percentage points) and D2 (+10 percentage points). This finding may be related to the decrease of tannin inhibitory effects on protein degradation and micro-organisms due to PEG. The negative effect of tannin on *in vitro* degradability of nitrogen was clearly demonstrated by Mathieu and Jouany (1993) in the case of tannin of chestnut. Several studies showed that adding PEG to acacia-based diets resulted in increased *in vivo* crude protein digestibility (Ben Salem *et al*., 2000, 2002; Moujahed *et al*., 2000). Ammonia-N uptake was improved (P<0.01) by the presence of B1 in D2 (+7.6 mg), comparatively to D1. This result confirms the trend of increased FOM observed between D1 and D2. The specific positive effect of PEG (P<0.01) observed between D2 and D3 (+4.3 mg) may be more related to the increase of FOM than to increased N degradability, since N is not limiting in the two diets. Higher OM fermentation may generate ATP which is the main motor for microbial yield (Demeyer, 1991). This results confirm the findings by Ben Salem *et al.* (2000, 2002) on *Acacia cyanophylla* supplemented with PEG-containing blocks. The authors found that including PEG resulted in increased microbial protein synthesis as indicated by the increased urinary excretion of allantoin. Additionally, these authors underline the advantage of including PEG in blocks in relation with microbial synthesis. Indeed, feed blocks, as compared to other means of administrating PEG, seem to be an appropriate way to ensure a slow release of PEG over the day and thus to obtain maximal microbial protein synthesis (Ben Salem *et al.,* 2000).

Intake and growth of lambs

Feed intake and lamb growth are presented in Table 5. The growth reached by lambs in D2, suggested that blocks allowed relatively the valorisation of the basal diet by enhancing microbial fermentation and improving N supply to animals. This finding may be the result of the effect of

supplements provided by the blocks, rather than a detanning effect. Consuming PEG block (D3) resulted in higher (P<0.05) acacia intake (+105 g DM) and higher daily gain (+21g/d). This result suggests an improvement of rumen fermentation and therefore, an increase of acacia digestion as a result of PEG supply. It confirms several earlier studies in which supplementing tanniniferous species with PEG resulted in improved animal growth (Ben Salem *et al*., 2000; Ben Salem *et al*., 2002). In our study, such improvement may be the result of higher intake, improved N digestibility in the rumen and probably in the gut, and enhanced microbial growth by PEG.

Table 5. Daily DM intake and weight gain

	Diets		SEM
	D2	D3	
DM intake			
Acacia*	432.4[b]	537.2[a]	47.54
Hay	245.3	228.1	11.01
Feed blocks	143.3	155.5	17.13
Diet*	821[b]	921[a]	52.09
Daily gain (g/day)**	33.7[b]	54.6[a]	7.28

D2: hay + acacia + B1; D3: hay + acacia + B2 (PEG).
[a, b, c] For the same line values with the same letter do not differ significantly, * P<0.05, **P<0.01.

Conclusions

It is concluded that supplementing acacia-based diets with feed blocks resulted in improved nutritive value of the basal diet allowed by enhanced microbial activity in the rumen. This was due mainly to block ingredients and their catalytic effects. PEG resulted in a specific beneficial effect on rumen fermentation, intake and lamb growth, probably by deactivation of acacia tannins. In the case of tannin-rich species, the studied feeding strategy based on block supplementation may only allow saving body mass of sheep and adding PEG in blocks permitted moderate performances.

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