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Effects of multinutrient blocks and polyethylene glycol 4000 supplies on intake and digestion by sheep fed *Acacia cyanophylla* Lindl. foliage-based diets

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Abstract

The effect of multinutrient block supply and polyethylene glycol 4000 (PEG) on intake, digestion and rumen fermentation was studied in sheep fed with air-dried *Acacia cyanophylla* foliage (acacia)-based diet. In Experiment I, six Noire de Thibar breed sheep (BW = 46 kg) were used in double 3 x 3 Latin square design. All diets included about 360 g of dry matter (DM) of oat-vetch hay and acacia ad libitum. Diet D0 was without a block supplement. Diet D1 included a urea-molasses-mineral block (B1). While D2 included another type of block (B2) that differed from B1 essentially by adding polyethylene glycol 4000. Each experimental period lasted 33 days (21 days for adaptation and two periods of 5 days for measurement separated by 2 days for rest). Feed intake, apparent digestibility of organic matter (OM), crude protein (CP) and crude fibre (CF) and retained nitrogen (Nr) were measured by total faecal collection. In Experiment II, four Noire de Thibar sheep (BW = 53 kg) fitted with rumen cannulae were fed sequentially D0, Dl and D2, respectively, to 90% of intake levels as measured in Experiment I on metabolic weight (MW) base. Fermentation parameters in rumen liquid (pH, NH_3-N, volatile fatty acid (VFA)) were measured at 0, 2, 5, 8, 10, 13, 16 and 21 h after the morning meal. Protozoal number and composition in rumen fluid were determined at 2 h sampling time. Solid digesta kinetics through the rumen was measured using chromium (Cr) mordanced acacia. The DM and CP degradation of acacia was determined using the nylon bag technique.

Acacia had a relatively high content of condensed tannins (41 g kg^{-1} of DM) and acid detergent lignin (176 g kg^{-1} of DM). B1 and B2 were high in CP (381 and 369 g kg^{-1} of DM, respectively) compared to acacia (127 g kg^{-1} of DM, 20% bound to fibre) and hay (75 g kg^{-1} of DM). The two kinds of blocks improved similarly ($P < 0.001$) acacia DM intake by 195 g. Block 1 increased ($P < 0.001$) only diet CP digestibility and Nr. Block 2 increased ($P < 0.05$) by a low extent DM and OM digestibility compared to D1, and remarkably ($P < 0.001$) CP digestibility and Nr compared to D0 and Dl. Block supplies considerably increased water intake.

Both B1 and B2 increased ($P < 0.001$) NH_3-N and VFA concentrations in the rumen liquid with a positive specific effect of B2 (PEG). VFA molar proportion was significantly modified by B1 and B2. B1 and B2 decreased acetate proportion and increased propionate and butyrate proportions as compared to D0 ($P < 0.001$). Protozoal number in rumen fluid was increased significantly by B1 and B2 ($P < 0.001$). PEG-containing block (B2) increased protozoal number as compared to B1. Both B1 and B2 increased ($P < 0.001$) solid outflow rate, with a specific increasing effect of B2 (D2) when compared to B1 (D1). Blocks supply did not modify in situ DM degradability of acacia, but B2 improved ($P < 0.05$) effective degradability of CP when compared to D0 and Dl which were similar. It is concluded that both B1 and B2 improved the nutritive value of acacia-based diet. A further positive effect was noted in D2 (PEG), especially for N metabolism.

Keywords : *Acacia cyanophylla* Lindl. ; tannin ; multinutrient block ; PEG ; intake ; digestion ; fermentation ; sheep

1. INTRODUCTION

Since several years shrubs and fodder trees such as *Acacia cyanophylla,* atriplex and cactus are widely established mainly in arid and semi-arid regions. In addition to their environmental and antierosive roles, these species generate substantial amounts of biomass which can be used for livestock feeding in these regions. *Acacia cyanophylla* Lindl. [syn. *Acacia saligna* (Labill.) H. wendl] is the most abundant shrubs species in Tunisia. In

arid and semi-arid and in some dry years in the northern Tunisia, this species is frequently harvested in winter and fed to sheep and goat.

Several studies noted that despite its relatively high crude protein content, *A. cyanophylla* foliage had a low nutritive value (Degen et al., 1995, 1997; Ben Salem et al., 1997a) and its consumption by sheep caused a decrease in feed intake, digestion and growth of animals (Reed et al., 1990). These negative effects were attributed to the presence of high levels of condensed tannins in acacia foliage. These secondary compounds which are synthesised by the plant and released during mastication affect their intake and diet digestibility (Leinmüller et al., 1991; Reed, 1995; Zimmer and Cordesse, 1996). It seems that the major antinutritive effect of condensed tannins is exerted on protein digestion and absorption by complexing them (Leinmüller et al., 1991), binding digestive enzymes (Daiber, 1975), depressing microbial activity in the rumen and by reducing duodenal digestion and absorption (McLeod, 1974). The improvement of the nutritive value of tannin-rich species must be viewed by reducing and/or eliminating inhibitory effects of tannins.

The few earlier national studies in this topic were dealing with the improvement of the nutritive value of acacia-based diets by energy and nitrogen supplies (Ben Salem et al., 1995), polyethylene glycol (PEG) treatment (Ben Salem et al., 1996) and by air drying foliage (Ben Salem et al., 1997a). The most encouraging results were obtained with PEG treatment which deactivate condensed tannin in acacia leaves and improved significantly diet nutritive value and sheep growth. PEG forms stronger complexes with tannin than tannin-protein complexes (Oh et al., 1980) and release protein from the complex owing to exchange reaction between PEG and the protein in the complex (Jones and Mangan, 1977). This exchange reaction may occur due to the stronger hydrophobic property of PEG. It has been suggested that the treatment of tannin-rich leguminous shrubs with PEG offered a viable technique to enhance their nutritive value and animal productivity (Pritchard et al., 1992; Silanikove et al., 1994).

The present work was designated to evaluate the effect of multinutrient block supply and PEG on intake, digestion, rumen fermentation and solid digesta outflow rate of *A. cyanophylla* Lindl. foliage-based diet in sheep.

2. MATERIAL AND METHODS

2.1. Plant material

A. cyanophylla Lindl. (acacia) branches were harvested in January 1995 from a 9-year-old plantation in Oued Zargua (north of Tunisia) which is a sub-humid region. Acacia leaves and twigs (maximum 4 mm in diameter) were separated from initial branches and air-dried in the shade for about 3 weeks. The dried material (85% DM) was homogenised and then stored in large perforated bags made of nylon fibres. Oat-vetch hay (70% of oat and 30% of vetch at seedling) was produced at the INAT-farm. It was chopped in order to avoid selection and loss from troughs.

2.2. Block manufacturing

The two types of multinutrient blocks involved in this study (Table 1) were made in March 1995. In block 1 (B1), all solid components were well homogenised with the liquid mixture composed of water dissolved urea, molasses and salt. Block 2 (B2) includes PEG-4000 which was completely dissolved in water and then added to the liquid mixture. The obtained homogenous material for both B1 and B2 was then heavily packed down in plastic moulds. Compacted blocks were taken out of moulds and air-dried in a shady site until they are sufficiently hard for transport and resistant for sheep consumption.

Table 1: *Ingredients proportions of blocks (percentage of dry matter)*

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

2.3. Animals, diets, experimental designs and measurements

2.3.1. Experiment I: intake, digestibility and nitrogen balance

Six adult Noire de Thibar breed male sheep (initial liveweight averaged: 46 kg) were used in a double 3 x 3 Latin square design. They were given 250 g of commercial concentrate and oat-vetch hay ad libitum for 1 month before the beginning of the experiment in order to homogenise their body states. Then they were housed individually in metabolism cages. Three experimental diets based on 400 g of oat-vetch hay (about 360 g of DM) and air-dried acacia leaves and twigs ad libitum were tested. Diet D0 did not include supplementation, while D1 and D2 included B1 and B2 (PEG), respectively. Animals were fed daily the experimental diets in two equal meals at 8:00 and 16:00 h and had free access to fresh water. Acacia offered was daily adjusted to allow about 20% of refusals, while B1 (D1) and B2 (D2) were available all the time in the troughs. Each experimental period lasted 33 days and was divided in 21 days for adjustment and two 5-day measurement periods separated by a rest period of 2 days.

Amounts of acacia offered and refused, blocks and water were weighted daily to determine voluntary intake (oat-vetch hay was entirely consumed). Diet digestibility was measured by total faecal collection. Daily samples of offered acacia and blocks and acacia refusals and faeces were taken. Dry matter content of these samples was determined by drying to a constant weight at 105°C in a forced air oven. The remaining quantities were stored, and at the end of each experimental period, aliquots (10%) of the daily collection were mixed and pooled by animal and then dried at 40°C for 48 h in a forced air oven. Dried samples were ground to pass through a 1 mm sieve and stored for later analysis. Urine excreted by each animal was collected in plastic buckets containing 100 ml of 10% H_2SO_4 solution. A 10% aliquot of urine was taken daily and stored at -10°C for later nitrogen analysis. For D2, dry matter and organic matter (OM) digestibilities were corrected to a PEG-free basis (assuming PEG was indigestible).

2.3.2. Experiment II: ruminal fermentation and passage rate of digesta

Four Noire de Thibar breed male sheep fitted with ruminal cannula were used (average initial liveweight, 53 kg). They were housed in individual pens and received sequentially the three diets through three successive periods (D0, D1 and D2, respectively). Sheep were fed twice per day (at 8:30 and 16:00 h) at 90% of the intake level determined in Experiment I. Offered quantities of acacia leaves and twigs and hay were adjusted according to sheep metabolic weight and the relative proportions of acacia, hay and blocks were adjusted to that measured in Experiment I. Weighted pieces of blocks were offered daily before the morning meal. Water was continuously available.

Measurements started after 21 days of adjustment. During day 22, 23 and 24 of each period, just before (0 h) and 2, 5, 8, 10, 13, 16, 21 h after the morning meal, ruminal fluid samples (50 ml) were taken through the cannulae using a 35 cm plastic tube. Ruminal pH was immediately measured using a combination electrode and samples were then strained through four layers of cheesecloth. Samples (18 ml) of rumen liquid were preserved for volatile fatty acid (VFA) and ammonia nitrogen (NH_3-N) analysis by adding 2 ml of conserving solution (1 g of mercuric chloride + 5 ml orthophosphoric acid, diluted to 100 ml with distilled water). Samples were then stored at -18°C pending analysis. At 2 h sampling time, 5-ml samples of unfiltered rumen content were mixed with 5 ml of fixing solution (50 ml of glycerol + 2 ml of formaldehyde, diluted to 100 ml with distilled water) and stored at 4°C for protozoa counting.

Chromium-mordanced acacia leaves and twigs prepared as described by Uden et al. (1980) was used to study rumen solid outflow. Chromium concentration was 8.5% of DM. Treated acacia (50 g of DM) was introduced into the rumen of each sheep before the morning meal. Digesta samples (50 ml of rumen fluid) were collected 2, 4, 6, 8, 10, 12, 24, 30, 36, 48, 56, 72, 80, 96 and 104 h following the morning meal. Samples were oven-dried and milled for Cr analysis.

In situ DM and crude protein (CP) degradability of acacia leaves and twigs was studied using the nylon bag technique described by 0rskov and McDonald (1979). About 3 g of ground acacia (2 mm screen) were introduced in nylon bags (50 µm pore size; 15 mg/ cm^2). Bags were attached to a 30 cm plastic tube with 200 g of plumb and incubated in the rumen for 3, 6, 12, 24, 48, 72 and 96 h. After withdrawal, the bags were washed in a domestic washing machine (3 mn x 5 mn), dried at 60°C for 48 h and then weighed. Bag residues were collected, milled and analysed for nitrogen content.

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2.4. Laboratory analysis and calculation

Feed, refusals and faeces were analysed for ash (550°C, 8 h) and nitrogen (N) contents by the Kjeldahl method (AOAC, 1984). Crude protein was calculated as 6.25 x N. Soluble fraction of N was determined according to the method proposed by Durant (Vérité and Demarquilly, 1978). 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). Crude fibre (CF) was determined for both feeds and faeces according to Weende procedure. Urine nitrogen was analysed by Kjeldahl method. Acacia condensed tannin (CT) concentrations were measured according to the vanillin-HC1 procedure described by Broadhurst and Jones (1978). Aqueous methanol (10 ml, 50%) was used to extract CT from 200 mg samples of air-dried acacia during 24 h at 4°C. After centrifugation (3000*t*/min), total condensed tannin were measured through absorbency determination using a spectrophotometer at 500 nm. (+)-Catechin (Sigma, lot 126H0276) was used as standard. Results were expressed as methanol-extractable catechin equivalent condensed tannins (g kg^{-1} of DM). Rumen VFA concentrations and molar proportions of individual VFA were analysed by gas chromatography (Jouany, 1982). Ruminal NH_3-N was determined by the method of Conway (1962). Protozoa number of ruminal fluid was determined using a counting cell (Hawskley, UK) as described by Prins (1967, cited by Demeyer, 1981). Chromium was analysed using atomic absorption procedure as described by Williams et al. (1962).

In Experiement II, solid outflow, solid rumen pool size and mean retention time were estimated using the following equation:

$$C = C_0 \, e^{-kt}$$

where C is the Cr concentration (µg g^{-1} of DM) at time t (h), C_0 the Cr concentration at 0 h, k the solid outflow rate (% h^{-1}). Rumen mean retention time T (h) was calculated as

$$T = \frac{1}{k} \ \text{(h)}$$

and solid rumen pool size S (g) as

$$S = \frac{Q}{C_0}$$

where Q is the quantity of Cr (g) introduced in the rumen.

Acacia DM and CP disappearance kinetics were fitted according to the model of Ørskov and McDonald (1979):

$$D_t = a + b(1 - e^{-ct})$$

where D_t is the degradation at time t, a the rapidly degraded fraction, b the slowly degradable fraction, and c the degradation rate of fraction b. Solid outflow rate (k) was used to determine effective degradability (D_e) of DM and CP according to Ørskov and McDonald (1979):

$$D_e = a + \frac{bc}{c + k}$$

2.5. Statistical analysis

The general linear procedure (GLM) of SAS (1985) was used to analyse data for Experiments I and II. The Duncan multiple range test was used to compare means of treatment for both experiments.

3. RESULTS

3.1. Chemical composition

Oat-vetch hay had a higher fibre (NDF and ADF) content than acacia except for ADL which was highest in acacia (Table 2). Compared to the forages, blocks are low in fibres. Acacia condensed tannin content was high (41 g kg^{-1} DM). Crude protein was the lowest in hay and the highest in blocks. B1 had a slightly greater CP content than B2 (381 vs. 369 g kg^{-1} DM, respectively). Acacia were relatively high in CP (127 g kg^{-1} DM). Important fraction of the acacia nitrogen was insoluble (824 g kg^{-1} of total nitrogen). This insolubility was associated to the high proportion of nitrogen bound to NDF (384 g kg^{-1} of total nitrogen) and to ADF (203 g kg^{-1} of total nitrogen).

Diet D0 was higher in OM and fibre (NDF and ADF) than D1 and D2, but was lower in CP (Table 2). Lignin content (ADL) was similar for the three diets. Chemical composition was comparable in blocks containing diets

(Table 2).

Table 2: *Chemical composition of the feeds and the diets (g kg^{-1} of dry matter)*[a]

Item[b]	*A. cyanophlla* foliage	Oat-vetch hay	Block 1	Block 2	D0	D1	D2
OM	880	911	705	720	892	854	855
CP	127	75	381	369	107	163	161
NDF	462	620	261	210	522	463	465
ADF	349	367	117	87	350	308	310
ADL	176	96	48	28	131	130	131
Condensed tannins[c]	41	-	-	-	-	-	-

[a]Calculated values for D0: acacia + hay, D1: acacia + hay + B1 and D2: acacia + hay + B2 (PEG). [b]OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.
[c] Expressed as grams of catechin equivalent per kilogram of DM.

3.2. Intake

Feed intake mean values are reported in Table 3. Supplementation with B1 and B2 increased significantly DM and OM intake ($P < 0.001$) of the whole diets. This increase was related to the block consumption and to a substantial increase in acacia intake ($P < 0.001$). Blocks, acacia and total diet DM intake values were similar for the supplemented diets (D1 and D2). Taking into account the composition of the blocks, sheep receiving D1 (B1) consumed 31 g of urea and those receiving D2 (B2) consumed 29.3 g of urea and 29 g of PEG. Blocks supply increased significantly ($P < 0.01$) water intake by 49.5 and 63.2% with D1 and D2, respectively.

Table 3: *Intake and digestibility of A. cyanophylla foliage-based diet supplemented or not with B1 or B2*[a]

Item[c]	Diets[b]			S.E.M.
	D0	**D1**	**D2**	
DM intake (g/day)				
Acacia	569.7 b	760.4 a	773.4 a	29.7
Blocks	-	271.9	260.9	11.8
Total diet	929.5 b	1392.1 a	1394.1 a	12.9
DM intake (g kg^{-1} LW$^{0.75}$)				
Acacia	34.3 b	44.2 a	44.4 a	2.0
Total diet	56.0 b	79.5 a	79.8 a	2.2
Water (g/day)	3784 c	5665 b	6175 a	201
Diet digestibility (g kg^{-1})				
OM*	517 ab	502 b	537 a	10.1
CP***	304 c	513 b	642 a	14.0
CF	409	399	406	19.1
DOMi (g kg^{-1} LW$^{0.75}$)***	25.7 b	34.2 a	35.7 a	1.0
DCPi (g/day)***	29.7 c	114.8 b	135.5 a	4.9

[a] Data in the same line with different letters (a, b, c) differ significantly at: *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$.
[b] D0: acacia + hay, D1: acacia + hay + B1, D2: acacia + hay + B2 (PEG)
[c] DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fibre; DOMi: digestible organic matter intake; DCPi: digestible crude protein intake.

3.3. Digestibility

Diet digestibility coefficients are given in Table 3. OM digestibility of D1 and D2 was comparable to that of D0, however a significant increase of OM digestibility was noted for D2 as compared to D1 ($P < 0.05$). CP digestibility of D0 was low. It was significantly increased ($P < 0.001$) by 68.4 and 110.8% for D1 and D2, respectively. CP digestibility was higher for D2 than for D1. CF digestibility coefficients were similar among the three diets.

Both in D1 and D2, the nutritive value of the diet as indicated by DOMi and DCPi values increased significantly

($P < 0.001$) compared to D0 (Table 3). A tendency of a not significant increase of DOMi was noted in D2 compared to D1; whereas D2 exhibited the highest DCPi ($P < 0.001$).

3.4. Nitrogen balance

Nitrogen balance is given in Table 4. Nitrogen intake increased ($P < 0.001$) in the supplemented diets (D1 and D2) compared to D0, but no differences were observed between Dl and D2. Faecal and urinary N losses ($P < 0.001$) were higher in D1 and D2 than in D0. Diet D1 gave the highest faecal nitrogen loss, but it was similar to D2 in urinary N excretion. Absorbed N increased significantly ($P < 0.001$) by 13.8 and 17.5 g in Dl and D2, respectively, which were statistically different. Nitrogen balance was negative in D0 (-0.39 g/day). In both D1 and D2, N retention was significantly increased ($P < 0.001$) by 4.5 and 8.1 g, respectively. Diet D2 exhibited the highest N retention.

Table 4: *Nitrogen balance in sheep fed A. cyanophylla foliage-based diet supplemented or not with B1 or B2*[a]

	Diets[b]			**S.E.M.**
	D0	**Dl**	**D2**	
N intake (g/day)	15.6 b	35.6 a	34.3 a	0.9
N excretion (g/day)				
Faeces	10.9 c	17.2 a	12.3 b	0.5
Urine	5.1 b	14.2 a	14.3 a	0.6
N absorbed (g/day)	4.6 c	18.4 b	22.0 a	0.7
N retained (g/day)	-0.4 c	4.1 b	7.7 a	0.7

[a]Data in the same line with different letters (a, b, c) differ significantly at: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[b] D0: acacia + hay, D1: acacia + hay + B1, D2: acacia + hay + B2 (PEG).

3.5. Ruminal fermentation

Mean daily values for rumen pH, VFA and NH_3-N are presented in Table 5 and Fig. 1. Overall rumen liquor-pH was not affected with block supplementation, whereas mean NH_3-N concentration in the rumen fluid increased significantly ($P < 0.001$) from 60.9 mg l^{-1} (D0) to 187.9 mg l^{-1} (D1). Diet D2 exhibited the highest NH_3-N (245.4 mg l^{-1}).

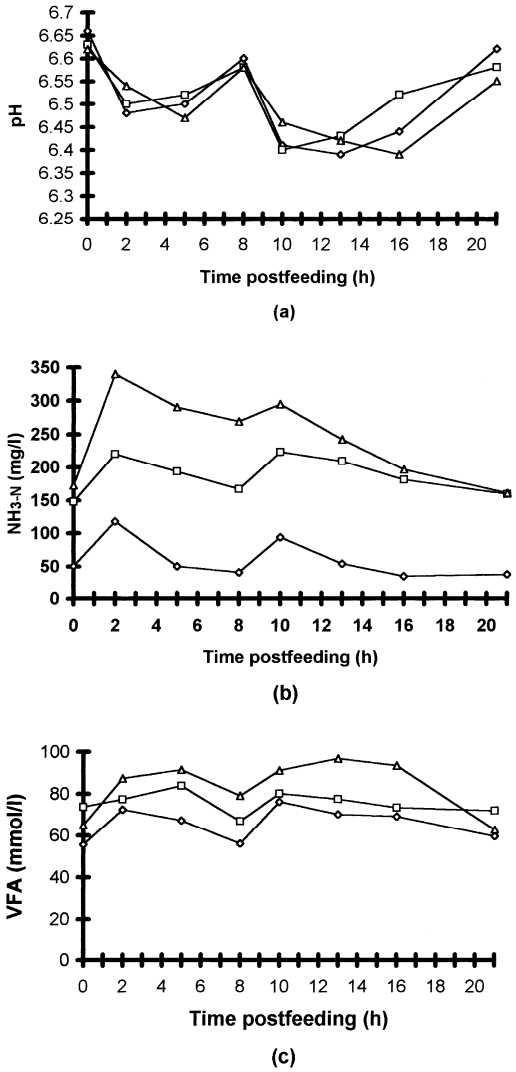
The VFA concentrations differ significantly ($P < 0.001$) between the three diets (65.6, 75.7 and 83.0 mmol 1^{-1} for D0, Dl and D2, respectively). Fermental trends were acetic for the three diets. Molar proportions of acetate decreased from 73.9% (D0) to 72.3 (D1) and 71.0% (D2). Differences between the three diets were significant ($P < 0.001$). When compared to D0, molar proportion of propionate (16.7%) exhibited a significant ($P < 0.001$) increase in supplemented diets which were similar and averaged 17.5%. The same tendency was noted for butyrate ($P < 0.001$). The ratio C2/C3 decreased slightly but significantly ($P < 0.001$) by supplementation and PEG (4.5, 4.2 and 4, respectively, for D0, D1 and D2).

Table 5: *Fermentation parameters in the rumen of sheep fed A. cyanophylla foliage-based diet supplemented or not with B1 or B2*[a]

	Diets[b]			**S.E.M.**
	D0	**D1**	**D2**	
pH	6.51	6.52	6.5	0.0
NH_3-N***	60.9 c	187.9 b	245.4 a	7.8
Total VFA (mmol 1^{-1})***	65.6 c	75.7 b	83.0 a	1.9
Acetate (mol%)***	73.9 a	72.3 b	71.0 c	0.2
Propionate (mol%)***	16.7 b	17.3 a	17.8 a	0.3
Butyrate (mol%)***	8.6 b	9.1 a	9.1 a	0.1
C2/C3[c,***]	4.5 a	4.2 b	4c	0.1

[a]Data in the same line with different letters (a, b, c) differ significantly at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[b] D0: acacia + hay, D1: acacia + hay + B1, D2: acacia + hay + B2 (PEG). [c] Acetate/propionate ratio.

Fig. 1: *(a) Daily change in pH, concentration of (b) NH_3-N and (c) volatile fatty acid (VFA) in the rumen of sheep given acacia and oat-vetch hay (D0: ◊) or supplemented with B1 (D1:□) or B2 (D2: Δ)·*



3.6. Protozoa

Protozoa number and composition are presented in Table 6. At 2 h sampling time, protozoa number in the rumen fluid increased significantly ($P < 0.01$) with blocks supply. The three diets were statistically different. Protozoal composition was also modified. *Holotricha* class proportion increased ($P < 0.01$) whereas *Entodiniomorpheida* one decreased ($P < 0.01$), but no differences were observed between D1 and D2.

3.7.Ruminal solid digesta kinetic

Data of solid digesta kinetic are given in Table 7. The particle outflow rate (k) increased ($P < 0.001$) from 3.27% h^{-1} (D0) to 4.04 (D1) and 4.67% h^{-1} (D2). The three diets were different. Ruminal retention time (T) in D0 (31.3 h) was lower ($P < 0.01$) than in D1 and D2 which were similar, despite the slight increase noted in D2. Sheep receiving D0 presented the lowest ($P < 0.05$) rumen solid pool size (S) (1163 g) compared to the supplemented diets. Difference between D1 and D2 was not significant.

3.8. In situ dry matter and nitrogen degradation

Degradation parameters of acacia-DM (Table 8) were not modified in supplemented diets. Acacia-N ones were

similar between D0 and D1, except for the constant rate of N degradation *c*, which was the highest with D1 (3.54% h^{-1}) between the three diets ($P < 0.05$). When compared to D0 and D1, higher N rapidly degraded fraction *a* ($P < 0.05$), higher N slowly degraded fraction *b* ($P < 0.01$), higher N potential degradability (*a* + *b*) ($P < 0.001$) and a slightly higher N effective degradability (D_e) ($P < 0.05$) were registered with D2 (B2, PEG). It is important to note that for both DM and N degradation kinetics, the rapidly degraded fraction *a* was high in the three diets.

Table 6: *Rumen protozoal number in the luminal fluid of sheep fed A. cyanophylla foliage-based diet supplemented or not with B1 or B2*[a]

	Diets[b]			**S.E.M.**
	D0	**D1**	**D2**	
Protozoa (x 10^5 per ml)***	0.93 a	1.5 b	1.69 c	0.09
Entodiniomorpheda (%)***	92.26 a	90.53 b	90.12 b	0.4
Holotricha (%)***	7.73 b	9.46 a	9.89 a	0.4

[a]Data in the same line with different letters (a, b, c) differ significantly at: *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$.
[b] D0: acacia + hay, D1: acacia + hay + B1, D2: acacia + hay + B2 (PEG).

Table 7: *Ruminal digesta kinetic in sheep fed A. cyanophylla foliage-based diet supplemented or not with B1 or B2*

	Diets[a]			**S.E.M.**
	D0	**D1**	**D2**	
Ruminal solid pool size (g DM)	1164 b	1722 a	2086 a	158
Solid outflow rate (% h^{-1})***	3.27 c	4.04 b	4.67 a	0.13
Ruminal mean retention time (h)**	31.3 a	25.2 b	21.7 b	1.1

[a] D0: acacia + hay, D1: acacia + hay + B1, D2: acacia + hay + B2 (PEG). *Data in the same line with different letters (a, b, c) differ significantly at $P < 0.05$. **Data in the same line with different letters (a, b, c) differ significantly at $P < 0.01$. ***Data in the same line with different letters (a, b, c) differ significantly at $P < 0.001$.

Table 8: *In situ dry matter and crude protein degradability of acacia on sheep fed A. cyanophylla foliage-based diet supplemented or not with B1 or B2*

	Diets[a]			**S.E.M.**
	D0	**D1**	**D2**	
DM degradation parameters[b]				
a (%)	28.8	31.0	30.7	0.9
b (%)	29.5	27.8	29.9	1.0
c (% h^{-1})	3.95	3.86	3.96	0.5
a + *b* (%)	58.3	58.8	58.1	2.4
D_e (%)	44.9	44.6	44.1	0.4
CP degradation parameters				
a (%)*	26.1 b	25.9 b	29.9 a	1.0
b (%)**	36.4 b	34.5 b	47.1 a	1.5
c (% h^{-1})*	2.43 b	3.54 a	1.89 b	0.4
α + b (%)***	62.5 b	60.4 b	77.1 a	1.1
D_e (%)*	41.7 b	41.8 b	43.6 a	0.6

[a] D0: acacia + hay, D1: acacia + hay + B1, D2: acacia + hay + B2 (PEG). [b]*a* is the immediate soluble fraction, *b* is the insoluble but fermentable fraction, *c* is the rate constant of degradation of *b*, *a* + *b* is the potential degradability and D_e is the effective degradability. Model used was $y = a + b(1 - e^{-ct})$.
*Data in the same line with different letters (a, b, c) differ significantly at $P < 0.05$. **Data in the same line with different letters (a, b, c) differ significantly at $P < 0.01$. ***Data in the same line with different letters (a, b, c) differ significantly at $P < 0.001$.

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4. DISCUSSION

4.1. Intake and digestibility

Voluntary DM intake of acacia was relatively low in the non-supplemented diet (D0). It was lower than that reported by Ben Salem et al. (1997b), who found that sheep receiving a high quality forage (Lucerne hay: 600 g of DM) and *A. cyanophylla* foliage ad libitum consumed 610 g of DM of acacia which represent 46% of the DM intake. However, our values are higher than those reported by Degen et al. (1997) who fed only *A. saligna* (= *A. cyanophylla)* to sheep (235 g DM) and by Reed et al. (1990) when acacia was associated to teff straw (170 g DM, 36% of diet). These reported results seem to indicate that acacia intake is somewhat related to the associated forage. In general, the low intake of acacia could be related to negative effect of condensed tannins (Reed et al., 1990; Degen et al., 1997). Tannins in the plant tissue may precipitate salivary glycoprotein causing an astringent taste in the mouth (Bate-Smith, 1973). Mitjavila et al. (1977) suggested that tannins slow down DM digestion in the rumen and reduce the intestinal permeability. This phenomenon reduces the passage of nutrients through gut and leads to a gut feel effect (Silanikove et al., 1996).

Block 1 supply resulted in increased DM intake of the total diet by about 50%, and acacia DM intake by 33%. The positive effect of blocks on poor quality forages intake was reported by several authors (Soetanto and Dixon, 1987; Habib et al., 1994). Multinutrient blocks supply could stimulate microbial growth and improve fibre degradation in the rumen due to the catalytic effect of urea, molasses and minerals (Chenost and Kayouli, 1997).

The incorporation of PEG in B2 had no effect on total diet or acacia intake. This trend is not consistent with several studies where PEG was shown to improve intake of tannin-rich forages (Pritchard et al., 1992; Silanikove et al., 1996). Ben Salem et al. (1996) found that adding PEG (two parts of PEG/one part of tannin) to sheep fed 400 g of barley and acacia foliage ad libitum had no effect on acacia intake. Our result could be explained by the combined effects of physical and chemical intake regulation. On one hand, the rumen might reached its greatest distension level by the sole effect of the other components present in the blocks, so supplementary increase in intake would be unlikely. On the other hand, the chemical factors might limit intake through absorption of fermentation end products (Faverdin et al., 1997). These explanations generally concerns conventional forages and may not extend to tannin-rich species. Regulation mechanisms of intake for those species when supplemented with blocks need further investigations.

Both B1 and B2 increased water intake. This finding is consistent with the results of El Khidir et al. (1989) who noted similar trend with low quality roughages supplemented with blocks. In our case, the results could be explained by an increase of the total DM intake and the presence of salt in blocks (Chenost and Kayouli, 1997). The noted specific positive effect of PEG on water consumption may be attributed to an eventual increase of the rate of fermentation in the rumen, because water intake seems to be the mechanism by which ruminants adjust the osmotic pressure in the rumen (Faverdin et al., 1997).

The supplementation of the diet with the two blocks affected substantially the CP digestibility, but had a slight effect on OM and no effect on CF digestibilities of the diet. When compared to D1, PEG in B2 (D2) had a significant positive effect on OM digestibility but no effect on CF digestibility. This result is not consistent with several published studies where PEG increased more clearly OM and fibre digestibility of tannin-rich species (Barry et al., 1986; Silanikove et al., 1996). The absence of effect of B1 and clear effect of PEG (B2) on OM and fibre digestibility might be explained by an increase of digesta transit and consequently a decrease of retention time in the digestive tract caused by block consumption (Experiment II). In addition, the supplementary intake of acacia provided supplementary condensed tannins, which might contribute to the persistency of the negative effect despite the block intake.

It has been largely shown that the main negative effect of tannins on digestibility was generally noted on protein (Barry and Manley, 1984; Nastis and Malechek, 1981; Ben Salem et al., 1997b). Condensed tannins of tree leaves affect dietary protein digestion by forming indigestible tannins-protein complexes and by inactivating digestive enzymes (Kumar and Singh, 1984). They also depress protein digestion and absorption in the gut (McLeod, 1974). This could explain the low CP digestibility and the negative nitrogen balance found in D0. The significant increase of CP digestibility of acacia-based diet (+68.4%) in D1 may be related to the intake of about 31 g of totally and rapidly degradable urea. In fact, if we evaluate CP digestibility of D1 without urea N using Table 4 data, the obtained value would be 19.4%. This result is lower than that of D1 and even that of D0. In this condition it is not possible to hypothesise a urea detanning effect as suggested by Kumar and Singh (1984).

The important positive effect of PEG on CP digestibility observed in D2 (+110.8 and 25.19% compared to D0 and D1, respectively) confirmed results of the few earlier studies which dealt with adding PEG to acacia species such as *Acacia aneura* (Pritchard et al., 1992) and *A. cyanophylla* (Ben Salem et al., 1996). Tannins in feed can be deactivated by PEG to which they bind more strongly than to protein (Oh et al., 1980). Furthermore, PEG supply could result in the release of protein from the complexes owing to exchange reaction between PEG and the protein in the complex (Jones and Mangan, 1977). Consequently, enzymatic and microbial inhibitory effects of tannins are reduced. In addition, PEG is likely to improve protein digestion and absorption in the gut (McLeod, 1974).

4.2.Nitrogen balance

The negative N retention in D0 (Table 4) is consistent with the results reported by Reed et al. (1990) and Degen et al. (1997) when *A. cyanophylla* was given to sheep. The increase of retained nitrogen (Nr) after B1 supply (D1) is related to the increase of N intake and digestibility. The increase in faecal N losses observed with D1 may indicate that a part of tannin-protein or tannin-ammonia complexes were not fully hydrolysed in the gastrointestinal tract, thereby preventing N absorption (Nuñez-Hernandez et al., 1991) because such increase was not observed with D2. In addition, the higher intake of acacia may increase the availability of free condensed tannins in the gastro-intestinal tract (Barry and Duncan, 1984) which bind to the gut epithelium and then reduce amino acid absorption (Wang et al., 1994). The relatively high urinary N losses in Dl may be the result of imbalance between nitrogen and energy in the rumen (Nastis and Malechek, 1981).

Nitrogen retention increased significantly when PEG is added (D3). The decrease of faecal N losses compared to Dl may be the result of higher digestion of protein in the rumen. This result confirms the earlier finding of Barry et al. (1986) and Nunez-Hernandez et al. (1991) when PEG is added to tannin-rich diets. However, our result is not consistent with those of these authors who found that PEG addition increased urinary excretion of N. Our results may indicate that the improvement of Nr with D2 was not fully related to the effect of PEG in the rumen, but also to an eventual positive effect of PEG on N digestion and absorption in the gut. In consequence, absorbed nitrogen with D2 may be of a higher quality compared to D1, probably due to enhanced microbial synthesis which provides well-balanced protein (essential amino acids). This hypothesis is confirmed by Nr/Na ratio (22.9 and 35.8% with D1 and D2, respectively) but needs further investigations.

4.3.Rumen fermentation

Overall NH_3-N concentration in D0 (60.9 mg 1^{-1}) is not limiting for microbial fermentation and synthesis. It was higher than the level of 50 mg 1^{-1} suggested by Satter and Slyter (1974) to maintain microbial growth in the rumen. This moderate NH_3-N concentration, despite the low solubility of acacia N and the tannin negative effects, may be ascribed to N recycling phenomenon. Tannin may increase the glycoprotein content and excretion of saliva which could lead to more N recycled to the rumen (Robbins et al., 1987). This hypothesis could explain the moderate but not very low values of OM and CF digestibility observed in Experiment I. Block 1 (D1) resulted in an important increase of NH_3-N concentration in the rumen liquor. This expected result is consistent with the general trends when low quality forages are supplemented with urea-containing blocks (Habib et al., 1994) and may be due to the high solubility and the rapid degradation of urea N. The significant increase of NH_3-N concentration in the rumen liquor noted in D2 (PEG) may indicate that acacia condensed tannins exerted an inhibitory effect on rumen protein degradation in both D0 and D1. The negative effect of *A. cyanophylla* condensed tannins on CP digestibility and NH_3-N was well demonstrated in sheep by Ben Salem et al. (1997b). The high concentrations of NH_3-N noted with both D1 and D2 confirmed results of Experiment I. The visible reduction of NH_3-N noted especially at 0, 8 and 21 h sampling time (Fig. lb) may indicate that an important part of degradable N is absorbed in the rumen and lost as urinary excretion (Na/ Ni: 51.3 and 64.1% with D1 and D2, respectively). In consequence Nr was low in both D1 and D2 (Nr/Ni: 11.7 and 22.9%, respectively). These trends generally characterise urea-rich diets.

Overall VFA concentration in the rumen liquor of sheep receiving D0 is moderate and showed an acetic trend. Block 1 supply increased significantly VFA concentration as it was found by some authors who supplemented low quality forages with multinutrient blocks (Kunju, 1986; Sudana and Leng, 1986). This result may be due to the increase of acacia intake and also to some block components, notably bran and molasses, as indicated by the observed higher molar proportions of propionate and butyrate.

Overall VFA concentration in rumen liquor increased significantly with PEG supply in D2 (B2) compared to D0 and D1. This result may indicate that condensed tannins in acacia exerted an inhibitory effect on carbohydrate fermentation in the rumen. Silanikove et al. (1996) observed a similar effect when goats fed *Quercus*

calliprunos, *Pistacia lentiscus* and *Ceratonia siliqua* received PEG. When compared to Bl (Dl), PEG (D2) did not modify considerably fermentation trends in the rumen. This result suggested that PEG had probably no impact on the use of metabolisable energy (ME). Waghorn et al. (1994) found that addition of PEG-3500 to sheep fed *Lotus pedunculatus* (5.5% CT) increased VFA concentration in the rumen but did not modify molar proportions of the major VFA compared to control.

The increase of intake and VFA concentration, the slight changes in OM digestibility and the absence of great changes in VFA molar proportions after B1 and especially B2 supplies may signify that the main respective effects of the two types of blocks in the rumen was the increase of the rate of fermentation.

4.4. Protozoa

Total protozoal number was very low in the rumen of sheep fed D0. It seems that acacia condensed tannins exerted a toxic effect on protozoa. Such effect was clearly demonstrated on sheep given *A. cyanophylla* by Ben Salem et al. (1997b). Block 1 supply significantly improved protozoal number in the rumen fluid. This result could be attributed to the improvement of nutritional balance in the rumen (Clarke, 1977) by nutriments supply and probably to starch (Mackie et al., 1978) accompanying bran. The higher proportion of Holotricha observed in D1 was probably related to molasses in feed blocks (Preston and Leng, 1980).

The increase in protozoal total number with PEG confirmed the hypothesis of the inhibition of protozoa by tannin in the rumen of sheep fed D0 and also D1. PEG seems to reduce this toxic effect by binding condensed tannin. It is opportune to note that effects of condensed tannins on protozoa are still controversed. Wang et al. (1994) studied the effect of PEG on protozoa in sheep fed *Lotus corniculatus.* They found that protozoa number increased in PEG sheep relative to control. After 22 days of treatment, the protozoa number had declined in PEG sheep and were not significantly different from that measured in control. In contrast, Chiquette et al. (1989) recorded an increase of protozoa in the rumen of sheep fed tannin-rich diet as compared to those fed on low-tannin diets. Moreover, El Hassen (1994) demonstrated that the toxic effect on protozoa of some tannins species was not related to tannins themselves but to the presence of saponines. Effects of tannins on protozoa populations in ruminants need further investigations.

4.5. Rumen solid particle kinetic

The low solid particle outflow rate (k = 3.2% h^{-1}) observed in D0 is consistent with data reported by Osuji and Odenyo (1997) on sheep fed teff straw and different browse species (2.2-3.4% h^{-1}). Block 1 (D1) increased significantly solid digesta outflow rate. This result agrees with the finding of Bandla and Gupta (1994) when supplementating low quality forage with blocks. This effect may be explained by higher fibre rate of degradation in the rumen allowed by the supplied energy, N and minerals (Chenost and Kayouli, 1997). On another hand, it is possible to suggest a physical effect of blocks by increasing the proportion of dense particles near the reticulo-omasal orifice, which resulted in increased amount of dry matter evacuated (Ulyatt et al., 1986).

The specific increase of solid digesta outflow rate related to PEG (D2) could be explained by a supplementary positive effect on the rate of degradation in the rumen as proved by VFA concentration. In addition one could suggest an eventual positive effect in the gut, where PEG would have reduced the gut feel effect by improving protein digestion and absorption (McLeod, 1974). It is important to underline that for both B1 (D1) and B2 (D2) supplies, the increase of the rate of solid particles outflow rate in combination with the presumed increase of the rate of fermentation in the rumen, may explain the only just slight increase in DM and OM digestibilities and the stability of CF digestibility. Otherwise, the digestibility of nutrients might decrease, since apparent digestibility is negatively correlated to digesta outflow rate (Ørskov and Ryle, 1990).

In Experiment II, water consumption and liquid outflow rate were not measured. Therefore, it is possible to suggest that increased water consumption noted in Experiment I may accelerate digesta outflow rate through a carrying phenomenon in both D1 and D2. This hypothesis is supported by the positive correlation between solid outflow rate and dilution rate in the rumen (Bergen et al., 1980). Moreover, Rémond et al. (1995) suggested that the increase of the rumen solid content may induce a decrease of rumen digesta retention time, since the amount of dry matter evacuated by each contraction of the reticulo-omasal orifice increases. In this connection, Nitsan et al. (1996) found that adding PEG to goat receiving *C. siliqua* increased DM content in the rumen. Suggesting a solidifying blocks and/or PEG effect on rumen digesta requires further investigations, mainly the determination of the rumen liquid volume.

4.6. In situ degradability

Block1 (D1) had no effect on DM and CP in situ degradability parameters of incubated acacia, except the N rate of degradation *c*, which increased. This result does not support the data reported in several studies when low quality forage is supplemented with blocks (Soetanto and Dixon, 1987; Habib et al., 1994). It is also difficult to interpret and should be considered with caution. In fact, the rapidly degraded fraction *a* is very high. This phenomenon is generally more frequent in leguminous than in grasses. Emanuele and Staples (1988) observed that the mean particles size of grasses was larger than that of leguminous forages after milling through a 2 mm screen. This observation leads to higher washing losses in leguminous forages and then to overestimated extent of degradation and an underestimated rate of degradation if the particles remaining in the bag have a slower rate of digestion than those lost. In consequence, one could think that incubated acacia should be milled through larger grinding screen.

PEG supply in B2 (D2) had no effect on DM in situ degradability. Therefore it increased significantly CP degradability of incubated acacia. This result may indicate that only a slight part of the increase of CP digestibility (Experiment I) and ruminal NH3-N concentration (Experiment II) could be explained by the increase of acacia CP-degradability due to tannin deactivating effect of PEG, and also that PEG effect is potentially of a higher importance ('α + b' higher than D_e). In Experiment I, positive PEG effect on CP-digestibility was more pronounced than in situ measurements. This may confirm the hypothesis of an eventual important positive effect of PEG on gut digestion and absorption.

It is important to note that in our study the contamination of residues by rumen microbes was not determined. Kammoun (1995) found that the D_e of CP in leguminous for which the CP/NDF ratio ranged from 0.2 to 0.5 (our case about 0.27) could be under estimated by about 10-20% when contamination was not considered. The lack of data concerning contamination of tannins rich leguminous especially, cannot allow to develop profoundly this aspect.

In conclusion, this study has shown that block supply improved the nutritive value of acacia-based diets by increasing intake and enhancing rumen fermentation, whereas PEG (B2, D2) seemed to reduce the inhibitory effects of acacia tannins in the rumen and probably in the gut resulting especially in enhanced Nr. The presence of tannins and the high level of lignin in acacia seem to limit fermented OM and hence energy availability in the rumen for the animal host both with D1 and D2. The studied feeding strategy could be advantageous in poor conditions but economical aspects should be considered. Effects of multinutrient blocks and PEG on microbial growth and animal performance in sheep fed *A. cyanophylla* foliage will be the subject of further research.

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