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Effect of Using Polyethylene Glycol or Sodium Bentonite on Performance of Sheep Fed *Acacia saligna.*

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Abstract: This study was carried out to evaluate the effects of adding polyethylene glycol (PEG 4000), sodium bentonite to *Acacia saligna* hay as a roughage in the diet to deactivate it's content of tannins. Twenty healthy growing Barki lambs averaged of 30.90±4.63 Kg body weight were randomly divided into four groups (five animals of each) in fattening trial lasted for 180 days. Nutrients digestibility, nitrogen balance, blood serum metabolites, rumen parameters, weight changes and average daily gain (ADG) were determined. Animal groups were fed on concentrate feed mixture (CFM)+ berseem hay (G1), CFM + acacia hay (G2), G2 diet + 5% PEG (G3) and G2 diet + 4% bentonite (G4). Results revealed that group one had higher values of digestibility coefficients for DM, OM, CP, CF, EE and NFE than the other groups. All animals were in positive nitrogen balance (from 3.99 to 12.13 g/h/d). The highest amount of nitrogen was in G1 and the lowest was G2. Ruminal total volatile fatty acids (TVFA's) and ammonia-nitrogen were increased to reach the peak value at 3hr post feeding. All serum blood metabolites were in normal ranges. Total dry matter intake was 1.69, 1.25, 1.39 and 1.42 Kg, for the experimental groups, respectively. Average daily gain was 149.22, 67.22, 79.32 and 78.00g/d for the experimental groups, respectively. Finally, G1 recorded the best digestibility coefficients, nitrogen balance, total dry matter intake, total digestible nutrients (TDN), digestible crude protein (DCP), ADG with normal blood parameters compared with the others. However, Adding PEG and sodium bentonite to Acacia diet could be used to increase feed intake utilization as result of tannins deactivation.

Key words: Tannins · PEG · Bentonite · Acacia · ADG

INTRODUCTION

Desert plants are characterized by limiting factors of animal productivity like high fiber content and anti-nutritional factors (lignin, tannins, alkaloids, oxalte,…). Palatable plants are available for grazing animal only at short periods (wet season). *Acacia saligna* is belonging to family Fabacea and characterized as evergreen perennial tree or shrub, drought tolerant and moderately salinity tolerant and considered as less palatable feed [1]. Feeding acacia as a sole feed resulted in negative growth rates due to high anti-nutritional factors especially lignin and tannins and condensed tannins [2] and [3]. Polyethylene glycol (PEG) is widely used to reduce the negative effects of tannins in feed stuffs. Using PEG in ration containing tannins may had some negative effects. It is very expensive especially in developing countries and it may reduce the effect of tannin on decreasing methane production and internal parasites [4-7]. So that, using alternative material will be favorable in animal feeding under desert conditions. Bentonite (as clay mineral) was used in animal nutrition mainly as feeds pelleting agents (binder), adsorbing agents to mycotoxins (aflatoxins), ammonia, pesticides, heavy metals, phenol and tannins [8-10].

The objective of this work was to investigate the possibility of using PEG or sodium bentonite as deactivation of tannins in *Acacia saligna as* roughage feed on Barki sheep preformance.

MATERIALS AND METHODS

Animals and Rations: Twenty healthy growing Barki sheep with an average body weight of 30.90±4.63 Kg and age nine months old were used in feeding experiment lasted for six months. Animals were fed in groups

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according to Kearl requirements [11], sixty percentage of energy as total digestible nutrients (TDN) was offered from concentrate feed mixture (CFM), while the roughage (berseem hay or acacia hay) was fed ad libitum. At the beginning, all animals were drenched drugs against internal parasites. Animals were divided randomly into four groups (five animals per group) as follows:

G1: CFM and Egyptian berseem hay
G2: CFM and acacia hay
G3: CFM and acacia hay+Polyethylene glycol 4000 (5 g/100g of dried acacia)
G4: CFM and acacia hay+sodium bentonite (4 g/100g of dried acacia).

The roughage was offered once daily, while concentrate mixture was offered twice daily, animals were watered twice a day. Amount of feeds was adjusted biweekly according to growth rate changes and feed efficiency was also calculated.

Nutrients Digestibility and Rumen Parameters: At the end of feeding experiment, four animals from each group were used for digestibility trail. Animals were kept in the metabolic crates for seven days as a preliminary period followed by seven days as a collection period. In the latter, feeds, water intake and refused feeds were recorded daily and composite samples were kept for further lab analysis. Fecal and urine samples (10%) were collected daily. At the end of collection period of the digestibility trial, rumen liquor was withdrawn using stomach tube before morning feeding (zero time), 3 and 6hr after feeding. The pH value was measured immediately using portable pH meter. The rumen liquor samples were filtered through three layers of cheese cloth. Then rumen liquor samples were put in plastic bottles and preserved by using drops of toluene and paraffin oil and kept at -20 °C for determination of total volatile fatty acids and ammonia-nitrogen.

Chemical Analysis: Proximate analysis for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash of feeds, feed refusals, feces and urinary nitrogen were determined according to the official methods [12]. Gross energy in feeds, feed refusals and feces were determined using bomb calorimeter (C200, IKA Works Inc., Staufen, Germany). Fiber fractions were estimated according to Van Soest and Robertson [13]. Total tannins (TT) was determined gravimetrically with copper acetate method according to Balbaa *et al*. [14]. Condensed tannins (CT) was determined by spectrophotometer according to Makkar *et al*. [15]. Rumen ammonia-N and TVFA's concentrations were determined using micro-Kjeldahl's method and steam distillation method as described by AOAC [12] and Annison [16], respectively.

Blood Parameters: Blood samples were collected from jugular vein puncture before feeding and were allowed to stand at room temperature for 1hr then centrifuged at 3000 rpm for 20 min to get serum samples and stored at-20°C for further analysis of blood metabolites using colorimetric methods (using commercial kits). Total protein and albumin were measured according to Gornal *et al*. [17] and Doumas *et al*. [18], respectively. Globulin was determined by difference. Blood urea nitrogen was determined according to Fawcett and Soctt [19]. Creatinine was determined according to Bartles *et al*. [20]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined according to Reitman and Frankel [21].

Statistical Analysis: Differences among groups was significantly checked using one way analysis of variance. Duncan's new multiple range test [22] was used to compare between different means. The General Linear Model (GLM) procedure of SAS [23] was employed.

RESULTS AND DISCUSSION

Chemical composition of acacia hay, berseem hay and CFM are presented in Table (1). The results revealed that acacia and berseem hay had comparable values of DM, CF, EE, NDF%. While, Acacia hay had higher values of OM, NFE, ADF, ADL, TT and CT%, than those in berseem hay. On the opposite, berseem hay had higher level of CP than acacia hay. The chemical composition of acacia was closely similar to El Share, [1] and Salem [24].

Digestibility Coefficients and Nutritive Values: Digestibility coefficients and nutritive values of rations are presented in Table (2). Values of DM and OM digestibility coefficients were higher ($p<0.05$) in G1 (67.02 and 70.10%) than that of G2, G3 and G4 which had similar values of DM and OM digestibility. Low digestibilities of DM and OM in the acacia groups may be due to the high content of ADL, TT and CT compared to G1 (Table 1). Similar results were obtained by Degen *et al*. [2], Youssef *et al*. [25] and Hassan [26].

Table 1:Chemical composition (% on DM Basis) of feed ingredients.

	Ingredients		
Item	Acacia hay	Berseem hay	CFM
DM	88.23	86.13	91.35
OM	92.24	85.21	86.95
CP	10.15	14.58	16.18
CF	31.88	29.72	8.26
EE	2.67	2.5	3.00
NFE	47.54	38.41	59.26
Ash	7.76	14.79	13.05
NDF	52.29	50.43	26.07
ADF	50.00	37.91	10.80
ADL	17.52	6.33	3.09
GE(Mcal/Kg)	4.53	3.97	3.99
Total tannins%	5.75	1.61	2.66
Condensed tannins%	4.27	nil	nil

Digestibility of CP were higher (p<0.05) in G1 and G3 (71.13 and 63.48%) and lower in G2 and G4 (51.45 and 50.50%), respectively. These findings agree with that reported by Silanikove, *et al*. [27] who found that binding PEG with tannins in the rumen (pH 5.5-7.0) lead to increase the CP digestibility and its utilization. Digestibility of CF was lower (p<0.05) in all treatments. This may be attributed to increase the levels of concentrate (about 60%) in the diets, while the animals fed on acacia digested CF lower than animals fed berseem hay. These results may be due to tannins inhibition to cellulolytic bacterial activity [28].

Digestibility of EE was high (p<0.05) in all groups except G2 (59.36%). This may be explained the negative effect of tannins on lipase activity post ruminally so low EE digestibility [29]. Binding tannins with PEG protect lipase from inactivation. Moreover, sodium bentonite is used in industrial application to adsorb lipase enzyme to increase its activity [30]. It is clearly to note that animals fed berseem hay (G1) recorded the highest digestibility of DM, OM, CP, CF, EE and NFE with significant (p<0.05) variation followed by G3 which fed acacia with PEG. The present results indicated that untreated acacia which had high levels of tannins reduce the nutrients digestibility. In this respect, Mousa [31] reported low digestibility of acacia and ascribed that to the inhibitory effect of tannin on ruminal microbial activity.

The nutritive values expressed as TDN% and DCP% were significantly (p<0.05) different among groups. The TDN% was high in animals fed berseem hay (62.99%) compared with the other groups (G2, G3 and G4) which were very similar (54.19, 53.09 and 52.34%, respectively). The DCP% in animals fed G1 diet was 10.93% followed by G3 (9.16%). whereas G2 and G4 had comparable values of DCP% (7.5 and 7.22%). These results may be due to the high utilization of CP. These findings agree with those reported by Youssef *et al.,* [25].

Nitrogen Utilization: Nitrogen utilization as nitrogen intake (NI), fecal nitrogen (FN), urinary nitrogen (UN), total nitrogen excretion (NE) and nitrogen balance (NB) are presented in Table (3). Nitrogen utilization g/h/d in terms of NI, FN, UN and NB were affected by type of roughages. Nitrogen intake ranged from 42.79 to 34.60g/h/d for all treatments. It was higher in animals fed berseem hay than other animal groups fed untreated or treated acacia. Fecal nitrogen was non-significantly (p>0.05) increased in G2 and G4 followed by G3 and G1.

Table 2: Nutrients digestibility and nutritive values of the experimental diets fed by sheep.

	experimental groups				
Item	G1	G2	G3	G4	±SE
Nutrients digestibility (%)					
DM	67.02^{a}	55.49^{b}	55.94^{b}	54.09^{b}	1.42
OM	70.18^{a}	58.67^{b}	57.82^{b}	57.53^{b}	1.45
CP	71.13^{a}	51.45^{c}	63.48^{b}	50.50^{c}	2.31
CF	47.24^{a}	33.44^{b}	34.23^{b}	34.44^{b}	1.76
EE	72.97^{a}	59.36^{b}	71.45^{a}	71.10^{a}	1.73
NFE	76.43^{a}	67.14^{b}	63.07^{b}	64.29^{b}	1.45
Nutritive values (%)					
TDN%	62.99^{a}	54.19^{b}	53.09^{b}	52.34^{b}	1.19
DCP%	10.93^{a}	7.50^{c}	9.16^{b}	7.22^{c}	0.40

a, b, c, means with different superscripts on the same row differ significantly (P<0.05).

G1: CFM + Berseem hay

G2: CFM + Acacia hay

G3: CFM + Acacia hay + 5% PEG

G4: CFM + Acacia hay + 4% bentonite

Table 3: Nitrogen utilization by sheep fed the experimental diets.[1]

	experimental groups				
Item	G1	G2	G3	G4	±SE
NI g/h/d	42.79	34.57	34.60	36.55	1.60
FN g/h/d	12.35	16.97	12.68	18.18	1.01
UN	18.13[a]	13.62[ab]	14.12[ab]	12.77[b]	0.83
NE	30.48	30.59	26.80	30.95	1.33
NB g/h/d	12.13[a]	3.99[c]	7.79[b]	5.61[c]	0.91
NB / NI %	28.65[a]	11.53[b]	22.75[a]	15.56[b]	1.99

a, b, c, means with different superscripts on the same row differ significantly (P<0.05).
1- Calculated from digestibility trail
Nitrogen intake (NI), fecal nitrogen (FN), urinary nitrogen (UN), total nitrogen excretion (NE) and nitrogen balance (NB)
G1: CFM + Berseem hay
G2: CFM + Acacia hay
G3: CFM + Acacia hay + 5% PEG
G4: CFM + Acacia hay + 4% bentonite

Table 4: Some rumen parameters of sheep fed the experimental diets.

		experimental groups				
Item	sampling time (h)	G1	G2	G3	G4	±SE
pH	0	6.95	6.79	6.88	6.69	0.10
	3	6.13	6.18	6.31	6.16	0.03
	6	6.41	6.25	6.20	6.32	0.10
	Mean	6.51	6.41	6.46	6.39	0.10
TVFA´s concentrations						
(m. equiv./100 ml)	0	3.24[b]	4.38 [a]	3.31[b]	3.80 [ab]	0.15
	3	8.44	6.98	7.58	7.62	0.30
	6	6.63[ab]	6.16[b]	7.40 [a]	5.72[b]	0.23
	Mean	6.10	5.84	6.10	5.71	0.28
Ammonia-N concentration						
(mg/100 ml)	0	14.03	17.12	15.60	2.22	0.84
	3	46.18[a]	26.80[b]	43.11[a]	24.71[b]	2.58
	6	38.24[a]	23.77[b]	37.94 [a]	22.12[b]	2.21
	Mean	33.08 [a]	22.22[b]	30.35[a]	20.67[b]	1.71

a, b, c, means with different superscripts on the same row differ significantly (P<0.05).
G1: CFM + Berseem hay
G2: CFM + Acacia hay
G3: CFM + Acacia hay + 5% PEG
G4: CFM + Acacia hay + 4% bentonite

These results clearly show that tannins protein complex change pathway of nitrogen excretion from urine to feces. These findings agree with noticed by Frutos *et al*. [32]. Nitrogen balance was the highest in sheep fed G1 followed by G3, G4 and G2 in descending order. These findings could be attributed to high CP content and digestibility of berseem hay in group one than other groups. The negative effect of tannins on NB was demonstrated in numerous studies on sheep and goats [2, 6].

Rumen Activity: Results of Table (4) illustrated some rumen parameters of sheep fed the experimental diets. The results revealed that the highest value of ruminal pH was recorded in G1 followed by G2, G3 and G4, respectively. Generally, pH values were lower at 3hr post feeding and tented to slightly increase at 6hr post feeding. This is may be due to high rumen fermentation. The lowest values of TVFA's were obtained before feeding and then increased to the maximum after 3hr post feeding. This increase may be caused a reduction in pH values. This coincides with the results of Khattab *et al*. [33]. Average values of TVFA's were higher in G1 and G3 which have the same value (6.10) followed by G2 and G4.

Ammonia nitrogen values differed significantly ($p<0.05$) among the groups being 33.08, 22.22, 30.35 20.67 mg/dl on average for G1, G2, G3 and G4, respectively, Ammonia-N was higher in the control group due to its higher nitrogen content and intake of berseem hay. The same trend was in G3 there higher concentration

Table 5: Serum blood metabolites of sheep fed the experimental diets.

Item	experimental groups G1	G2	G3	G4	±SE	Normal Range
Total Protein (g/dl)	7.77	7.44	7.59	7.25	0.16	(6.0-7.9)
Albumin (g/dl)	3.10	3.11	3.21	3.02	0.04	(2.4-3.0)
Globulin (g/dl)	4.68	4.33	4.38	4.23	0.17	__
BUN (g/dl)	29.28	30.318	33.94	30.62	0.72	(10-35)
Creatinine (mg/dl)	0.98^{b}	1.39^{a}	1.02^{b}	1.07^{b}	0.06	(0.9-1.9)
AST (IU/L)	84.34^{a}	63.68^{b}	72.10^{b}	63.18^{b}	2.82	(60-280)
ALT (IU/L)	24.90	23.03	21.10	24.55	0.60	(22-38)

a, b, c, means with different superscripts on the same row differ significantly (P<0.05).

G1: CFM + Berseem hay

G2: CFM + Acacia hay

G3: CFM + Acacia hay + 5% PEG

G4: CFM + Acacia hay + 4% bentonite

might be related the PEG binding with tannins so the solubility and fermentation of nitrogen will be available to rumen microbes. The higher solubility of CP with PEG than untreated acacia was reported by Hassan [26]. Similar results were obtained by Yossef *et al*. [25] who reported that ammonia-nitrogen and TVFA's concentrations at 4hr after feeding of sheep fed on acacia without and with PEG were 27.3 and 30.10 mg/100ml; 9.44 and 11.15 m. equiv./100 ml, respectively. On the other hand, the lower ammonia-N concentration was lower in G2 may be due to the binding of tannins protein and inhibition of fermentation enzymes [34]. Moreover, Hassan [35] found that ruminal microbial protein synthesis requires an adequate supply of nitrogen to achieve maximal efficiency. McMeniman *et al*. [36] reported that lower nitrogen content uncoupled fermentation could occur. In the same trend, the mode of action in G4 using bentonite tended to low ammonia-N concentration due to the adsorbed of ammonia into clay [8].

Blood Parameters: It was evident that deactivation tannins in *Acacia saligna* by using PEG or bentonite affecting blood chemistry of the experimental animals as illustrated in Table (5). Generally, all the data of blood metabolites were in the normal range values according to Jackson and Cockcroft [37]. Concerning serum protein profile there are insignificant (p>0.05) differences among all groups and ranged from 7.77 to 7.25, from 3.21 to 3.02 and from 4.68 to 4.23 g/dl for total protein, albumin and globulin, respectively. These results may be due to no deficiency in protein and all animals were on positive nitrogen balance. Serum creatinine was significantly (p<0.05) influenced by the dietary roughage. The highest serum creatinine level was reported in animals fed on G2 (1.39 g/dl) while the lowest one was in G1 (0.98 g/dl). These results could be explained by the fact that acacia had high content of tannins. This finding agreed with that reported by Brenner et al., [38] who found that when creatinine levels increased indicating impairment of renal functions. Serum urea nitrogen (BUN) was non-significantly (p>0.05) affected by the type of roughage, being 29.28, 30.32, 33.94 and 30.62 g/dl for G1, G2, G3 and G4 respectively. Slightly increases of creatinine in acacia groups were reflected on higher value of BUN in acacia groups than berseem hay (G1). Although berseem hay had higher values of CP than that of acacia, Samanta *et al*. [39] reported that plasma BUN reflects the dietary CP intake. Romeroe *et al*. [40] also noticed that sheep fed with tanniferous diet had lower blood BUN level than sheep fed low dietary tannins. Serum enzymes revealed that Aspartate aminotransferase (AST) was significantly (p<0.05) influenced by dietary roughage, were 84.34, 63.68, 72.10 and 63.175 IU/L for G1, G2, G3 and G4 respectively. Generally, AST was within normal range (49-123 IU/L). These findings agreed with those reported by EL-Essawy *et al*. [41]. Ibrahim, [42] reported that goats fed on *Acacia saligna* had fluctuated AST values among time (50 weeks) even it was ranged for the control group from 30.33- 89.00 IU/L. While Alanine aminotransferase (ALT) was insignificant (p>0.05) and ranged from 21.10 to 24.90 IU/L. Similar results were determined by Ibrahim [42]. The present results could be explained according to the hypothesis of Acamovic and Brooker [43] who reported that there is considerable interaction between ingested plant secondary metabolites (PSM) or phytochemicals and tissues, enzymes and other compounds within animals. Makkar *et al*. [44] assumed that the inhibitory or stimulatory effect of tannins on enzyme activity may result from a change in the

Table 6: Dry matter intake, water intake, weight changes and average daily gain of sheep fed the experimental diets.

	experimental groups				
Item	G1	G2	G3	G4	±SE
Roughage intake, kg	0.671	0.477	0,507	0,539	__
Concentrate intake, kg	1,023	0,772	0,881	0,878	__
Total DM intake, kg	1,69	1,25	1,39	1.42	__
Free water intake, L	6.16	4.01	4.95	4.97	__
Initial weight, kg	30.94	31.1	30.07	31.50	1.04
Final weight, kg	57.80[a]	43.20[b]	44.35 [b]	45.54 [b]	2.10
Total gain, kg	26.86[a]	12.10[b]	14.28 [b]	14.04 [b]	1.66
Average daily gain, g/d	149.22[a]	67.22[b]	79.33[b]	78.00 [b]	9.23

a, b, c, means with different superscripts on the same row differ significantly ($P<0.05$).

G1: CFM + Berseem hay

G2: CFM + Acacia hay

G3: CFM + Acacia hay + 5% PEG

G4: CFM + Acacia hay + 4% bentonite

conformation of the enzyme in the presence of tannins leading to a variable of substrate at the catalytic site of the enzyme. In addition, Mousa [31] reported similar results, that there are non of activities of serum ALT and AST of sheep fed acacia.

Dry Matter Intake and Average Daily Gain: Feed intake, average daily gain (ADG) and free water intake are presented in Table (6). It is clear that dry matter intake (DMI) of roughage and concentrate feed mixture (CFM) revealed that animals fed berseem hay (G1) recorded the highest total dry matter intake (TDMI) of roughage and CFM, followed by those in G4, G3 and G2 in descending order. Generally, the highest TT and CT in the acacia groups which reduced feed intake or feed palatability due to the interaction of tannins with saliva protein in mouth resulting astringency taste and binding with digestive enzymes a long total digestive tract so that low digestibility of DM and OM [32]. While, Adding PEG resulted in increasing DMI from *Acacia saligna* [26]. Free water intake was higher in the control group (6.16 L) than the other groups (averaged 4.64 L) which related to high DMI and high ash contents of CFM and berseem hay in G1 (Table 1) than other groups. Data in Table (6) showed that the average daily gain (ADG) was significantly ($p<0.05$) high in animals fed G1 (149.22g/d) followed by animals fed treated acacia with PEG (79.32 g/d) then acacia with bentonite G3 (78.00g/d), while the lowest one was in untreated acacia G2 (67.22 g/d). Generally, growth rate of sheep fed *Acacia saligna* is low and sometimes can be negative [3].

Moreover Ben salem *et al*. [45] reported that DMI and average daily gain (ADG) were 626, 1042 and 1012 g; 15, 54, 28 g/d. for sheep fed on acacia with barley; with atriplex and with atriplex and cactus, respectively. Similar, results were obtained by Younis *et al*. [46] and Youssef *et al*. [25].

CONCLUSION

Finally, using berseem hay as a source of roughage recorded the best digestibility coefficients, nitrogen balance, total dry matter intake, total digestible nutrients (TDN), digestible crude protein (DCP), ADG with normal blood parameters, followed by G3 then G4 and the lowest was G2. Using bentonite as deactivation material may have some opportunities like PEG especially with the low price of bentonite and its activity to adsorb other harmful substances (mycotoxins, pesticides, ammonia) which may be occurred in feedstuffs.

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