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REGULAR ARTICLES

Growth of sheep fed tannin-rich *Albizia gummifera* with or without polyethylene glycol

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Abstract Twenty-four intact male Bonga lambs were studied in a 100-day experiment to evaluate the effect of feeding tannin rich leaves of *Albizia gummifera* (AG) on nutrient utilization, growth performance, and carcass composition. The dietary treatments consisted of hay alone (T_1, control), AG at 30 % of control diet + T_1 (T_2), and T_2 + polyethylene glycol 6000 (PEG) (T_3, 40 mg PEG, 1 kg AG/head on a dry mass (DM) basis. The lambs were individually fed at 50 g DM/kg live weight. In the last 10 days of the experiment, all animals from each treatment were harnessed with feces collection bags. At the end of the experiment, lambs were slaughtered after overnight fasting for measurements of carcass characteristics. Crude protein, neutral detergent fiber, and condensed tannin contents of AG were 300, 586, and 108 g/kg DM, respectively. Lambs fed AG with PEG had higher ($P<0.001$) feed intake, digestibility, carcass weight, and weight gain compared with other treatments. Although lambs fed diet T_2 were able to utilize AG, the efficiency of its utilization was lower ($P<0.001$) than the PEG-treated groups. The improvement in nutrient intake and digestibility, carcass weight, and growth performance of lambs supplemented with PEG emphasizes the negative effect of tannins on livestock performance. Leaves of AG enhance digestibility and performance in tropical hay-fed sheep, and the addition of PEG further improves this effect, likely due to the binding of tannins.

Keywords Carcass characteristics · Growth · Polyethylene glycol 6000 · Sheep · Tannin

Introduction

Feed supply is the most important factor influencing the success of global sheep production. In the tropics, poor quality natural pastures and crop residues are major components of sheep feed (Abubakr et al. 2013; Yisehak et al. 2014). These native pastures and crop residues are generally low in energy and protein content. However, the fiber content (neutral detergent fiber over 70 %) in these feedstuffs is usually so high as to reduce intake and digestibility (Belachew et al. 2013).

The foliage of multipurpose tanniferous species (MPTS) has been suggested to be a reliable substitute either as sole feed or as protein supplements (Yisehak et al. 2012, 2014). MPTS have been used to alleviate crude protein (CP) deficiency in fibrous feeds, reduce rumen acidosis and other health-related problems, and improve intake and productivity of animals (Yisehak et al. 2012). However, the presence of secondary plant compounds, such as condensed tannins (CT), in MPTS could present major constraints to their use (Lamy et al. 2011).

The effects of supplementing high concentrations of condensed tannins (CT) (>50 g CT/kg dry mass (DM) on ruminant nutrition have been studied for several years (Makkar 2003) and are often considered to have a negative impacts. However, there is little information on the ability of sheep breeds to cope with tannin rich diets, such as *A. gummifera* with or without polyethylene glycol 6000 (PEG) (Yisehak et al. 2014). As the inclusion rate of PEG in sheep diets is relatively small (40 mg PEG/kg CT on DM basis), it is particularly suited for use by smallholder farmers. For instance, the increased growth and carcass weight of sheep fed grass-based diets after dietary inclusion of *A. gummifera*

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leaves (rich in CT and CP) can enhance the profits of smallholder farmers in many tropical countries. Therefore, the present study was undertaken to investigate such effects in Bonga sheep breed of Ethiopia.

Materials and methods

Experimental animals, diets, and sampling

Twenty-four intact lambs of the Bonga sheep breed of Ethiopia, with initial body weight and age (mean and S.E.) of 20.5±0.18 kg and 0.83±0.26 years, respectively, were employed in this study. The lambs were randomly divided into three equal groups and assigned to one of the three treatments (after a period of 4 weeks of adaptation and health checks). The treatment groups were control diet (T_1), 30 % tannin rich test diet+T_1 (T_2), and T_2+PEG (T_3).

The lambs were fed on natural pasture hay (comprising a mixture of about 52 % Poaceae, 30 % Asteraceae, 17.5 % Fabaceae, 0.5 % Cyperaceae and Juncaceae) as a control or basal diet. Fresh leaves of *A. gummifera* that were hand-plucked from farm-grown trees and dried under shade were incorporated in the test diet. The total mass offered per day was 50 g DM/kg LW (dry matter/kg live weight) (Osuji et al. 1993). The initial body weight of all lambs was recorded in the feeding trial. Animals were then weighed weekly and the amount of feed offered was adjusted according to changes in their body weight. The amount of feed offered and refused was measured on a daily basis throughout the 100 day experiment. *A. gummifera* leaves were supplied at 08:00 prior to provision of the basal diet at 10:00 in a separate feeding trough. The sheep had free access to clean drinking water and mineral licks. PEG was dissolved in clean drinking water to a concentration of 0.5 g PEG/ml (Yisehak et al. 2014), and the solution were offered to every sheep in the T3 group in the proportion 40 mg PEG to 1 kg of *A. gummifera*. During the last 10 days of the experiment, total collection of feces was carried out by harnessing feces collection bags to all animals. Samples of feces were weighed, subsampled (10 %), and composited by animal, ground (1 mm screen) and kept frozen (−20 °C) until analysis. Frozen feces were allowed to thaw, mixed/agitated, subsampled, and oven-dried at 60 °C for 48 h (Osuji et al. 1993). The dried feces were ground in a Wiley mill through a 1-mm screen and stored in an airtight plastic container at room temperature (avg. 20 °C) until analysis.

Apparent digestibility coefficients ((nutrient intake − fecal excretions)/nutrient intake)×100 %)) were calculated for each animal (Farkhanda et al. 2013). The feed conversion efficiency (FCE) was calculated as the ratio of average daily body weight (BW) gain to daily DM intake (Yeaman et al. 2013). The metabolizable energy intake (MEI) (kJ/kg $BW^{0.75}$) was estimated according to Luo et al. (2004) as: 533+(43.2×ADG (g/kg $BW^{0.75}$)).

Slaughtering and carcass evaluation

At the end of experiment, the sheep were fasted overnight, weighed, and slaughtered to determine carcass composition (SPS-LMM 2006). Immediately after slaughter, nonedible carcasses (skin, head, blood, feet, lung, spleen, testes, pennies, pancreas, and trachea) were removed and weighed. The contents of the digestive tract were washed and weighed separately to obtain the weight of the empty stomach and postruminal tract. The dressing percentage (hot edible carcass weight/empty body weight)×100) for each animal was determined based on the measured empty BW.

Chemical analysis of feed and fecal samples

The leaves of *A. gummifera* and the basal diet were analyzed for DM, CP, crude ash, and ether extract (EE) (AOAC 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined sequentially (ADF after NDF, two different methods were used on the same laboratory subsample). Lignin content was determined by solubilization of cellulose with 72 % H_2SO_4. The content of hemicellulose was calculated as NDF − ADF and cellulose as ADF − lignin (Van Soest and Robertson 1985).

Determination of total extractable CT was based on oxidative depolymerization of CTs in butanol-HCl reagent using 2 % ferric ammonium sulfate in 2N HCl catalyst (Porter et al. 1986).

Chemical analysis of the dried feces samples (except N) followed standard procedures of the AOAC (2005). Nonoven-dried but thawed and well-mixed feces were used directly for N analyses. All chemical analyses were carried out in triplicate.

Statistical analysis

The data were subjected to one-way analysis of variance following general linear model procedures contained within the Statistical Analysis System software (SAS 2010, version 9.3). Differences between treatment means were compared using Duncan's multiple range test. Differences were deemed significant when $P<0.05$, whereas $0.05<P<0.10$ was considered to show a statistical tendency for difference. Levene's test was carried out to detect the homogeneity of experimental variances. Based on this test, there was no evidence that the residuals (i.e., errors) were not normally distributed.

Table 1 Chemical composition of feedstuffs offered to sheep (g/kg DM)

Feed stuff	DM	OM	Ash	CP	EE	CF	NDF	ADF	ADL	HC	CLL	CT
AG	908	950	50	300	10	380	586	451	189	135	262	108
Hay	895	884	116	84	14	365	638	516	123	122	393	BDL

AG *Albizia gummifera*, *DM* dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *CF* crude fiber, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *ADL* acid detergent lignin, *HC* hemicelluloses, *CLL* cellulose, *BDL* below determination limit

Results

The chemical compositions of the feedstuffs included in the trial are presented in Table 1. Although the *A. gummifera* leaves contained a high amount of CT (108 g/kg DM), its CP content was 275 % higher than the basal feed.

The mean daily DM intake (DMI, Fig. 1) during the first 90 days of feeding was higher for the lambs supplemented with either *A. gummifera* (T_2) or *A. gummifera* + PEG (T_3) compared to the control diet, i.e., hay alone, (T_1) ($P<0.01$) (Table 2). Similar to DMI, the average daily weight gain (ADG) tended to differ among the treatments, with the highest and lowest ADG values obtained for T_3 and T_1, respectively (Fig. 2).

Lambs fed T_3 showed an increased DMI by 68 % (1,060 g/day) compared to T_1 (630 g/day). Similarly, *A. gummifera* supplementation (T_2) improved the CP intake by 260 % over CP intake for T_1. *A. gummifera* supplementation also improved the NDF intake in T_2 (560 g/day) compared to T_1 (400 g/day) ($P<0.01$; 44 %). The PEG-supplemented lambs had the highest ($P<0.001$) ADG, followed by T_2, while the lowest ADG was recorded for T_1 ($P<0.001$). The lowest FCE (0.48) and protein efficiency ratio (PER) (0.04) were obtained for T_1, whereas the highest FCE and PER values were recorded for T_3 (3.35 and 0.21, respectively) ($P<0.001$).

Similar to nutrient intake, digestibility coefficient values were higher ($P<0.05$) for lambs that received T_2 or T_3 compared to the T_1 group (Table 3). Although lambs had better digestibility coefficients under T_2, supplementation of PEG further increased apparent digestibility of all nutrients determined under T_3 ($P<0.01$).

The mean values of edible and nonedible carcass components were higher ($P<0.05$) for lambs supplemented with *A. gummifera* with or without PEG compared to those receiving T_1. The highest carcass weight (16.25 kg) and dressing percentage (42.81 %) were obtained under T_3 ($P<0.05$) (Table 4).

Discussion

The CP content calculated for *A. gummifera* was much higher than the minimum CP level required for optimum functioning of the rumen (70 g CP/kg DM, Belachew et al. 2013). The crude protein content of *A. gummifera* also fulfilled the minimum CP concentration required for growth and multiplication of rumen bacteria in ruminants. Although feeds containing less than 70 g CP/kg DM cannot provide the minimum ammonia levels required by rumen microorganisms to support optimum activity, leaves of *A. gummifera* may be a suitable supplement for low CP feeds provided that the CT was deactivated biologically.

Although the Van Soest procedures are not the most precise method for NDF and ADF determination in tanniferous forages (Makkar 2003), the feedstuffs utilized in the present study contained relatively high NDF contents (average >580 g/kg DM). Tanniferous forages with lower NDF contents (200–350 g NDF/kg) have been shown to promote high digestibility of nutrients (Yisehak et al. 2014). Therefore, the

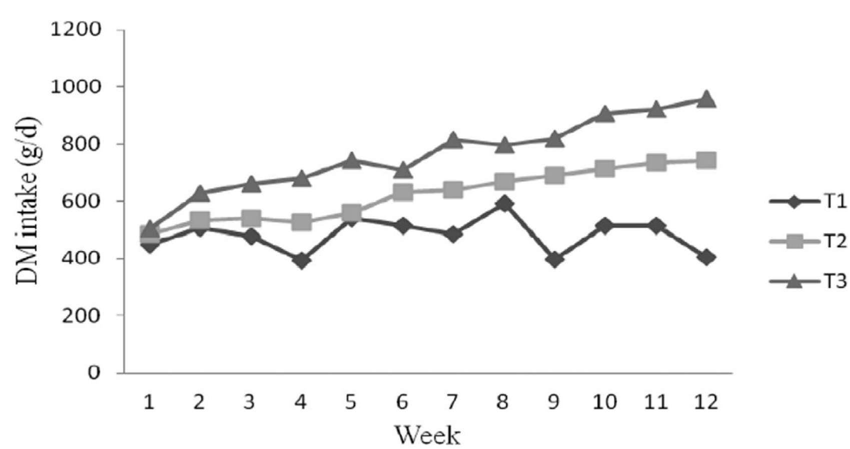

Fig. 1 Average weekly dry matter intake of sheep (g/day); *T1* treatment 1, *T2* treatment 2, *T3* treatment 3

Table 2 Nutrient intake, live weight gain, and protein efficiency ratio of lambs supplemented *A. gummifera* with or without polyethylene glycol during collection period (N=8)

Nutrient intake	Treatment (T), mean			SE	*P* value	%Diff		
	T_1	T_2	T_3			T_1, T_2	T_1, T_3	T_2, T_3
DM (g/day)	630^c	910^b	1060^a	2.42	***	44	68	17
CP (g/day)	50^c	180^b	200^a	4.55	***	260	300	11
EE (g/day)	9.0^c	11.0^b	13.0^a	0.24	*	22	44	9
OM (g/day)	560^c	810^b	940^a	1.86	**	45	68	16
ME (MJ/day)	0.66^c	1.14^b	2.04^a	0.05	***	73	209	79
NDF (g/day)	400^b	560^b	650^a	1.23	***	40	63	16
ADG (g/day)	3.0^c	14.0^b	35.0^a	2.44	***	367	1,067	150
FCE (%)	0.49^c	1.56^b	3.32^a	0.03	***	218	578	113
PER	0.06^c	0.08^b	0.18^a	0.01	***	30	192	125

ME metabolizable energy, *ADG* average daily body weight gain, *FCE* feed conversion efficiency (%, g LW gain/g DM intake×100), *PER* protein efficiency ratio (g ADG/g CP intake), *SE* standard error of means, *%diff*, percent difference between net increment or reduction minus the base times 100 divided by the base

*P<0.05; **P<0.01; ***P<0.00

[a,b,c] Same row with different letters point to statistical significance

minimum NDF content of a ration should be in the range 270–300 g/kg (Krizsan and Huhtanen 2012).

High CT levels are highly detrimental as they reduce the digestibility of fiber in the rumen (Lamy et al. 2011) by inhibiting the activity of bacteria and anaerobic fungi (Alonso-Díaz et al. 2010) and also lead to reduced intake (Lamy et al. 2011). Even though the *A. gummifera* leaves had a CT level above the threshold value that can affect sheep, the results of this study suggest that sheep in tropical regions are tolerant to tannin rich diets. Further, the inclusion of PEG in the *A. gummifera* leaf diet appeared to improve its utilization by neutralizing the negative effects of CTs in sheep.

The remarkable improvement in DMI under the *A. gummifera* diet with PEG addition (T_3) highlights the negative effects of CT on DMI (Fig. 1). The lower DMI of lambs receiving T_2 without PEG as compared to T_3 might principally stem from the inhibitory effects of a high CT content on digestion, with palatability having a minor influence on DMI.

The increased DMI under *A. gummifera* leaf supplementation (Table 2) may be attributed to the higher intake of CP, leading to more efficient fiber utilization in the total diet.

Supplementation of *A. gummifera* leaves at levels of 30 % of the basal diet (i.e., T_2 and T_3) did not cause toxicity problems or death of lambs within the 100 days feeding period. This might reflect the natural adaptation of tropical sheep to tannin rich diets. Moreover, the better feed intake and growth performance of lambs receiving the *A. gummifera* diet

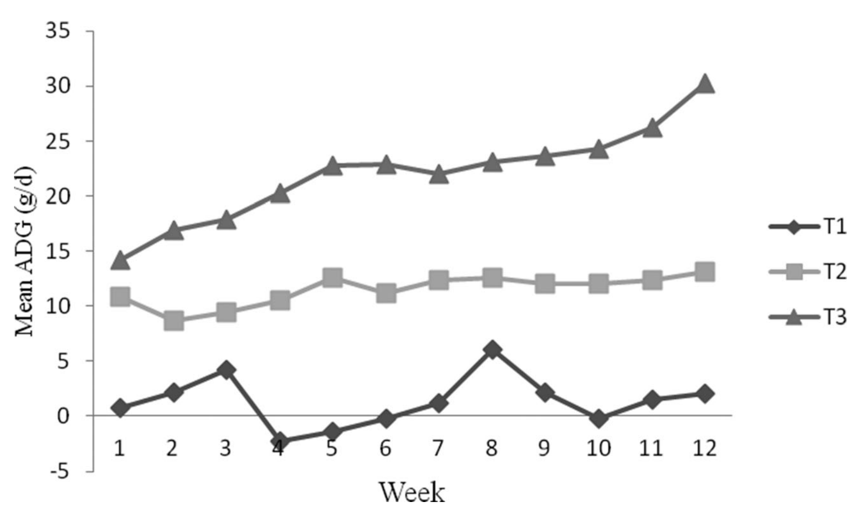

Fig. 2 Average weekly liveweight gain of sheep (g/day); *T1* treatment 1, *T2* treatment 2, *T3* treatment 3

Table 3 Least square mean values for apparent nutrient digestibility coefficients in lambs fed a grass hay-based diet with or without *A. gummifera* leaves with or without polyethylene glycol 6000 (N=8)

Nutrients	Treatment, mean (%)			SE	*P* value	%Diff		
	T_1	T_2	T_3			T_1, T_2	T_1, T_3	T_2, T_3
Dry matter	52^{c}	59^{b}	68^{a}	1.64	***	14	31	15
Organic matter	56^{c}	66^{b}	71^{a}	1.91	***	18	27	8
Crude protein	59^{c}	68^{b}	75^{a}	1.82	***	15	27	10
Ether extract	53^{c}	63^{b}	70^{a}	2.21	***	19	32	11
Acid detergent fiber	44^{c}	49^{b}	52^{a}	1.10	***	11	18	6
Neutral detergent fiber	47^{b}	53^{b}	60^{a}	0.99	***	13	28	13

SE standard error of means

***P< 0.001

[a,b,c] Same row with different letters point to statistical significance

with PEG could be linked to the ability of PEG to bind CTs as a replacement for protein and displace protein from tannin: protein complexes.

Table 4 Weights of carcass components of lambs fed a grass-based diet with or without *A. gummifera* leaves with or without polyethylene glycol

Traits	Treatment, mean			SE	*P* value
	T_1	T_2	T_3		
(a) Edible carcasses					
Hot carcass weight (kg) (Empty body weight)	13.55^{c}	14.30^{b}	16.25^{a}	0.30	**
Liver (g)	221.72^{b}	245.33^{b}	269.64a	0.59	**
Heart (g)	91.06^{c}	99.67^{b}	107.55^{a}	1.60	**
Empty gut (kg)	3.03	3.20	3.51	0.12	NS
Kidney without fat (g)	138.09	141.68	149.70	2.10	NS
Heart without fat (g)	78.05^{b}	85.01^{a}	86.05^{a}	0.95	*
Tail fat (g)	283.37^{c}	307.08^{b}	380.67^{a}	9.16	***
Rib fat (g)	2.50^{c}	6.30^{b}	13.50^{a}	1.02	***
Pelvic fat (g)	3.65^{c}	15.10^{b}	22.61^{a}	0.80	***
Dressing percentage	28.73^{b}	33.68^{b}	42.81^{a}	1.70	**
Intestinal fat (g)	129.08^{b}	167.68^{a}	172.21^{a}	4.34	***
(b) Nonedible carcass					
Skin (kg)	3.56^{b}	4.03^{a}	4.10^{a}	0.06	NS
Spleen (g)	49.12^{c}	52.10^{b}	57.56^{a}	0.87	***
Testicle (g)	220.54^{b}	237.86^{b}	306.30^{a}	9.83	***
Head (kg)	2.25^{c}	2.60^{b}	2.80^{a}	0.05	**
Blood (kg)	1.76	1.79	2.06	0.03	NS
Lung (g)	426.13	436.68	456.63	12.22	NS
Full gut (kg)	7.50^{c}	8.67^{b}	11.67^{a}	0.37	***

SE standard error of means, *NS* non significance difference (P>0.05)

*P<0.05;**P<0.01;***P<0.001

[a,b,c] Same row with different letters point to statistical significance (P<0.05)

The highest ADG recorded for T_3 over T_2 or T_1 might be due to increased nutrient concentrations obtained through intake of *A. gummifera* with PEG. The present study also confirmed that supplementation of a nutritionally poor roughage diet with CT and CP rich leaves markedly improved the ADG of lambs. The difference in ADG might be partly related to a higher intake of N and energy.

The higher FCE in lambs receiving T_2 and T_3 compared to T_1 could be associated with better utilization of the essential nutrients. Higher FCE values have been shown to follow better growth performance of an animal. The highest FCE recorded for the lambs receiving PEG may be attributed to deactivation of the CTs, which in turn allows animals to utilize potential nutrients from the tannin rich diet.

The lowest digestibility coefficient for nutrients in T_1 (41–56 % indigestibility) compared to T_2 (32–51 %) or T_3 (25–48 % indigestibility) might be partly explained by the high fiber and relatively low metabolizable protein content in the T_1 diet (Table 3). Tannins depress the fiber and N digestibility of MPTs (Makkar 2003). The lower digestibility of CP in the T_1 compared to T_2 or T_3 might not only be related to the lower CP content of the basal diet but also lower digestive utilization of N or higher microbial synthesis in the lower gut. There was a great improvement in CP digestibility under T_2 compared to T_1, which may indicate that the studied sheep breed has developed adaptative mechanisms to CT rich diets in a tropical environment. The lower CP digestibility in T_2 compared to T_3 might be explained the fact that tannins reduce protein digestion by forming complexes with dietary protein and digestive enzymes in T_2, while energy digestibility in the rumen is reduced by inhibition of microbial enzymes by CT (Lamy et al. 2011). The further improvement in CP digestibility in T_3 may be attributed to the tannin deactivating effects of PEG.

The relatively high dressing percentage values in T_3 over T_2 in the present study indicate that treating CT rich diets with PEG positively improves the growth performance and weight addition of sheep. The higher dressing percentage values in T_2 over T_1 imply that supplementation of sheep with a protein and CT rich diet source has nutritional potential to improve carcass parameters through improved feed intake and digestibility of the basal diet. This may further indicate that the Bonga sheep breed of Ethiopia is better able to convert a CT-rich diet to carcass parameters. Moreover, it has previously been shown that by improving the feeding value of a diet, it is possible to obtain heavy and fleshy carcasses (Alexandre et al. 2010). Polyethylene glycol 6000 supplementation to the tannin rich diet improved the dressing percentage of the lambs from 29 to 43 %. The ordinary dressing coefficient for sheep generally falls between 40–50 % (Hirut et al. 2011).

Conclusion

The *A. gummifera* leaf diet has a fairly good feeding value for sheep that are naturally adapted to tannin-rich feed resources. However, its feeding value is further improved when its tannin content is biologically inactivated using a tannin-deactivating agent such as polyethylene glycol 6000. Thus, polyethylene glycol 6000 could potentially be used by smallholder farmers in the tropics to detannify the new and potential tanniferous foliages prior to feeding sheep or other classes of ruminants so as to improve nutrient utilization and protect animals from long-term effects of condensed tannins.

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Conflict of interest The authors declare that they have no conflict of interest.

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