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Research Article

In vitro Dry Matter Digestibility and *in vitro* Gas Production of Some Acacia Seeds Treated with Sodium Hydroxide and Poly Ethylene Glycol

Balgees, A. Atta Elmnan and Moawia Mohamed Sharaf Ali

Department of Animal Nutrition, Faculty of Animal Production, University of Khartoum, 13314 Khartoum, Sudan

Abstract

Objectives: The study was carried out to determine the nutritive value and the effect of sodium hydroxide (NaOH) and poly ethylene glycol (PEG) treatments on *in-vitro* dry matter digestibility (IVDMD) and gas production parameters of some Acacia seeds. **Materials and Methods:** Seeds of *Acacia mellifea* (*A. mellifea*), *Acacia senegal* (*A. senegal*), *Acacia tortillis* (*A. tortillis*), *Acacia seyal* (*A. seyal*) and *Acacia nilotica* (*A. nilotica*) were collected from El-Gadaref State-East Sudan. Samples of each seeds were milled, and divided into 3 groups and assign to one of the three treatments; untreated seeds (control seeds), 0.2% PEG treatment (PEG seeds) and 0.2% NaOH treatment (NaOH seeds). **Results:** The results revealed that all of acacia seeds under study contained appreciable amount of crude protein (CP), which range from 16.68 to 22.57%. *A. mellifea* seeds had higher level of CP, lower level of crude fiber (CF) and tannin than the other seeds. Contrary *Acacia nilotica* seeds contained high level of CF, tannin and low amount of CP. PEG seeds and NaOH seeds had higher IVDMD and gas production than control seeds. The correlation study indicated that the IVDMD of acacia seeds were positively correlated with CP and negatively with tannins and CF content. **Conclusion:** It is concluded that the PEG and NaOH treatments had a great potential to improve the IVDMD and gas production for acacia seeds with superiority to the PEG treatment.

Key words: *A. mellifea*, *A. senegal*, *A. tortillis*, *A. seyal*, *A. nilotica* seeds, sodium hydroxide and poly ethylene glycol, *In vitro* dry matter digestibility, gas production

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Corresponding Author: Balgees, A. Atta Elmnan, Department of Animal Nutrition- Faculty of Animal Production, University of Khartoum, 13314 Khartoum, Sudan

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Providing feed throughout the year is a major constraint in animal production of the tropics. Natural pasture especially during the dry season and crop residues have low nutritive value in term of low crude protein, digestible nutrients and high fiber[1]. Sudan has huge numbers of livestock and browsing trees which are important source of forage for small ruminant and camel. Nowadays, fodder trees and shrubs considered as promising energy and protein sources to be utilized in the different animal production system. They have high potential to fill the gap of the shortage and nutritional deficiencies during dry season on small holder farm[2]. Tropic areas have a large number of legume fodder trees; their seeds could enhance the consumption of poor quality roughage for animals during dry season. However; the majority of these seeds contain anti-nutritional agents such as tannins which are unpalatable or toxic for animals and rumen microorganisms, thus limiting their use as feeds for animals[3,4].

Acacia seeds are promising feed in the tropics due to its capability to thrive in dry zones of the world[5] but the presence of tannin could be the major constraints to be used as feedstuff. The basic anti-nutritional factor in Acacia species and many other browse species are condensed tannins (CT)[6]. Tannins are a polyphenolic compound with high molecular weights and a various complexity[7]. There are different recommended treatments to eliminate the effect of high content of condensed tannin in browse species. For instance, PEG or NaOH treatment which are effectively bind with condensed tannin have been suggested by many researchers[8].

The study was carried out to determine the nutritive value and to assess the effect of PEG or NaOH treatment on *in vitro* dry matter digestibility and gas production of some acacia seeds collected from El-Gadaref State- East Sudan.

MATERIALS AND METHODS

Site of the study: The study was carried out at the laboratory of Department of Animal Nutrition, Faculty of Animal production, University of Khartoum.

Samples collection and preparation: Seeds samples were collected from 10 trees of five Acacias Spp. from EL-Gedaref State-Sudan (Table 1). Seed samples were cleaned by removing foreign particles then about 5000 g of each seeds were weighed, milled by a laboratory miller to 1 mm screen and kept at room temperature for further analysis and treatments. Then seed samples for each spp. were divided into 3 groups and each one was treated with PEG and NaOH at zero level for making control seeds, 0.2% of PEG glycol for making PEG seeds and 0.2 sodium hydroxide for making NaOH seeds. Each treatment had 4 replicates.

Table 1: Botanical and local name of trees sample

Botanical name	Local name
A. mellifera	Ketir
A. senegal	Hashab
A. torrtlis	Seyal
A. seyal	Taleh
A. nilotica	Sonut

Chemical analysis: The chemical composition of the seeds [crude protein (CP), crude fiber (CF), ether extract (EE) and ash] was analyzed according to AOAC[9]. Nitrogen free extract (NFE) was calculated as follow:

$$\text{NFE\% (DM)} = \{\text{DM}-(\text{EE\%}+\text{CP\%}+\text{CF\%}+\text{ash\%})\}$$

Determination of tannin: The total condensed tannin was determined by the butanol-HCl methods[10]. The reagent was prepared just at need by mixing equal volumes of 1% vanillin methanol and 8% HCl methanol. It was discarded if color appeared. Catechin was used to prepare the standard curve; this was done by adding 600 mg of catechin to 100 mL of 1% HCl methanol. From this stock solution various dilutions were prepared. 5 mL vanillin/HCl reagent was added to 1 mL of each dilution, incubated at 30°C for 20 min. After that the absorbance was read using spectrophotometer (DR3 spectrophotometer) at 500 nm. The absorbance was plotted against catechin concentration.

Weighted of 0.2 g of the sample was placed in a tube, then 10 mL of 1% HCl/methanol was added. The test tube was capped and continuously shaken for 20 min and then centrifuged at 2500 rpm for 5 min. One milliliter of the supernatant was pipetted into each of the tube and then proceeding as was described in the standard curve above. For zero setting prior absorbance was read,1 mL blank solution was mixed with 5 mL (4%) concentrated HCl in methanol and 5 mL of vanillin in a test tube. The absorption was read at 500 nm and the concentration of condensed tannin as catechin equivalent was calculated as follows:

$$\text{Tannin (\%)} = \frac{C \times V \times 100}{W}$$

Where:

C = Concentration corresponding to the optical density
V = Volume of extract (mL)
W = Weight of the sample (mg)

***In vitro* dry matter digestibility (IVDMD):** Dry matter samples from each sample were subjected to 48 h microbial digestion period with Mc Douglass buffer, rumen fluid mixture in sealed plastic bottles, followed by 48 h digestion with pepsin in week acid[11].

***In vitro* gas production:** The *in vitro* gas production was carried out using the method described by Menke and Steingass[12]. Rumen fluid was collected into pre-warmed insulted bottles from a healthy adult steer weighing 250-300 kg, fed on sorghum straw and commercial concentrate mixture diet. It was strained trough 4 layers of cheesecloth and stored in thermos container saturated with carbon dioxide (CO_2) and maintained at 39°C. Then well mixed CO_2 flushed rumen fluid was added to the buffer solution (1:2 v/v), which was maintained in a water both at 39°C. Buffered rumen fluid (30 mL) was pipetted into each syringe, containing the feed samples and the syringes were immediately placed into the water bath at 39°C. Two syringes with only buffer rumen fluid were incubated and considered as blanks. The syringes were gently shaken every 2 h and the incubation terminated after recoding the 72 h gas volume. The gas production was recorded after 3, 6, 12, 24, 48 and 72 h of incubation. Total gas values were corrected for the blank incubation and reported gas values are expressed in mL mL^{-1} 200 mg of DM.

Statistical analysis: Data obtained was subjected to one way analysis of variance (ANOVA) for a completely randomized design (CRD)[13]. Where the F test was significant; the treatment means were compared using least significant different (LSD). A p-value used for the determination of statistical significance was 0.05.

RESULTS AND DISCUSSION

Chemical composition: Table 2 shows the results of chemical compositions of seed samples under study. The dry matter of different seeds was rich in CP content, which ranged from 16.68 to 22.57%. Therefore, these seeds could be used as protein source to improve the consumption of poor quality roughages for animals during dry season. Among the seeds under study, *A. mellifera* seeds had the highest amount of CP, this result was similar to a previous study conducted by Adewusi *et al.*[14] who stated a range of 15.6-24%.

Analysis of the data indicated that there was a significant ($p\leq 0.05$) variation among the seeds on EE level. *A. mellifera* seeds contained high percent of EE compared with the rest of investigated seeds. The present finding of EE was higher than those reported by Elginaid[15] who mentioned a range of 0.84-3.90% for acacia seeds.

Low ash content of acacia seeds was observed in the current study. These results are different from the results obtained by Adewusi *et al.*[14] who reported a range of 6.4-9.6%. This difference between two studies could be attributed to the type of soil[16].

Although all seeds under study had high content of NFE, *A. seyal* recorded the highest value. Similar result was obtained by Adewusi *et al.*[14] who reported that *A. seyal* contained 54.8% of NFE. The high level of NFE of acacia seeds indicated that it can be ranked and used as a carbohydrate rich feed for animals.

Range of CF content of seeds under study was lower than the results of previous study conducted by Kebede *et al.*[17] who reported a range of 19.54-31.11%. *A. nilotica* seeds contained higher amount of CF than the other seeds under study. These variations could be attributed to the positive correlation between total condensed tannins and CF content[18,19]. Therefore, the higher amount of tannin content was noted for *A. nilotica* seeds than other seeds. This result was near to previous study conducted by Khazaal and Orskov[18] who reported a value of 8.2%.

***In vitro* dry matter digestibility:** The results of *in vitro* dry matter digestibility (IVDMD) for different seeds were given in Table 3. The treated seeds had higher IVDMD than untreated (control) seeds, this result could be due to reaction between PEG or NaOH with tannin, which provide a suitable

Table 2: Chemical composition and tannin content (%) of some *Acacia* seeds

Sample	DM	CP	EE	CF	Ash	NFE	Tannin
A. mellifera	95.68^a	22.57^a	8.89^a	12.54^d	4.35^c	47.33^e	4.20^d
A. senegal	95.51^b	21.61^b	4.75^c	15.48^c	5.45^a	48.22^d	4.70^d
A. torrtlis	93.18^c	21.53^b	3.96^d	14.16^c	3.55^d	49.98^b	5.27^c
A. seyal	95.84^a	18.30^c	4.15^{cd}	15.80^c	4.80^b	52.79^a	6.32^b
A. nilotica	95.63^b	16.68^d	5.27^b	20.59^a	4.20^c	48.89^c	7.60^a
SEM	0.12	0.18	0.06	0.10	0.11	0.15	0.04

CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, Ash: Ash content, Tannin: Tannin content, a-d: Values in the same column with different superscript are significantly different ($p\leq 0.01$) and SEM: Standard error means

Table 3: Effect of NaOH and PEG treatments of some *Acacia* seeds on *in vitro* dry matter digestibility (%)

	Treatments		
Acacia spp.	Control	NaOH	PEG
A. mellifera	43.40[b]	57.30[b]	66.00[d]
A. senegal	43.30[b]	58.80[a]	75.30[a]
A. torrtlis	45.50[a]	56.70[c]	72.40[b]
A. seyal	42.70[c]	54.90[d]	69.00[c]
A. nilotica	42.20[cd]	51.00[e]	60.40[e]
SEM	0.42	0.19	0.23
Over mean of treatments	43.42[c]	55.74[b]	68.62[a]

[a-d]Values in the same column for acacia seeds with different superscripts are significantly different ($p \leq 0.05$) and SEM: Standard error of the means. [a-c]Values in the same row for treatment with different superscripts are significantly different ($p<0.05$)

environment for microbes to ferment feed materials[20]. Among the un-treated samples, the highest value of IVDMD was acquired by *A. torrtlis*, while the lowest value recorded by *A. nilotica*. This result could be justified by the low CP, high CF and tannin content of *A. nilotica* seeds. Odenyo *et al.*[21] found that there was a positive correlation between CP content and IVDMD of some browses. Also Adewusi *et al.*[14] reported that the increase in CF content in diet resulted in decreases in DM digestibility.

Gas production parameters: Results of gas production during fermentation period indicated that the cumulative volume of gas production increased with increasing incubation time. Figure 1 shows the gas production (mL/200 mg DM) of control samples at different time of incubation period. For the control samples, at the end of incubation time (72 h) *A. mellifera* seeds produced more gas compared with other samples. This result could be attributed to relatively low phenols compound and CF in these species[22].

The highest gas production for treated seeds was recorded by *A. mellifera* for NaOH treatment and *A. Senegal* for PEG treatment (Fig. 2, 3), respectively. *A. senegal* seeds at different incubation time acquired the highest gas volume among the different species after treated by PEG.

The gas production of all samples slightly increased from 3-12 h, then increased exponentially from 12-72 h for PEG and NaOH treatments. Among the treated seeds *A. nilotica* seeds recorded low gas volume, this might be explained by the presence of high tannin and CF content in this seeds. Feeds contain high amount of fiber are known to be less degradable than feeds that contain high amount of soluble carbohydrates[2,23,24]. The adverse effect of the cell wall constituent on gas production may be due to the reduction of the microbial activity through increasing un-favorable environmental conditions[25].

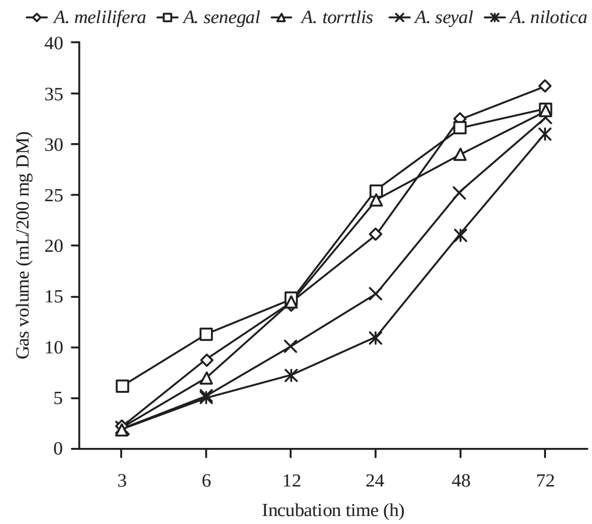

Fig. 1: Gas Production (mL/200 mg DM) of untreated samples

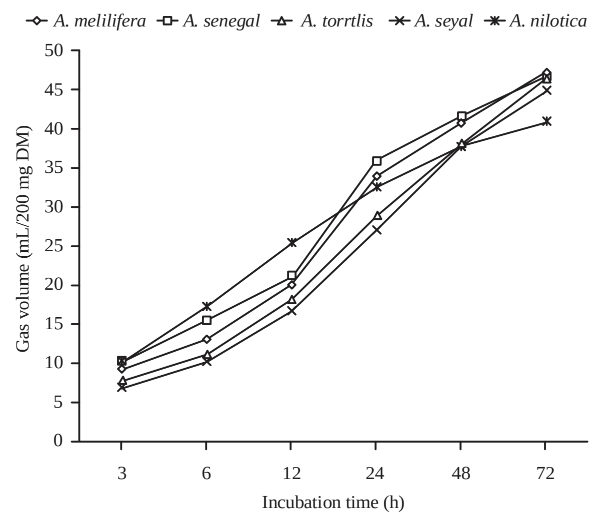

Fig. 2: Gas production (mL/200 mg DM) of seed samples treated with NaOH

The fermentable fractions of gas production of different sample species were presented in Table 4. Among untreated seeds the *A. senegal* gained the high fermentable fraction (b) and potential gas production (a+b), this result may be due to its high CP content. Atta Elmnan and Alamin[26] reported that there is a positive correlation between CP content and gas production.

The fermentable fraction (b) and potential gas production (a+b) were higher for treated seeds than the control seeds. This result could be attributed to the effect of PEG and NaOH on the phenolics compound that complex with protein. Since protein molecule becomes free, the microbe easily will ferment protein which lead to increase the production of gas.

Table 4: Effect of NaOH and PEG treatments of some Acacia seeds on gas production parameters

	A			b			a+b			c		
Treatment	Control	NaOH	PEG	Control	NaOH	PEG	Control	NaOH	PEG	Control	NaOH	PEG
A. mellifera	-1.00^{l}	3.50^{fg}	9.80^{a}	35.50^{h}	44.80^{e}	51.20^{c}	34.50^{i}	48.30^{f}	61.00^{c}	0.04^{d}	0.04^{d}	0.02^{f}
A. Senegal	2.23^{i}	4.40^{d}	8.40^{b}	36.90^{g}	42.80^{f}	59.60^{b}	38.80^{h}	47.90^{f}	68.10^{a}	0.04^{d}	0.05^{c}	0.03^{e}
A. torrtlis	-3.00^{m}	3.80^{ef}	6.30^{c}	36.60^{g}	46.90^{d}	62.70^{a}	34.20^{i}	50.80^{e}	69.50^{a}	0.06^{b}	0.03^{e}	0.03^{e}
A. seyal	0.50^{k}	2.90^{h}	3.20^{gh}	35.40^{h}	38.50^{g}	60.10^{b}	26.70^{j}	42.40^{g}	63.30^{b}	0.02^{f}	0.03^{e}	0.04^{d}
A. nilotica	1.40^{j}	4.10^{de}	-1.10^{l}	23.90^{i}	35.60^{h}	59.40^{b}	25.00^{k}	39.80^{h}	58.20^{d}	0.02^{f}	0.07^{a}	0.03^{e}
SEM±	0.07	0.23	0.24	0.04	0.07	0.23	0.24	0.04	0.07	0.23	0.24	0.03

a: Readily fermentable fraction; b: slow fermentable fraction, a+b: Potential fermentable, c: Rate of fermentation, a-m: Values with different superscripts are significantly different ($p \leq 0.01$) and SEM: Standard error means

Table 5: Correlations between crude protein, crude fiber, tannin and IVDMD

	CF	Tannin	IVDMD	CP
CP	-0. 93**	-0.84**	0.97**	1
IVDMD	-0.90**	-0.92**	1	
Tannin	0.80**	1		
CF	1			

CP: Crude protein, CF: Crude fiber, IVDMD: *in vitro* dry matter digestibility **Correlation is significant at the 0.01 level

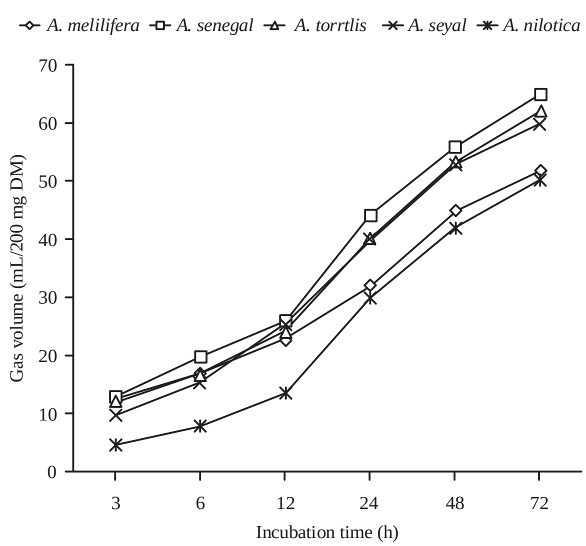

Fig. 3: Gas production (mL/200 mg DM) of seed samples treated with PEG

This result agrees with an earlier study conducted by Singh *et al.*[8]. Moreover, PEG seeds gained higher fermentable fraction (b) and potential gas production (a+b) than NaOH seeds. This result may be due to the high affinity of PEG which breaks down formed tannin- protein complexes[27,28]. The rate of fermentation (c) was higher for the NaOH treatment than the control and PEG treatment. This result could be attributed to the fact that PEG had no effect on the rate of fermentation. Similar results were reported by Kamalak *et al.*[28,29] and Getachew *et al.*[30] who concluded that the PEG supplementation had no effect on the rate of fermentation (c).

Table 5 shows the Pearson Correlations between CP, CF, tannin and IVDMD. Total condensed tannins were negatively correlated with CP, IVDMD and positively with CF. Moreover, the digestibility of acacia seeds was positively correlated with CP content and negatively with CF. The same results were demonstrated by Adewusi *et al.*[14] and Odenyo *et al.*[21], who found that there was a positive correlation between CP content and IVDMD of some browses. High level of tannin causes over protection of protein and attachment of microbes to feed particles resulting in low utilization of nitrogen and hence decreases the IVDMD[31].

CONCLUSION

According to the current study; *A. mellifera* seeds contained high level of CP, EE, low content of CF and tannin compared with other seeds. Contrary, *A. nilotica* seeds contained low level of CP, high content of CF and tannin compared with other seeds. Although all of the Acacia seeds under study had appreciable amount of nutrients, the presence of tannin may limit their use as animal feed. The present study indicated that PEG and NaOH treatments had great potential to improve the IVDMD and gas production for acacia seeds with superiority to the PEG treatment. Therefore, in order to alleviate some of the shortage and nutritional deficiencies during dry season, the present study suggested that the acacia seeds should be treated with PEG to eliminate the effect of tannin.

SIGNIFICANCE STATEMENT

This study discovers the positive effect of PEG and NaOH treatment that can beneficial to eliminate tannin effect of some acacia seeds. Moreover, this study determined the nutritive value of some negligible acacia seeds (*Acacia mellifea* and *Acacia tortillis*). Moreover, this study will help the producer of animal production at El-Gadaref State-East Sudan to use the abundant amount of these seeds effectively during dry season.

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