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Nutritive Value Assessment of *Acacia* Species Using Their Chemical Analyses and *in Vitro* Gas Production Technique

Al-Soqeer A.A.

Department of Plant Production and Protection,
Faculty of Agriculture and Veterinary Medicine, Qassim University
Buriedah-51452, P.O. 6622, Saudi Arabia.

Abstract: To evaluate the nutritional value of the fresh shoots of 14 *Acacia* species (*A. coriacea, A. cuthbertsonii, A. ineguilatera, A. iteaphylla, A. kempeana, A. ligulata, A. microbotrya, A. nilotica, A. oswaldii, A. pruinocarpa, A. saligna , A. sclerosperma, A. seyal* and *A. Victoria*) that imported to be cultivated in Saudi Arabia, this study aimed to determine the potentiality of these species as ruminant food resources in comparison with alfalfa hay and wheat straw. Chemical analysis and *in vitro* gas production technique were used as the base for that evaluation. The crude protein content (CP) among tested *Acacia* spp. ranged from 8.0 to 16.7 % and it was comparable in *A. iteaphylla* with that of alfalfa hay, the values were 16.7 and 17.1% in *A. iteaphyllaI* and alfalfa hay, respectively. Among the tested *Acacia* spp, *A. ineguilatera* had the highest condensed tannins (CT) value, which ranged from 10.4 to 77.0 mg/g DM. Metabolizabole energy (ME, MJ/ kg DM) of tested *Acacia* spp. ranged from 4.35 to 6.69 MJ/ kg DM, which could supply the animals with the 53-84% of ME as in alfalfa hay. The CT/CP ratio in *Acacia* spp. found to have a negative correlation (r= 0.72, P <0.001) with either organic matter digestibility (OMD, %) or with ME (r= 0.80, P <0.001). To ease the evaluation of nutritive value of *Acacia* spp. the following equations might be applied, OMD (%) = 36.5 + 0.02CP + 0.3NDF - 0.75CT; (r = 0.75; P < 0.001); ME (MJ/kg DM) = 5.8 + 0.14CP + 0.02NDF - 0.72CT; (r = 0.79; P <0.001), where CP and NDF are percentage of DM and CT as mg/g DM.

Key words: Acacia species; chemical composition; nutritive assessment; gas production technique

INTRODUCTION

Browse legumes are commonly used to overcome the low nitrogen content of ruminant diets in tropical regions that is caused by the high cost of protein sources and their demand as human food[18]. Leguminous trees and shrubs are widely used as fodder for livestock in the tropics and subtropics of the world, and only a few of the 900 *Acacia* genus are extensively cultivated for fodder[11]. During the prolonged season of about 8 months in a year especially draught years, *Acacia* species server as source of much need nutrients to domestic herbivores. Browse legumes are a very heterogeneous group of plants, with crude protein (CP) ranging from 81 to 306 g/kg dry matter (DM), with variable rumen degradable and intestinally digestible fractions[19]. Moreover, browse legume species have a substantive content of fermentable carbohydrate[38,21,12] that yields volatile fatty acids as an energy source for the animal.

The nutritive value of browse legumes depends on their nutrient composition, ruminal and post-ruminal digestibility, and on the presence of secondary compounds that may interact with the rumen microbial population, thereby limiting nutrients utilization[22,7].

The Fermentation pattern of forages can be estimated *in vitro* by gas production techniques[27,39]. Moreover, Menke and Steingass[27] found a strong correlation between metabolizable energy (ME) value measured *in vivo* and predicted from 24 h in *vitro* gas production and chemical composition of feeds.

The objective of this study was to assess the nutritive value of 14 *Acacia* species as potential new fodder resources for ruminants in comparison with alfalfa hay and wheat straw by chemical composition, tannins content and degradation kinetics using gas production technique, in order to evaluate its effectiveness in ruminants' diet and their value in designing feed management strategies.

MATERIALS AND METHODS

Samples Collection and Preparation: Fresh shoots (leaves and twigs) of fourteen *Acacia* species namely

Corresponding Author: Al-Soqeer A.A., Department of Plant Production and Protection, Faculty of Agriculture and Veterinary Medicine, Qassim University, Buriedah-51452, P.O. 6622, Saudi Arabia
E-mail:wasmy84@hotmail.com

A. coriacea, A. cuthbertsonii, A. ineguilatera, A. iteaphylla, A. kempeana, A. ligulata, A. microbotrya, A. nilotica, A. oswaldii, A. pruinocarpa, A. saligna , A. sclerosperma, A. seyal and *A. victoria* were collected from Prince Sultan Research Center for Environment, Water and Desert, King Saud University (Al-Riyadh-Saudi Arabia). Adequate quantities of samples were hand collected (about 1 kg) from at least 10 different trees under each specie, then pooled and dried at 60 °C in force draught oven for 48 h. Dried samples were ground with a 1 mm screen and stored until further analysis.

Laboratory Analysis: Organic matter (OM) and CP in *Acacia* species, alfalfa hay and wheat straw were determined according to AOAC[3]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as described by Van Soest[42].

Extraction and Determination of Condensed Tannins: An approximately 0.1 g (1.0 mm sieve) was extracted thrice with mixture of 10 ml of acetone/ water (7:3, v/v) in water both at 30 °C. The tubes were centrifuged at 2000 xg for 15 min. After extraction the supernatant was transferred to a 10 ml volumetric flask and the lower layer was discarded. The combined aqueous fraction was made up to 10 ml with water, and stored for quantification of the condensed tannins (CT). The CT was determined using butanol/Hcl assay[34], by adding 0.25 ml of the aqueous extract to 6 ml of *n*-butanol/Hcl (95:5, v/v). Then it was vortexed and heated at 95 °C on a water bath for 1 h. The absorbance of the red anthocyanidin products (i.e. condensed tannins) was measured at 550 nm.

In Vitro Gas Production Kinetics of Tested Roughages: The *in vitro* incubation system (gas method) as described by Menke[26] was used to measure gas production of tested roughages. The rumen liquor was obtained via the rumen cannulae of 2 sheep receiving alfalfa hay *ad lib*. Buffered rumen liquor (2:1 v/v) was prepared as described by Menke and Steingass[27]. About 200 mg DM of the *Acacia* species, alfalfa hay and wheat straw were weighed into calibration syringes (60 ml). Syringes pistons were lubricated with Vaseline to ease their sliding and to prevent escape of gas. The syringes were pre-warmed (40 °C) before the injection of 30 ml of rumen liquor-buffer mixture into each syringe, followed by incubation in a water bath (39± 0.1 °C). The syringes were gently shaken for 30 min. after the start of incubation and then every hour during the first 10 h of incubation. Reading of gas values were recorded after 2, 4, 6, 12, 24, 48 and 72 h of incubation. Data for gas production were fitted to an exponential equation as proposed by Ørskov and McDonald[33]: $GP = b\,(1-exp^{-ct})$, where GP is gas production (ml) at time t and $a+b$ is the gas potential production and c is the rate of gas production (ml/h).

Estimation of Nutritive Value: The organic matter digestibility (OMD. %) and metabolizable energy (ME, MJ/ kg DM) were calculated according to the following equations[27]:

OMD (%) = 14.88+0.889GP+0.45CP+ 0.0651 XA
ME (MJ/kg DM) = 2.2+ 0.136 GP+ 0.057CP, where
GP= accumulated gas production after 24 h incubation (ml/ 200mg DM)
CP= crude protein (% of DM)
XA= ash content (% of DM)

Statistical Analysis: Data were subjected to analysis of variance (ANOVA) using the Statview/SAS Institute, Inc.[37] Significant differences between individual means were identified using least significance difference (LSD) multiple range test.

RESULTS AND DISCUSSION

Chemical Composition: The CP content among tested *Acacia* spp. ranged from 8.0 to 16.7 % and it was comparable in *A. iteaphylla* with that of alfalfa hay, the values were 16.7 and 17.1% in *A. iteaphyllaI* and alfalfa hay, respectively. Meanwhile, wheat straw had the lowest CP content among tested feedstuffs. Fiber fractions (NDF, ADF and ADL) were higher in wheat straw than that of other tested feedstuff. The values of NDF, ADF and ADL for *Acacia* spp. were ranged from 33.6 to 56.0%, 20.9 to 45.0% and 4.2 to 15.0%, respectively. Alfalfa hay had an intermediate value for the fiber fractions (Table 1). Among the tested *Acacia* spp, *A. ineguilatera* had the highest CT value, which ranged from 10.4 to 77.0 mg/g DM. The CT was not detected in either alfalfa hay or wheat straw.

Feedstuff Degradation Kinetics: Degradation kinetics of *Acacia* spp, alfalfa hay and wheat straw are presented in Table 2. The degradable fraction (*b*, ml gas/ 200 mg DM)) for *Acacia* spp ranged from 15.9 to 39.07 ml/200 mg DM. Meanwhile, the *b* fraction in alfalfa hay had no significant differences (P > 0.05) compared to *A. kempeana, A. nilotica* and *A. seyal*, the values were 38.30, 39.07, 36.93 and 39.46 ml/200 mg DM, respectively. Wheat straw had an interment value of *b* fraction comparing to that of tested *Acacia* species (Table 2). The constant degradation rate (*c*, ml/h) was highest for *A. prainocara* and the lowest value found

Table 1: Chemical composition of different *Acacia* spp, alfalfa hay and wheat straw

	OM[1]	CP	NDF	ADF	ADL	CT[2]
	------% of dry matter------					mg/ g DM
A. coriacea	83.3	12.2	55.8	33.6	10.5	11.7
A. cuthbertsonii	84.2	9.6	44.0	37	11.9	65.1
A. ineguilatera	82.0	8.0	56.0	45	15	77
A. iteaphylla	83.6	16.7	51.2	40.4	12.5	40.1
A. kempeana	81.5	12.2	53.5	40.3	10.5	31.1
A. ligulata	84.8	8.4	39.5	27.5	14.1	62.1
A. microbotrya	84.4	13.1	43.0	34.8	8.4	57.8
A. nilotica	88.0	13.8	42.6	35.4	6	15.7
A. oswaldii	89.1	11.3	36.7	22.6	8.3	55.4
A. pruinocarpa	82.1	10.9	47.3	33.4	15.6	25
A. saligna	89.7	12.6	33.6	20.9	9.5	40.1
A. sclerosperma	87.7	14.4	43.0	32.1	9.4	33.7
A. seyal	90.7	14.4	34.7	27	4.2	10.4
A. victoria	88.4	12.4	44.6	31.3	7.3	19.3
Alfalfa hay	88.0	17.1	43.1	25.1	12	ND[3]
Wheat straw	84.1	3.9	75.3	46.2	15.8	ND

[1]OM, organic matter; CP, crude protein; NDF, Neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.
[2]Condensed tannins expressed as mg anthocyanidin equivalent per g DM.
ND = Not detected

in *A. cuthbertsonii* among tested *Acacia* spp, and the values were 0.112 and 0.021. The *c* values for alfalfa hay and *A. pruinocarpa* were not significantly (P > 0.05) different.

Assessment of Nutritive Value: Calculated ME (MJ/ kg DM) for tested species of *Acacia*, alfalfa hay and wheat straw are shown in Table 2. Alfalfa hay had the highest ME, meanwhile organic matter digestibility (OMD) was highest in alfalfa hay and it was ranged from 25.4 to 39.7% in tested *Acacia* spp. (Table 2).

Table 3 illustrates the correlation between ME and OMD with CP, fiber fractions, CT and CT:CP ratio of Acacia spp. The ME and OMD were positively correlated (P <0.01) with CP content. Negative correlations were detected between either ME or OMD and fiber fractions, however, no significant difference was found except with ADL content. The CT content and increasing the ratio between CT:CP had a highly negative correlation (P <0.001) with either ME or OMD (Table 3).

The following equations were predicted to estimate the OMD and ME of tested *Acacia* spp. using their chemical composition

OMD = 36.5 + 0.02CP + 0.3NDF - 0.75CT; (r = 0.756; P < 0.001) .... equation 1,

ME = 5.8 + 0.14CP + 0.02NDF - 0.72CT; (r = 0.798; P <0.001) ……… equation 2; where OMD, ME, CP, NDF and CT are organic matter digestibility (%), metabolizabole energy (MJ/kg DM), crude protein (% of DM), neutral detergent fiber content (% of DM) and condensed tannins (mg/g DM), respectively.

Discussion: In the current study, the variation in chemical composition among tested *Acacia* species may be partly due to genotypic factors which control accumulation of forage nutrients. Accumulation of nutrients in plants is a property of species and varies among species and genera[3.,35]. Chemical composition of *A. nilotica* and *A. senegal* in the current study were differ than the results reported by Rubanza[35]. They found that CP, NDF, ADF and ADL were 17.6, 22.2, 13.4 and 5.5; 14.5, 25.0, 14.8 and 6.4 for *A. nilotica and A. Senegal,* respectively. Similar trend was observed when the chemical composition of *A. saligna* compared with the results of Salem[36]. Differences in CP contents within species could be attributed to stage

Table 2: Gas production constants (ml/200 mg DM) and predicted metabolizable energy (ME, MJ/Kg DM) and digested organic matter (OMD, %) of different *Acacia spp*., in comparison with alfalfa hay and wheat straw

	Gas production constants			
	$a+b^1$(ml/200 mg DM)	c(ml/h)	ME	OMD
A. coriacea	24.17[cd]	0.064[bc]	5.54[d]	32.21[d]
A. cuthbertsonii	20.58[de]	0.021	4.35[f]	25.36[f]
A. ineguilatera	15.38[f]	0.057[c]	4.26[f]	25.36[f]
A. iteaphylla	26.56[cd]	0.038[d]	5.32[de]	29.07
A. kempeana	39.07[a]	0.053[cd]	6.69[b]	39.72[b]
A. ligulata	18.21[ef]	0.064[bc]	4.61[ef]	27.57[ef]
A. microbotrya	18.10[ef]	0.075[b]	5.03	28.48
A. nilotica	36.93[a]	0.052[cd]	6.42[bc]	37.30[bc]
A. oswaldii	19.2	0.065[bc]	4.96	28.72
A. pruinocara	23.37[d]	0.112[a]	5.80[cd]	34.35[cd]
A. saligna	26.72[cd]	0.050[cd]	5.47[de]	31.59[de]
A. sclerosperma	27.31[c]	0.061[bc]	5.98[cd]	34.25[cd]
A. seyal	39.46[a]	0.038[d]	6.11[c]	35.08[cd]
A. victoria	25.24[cd]	0.065[bc]	5.59[d]	32.42[d]
Alfalfa hay	38.33[a]	0.112[a]	8.08[a]	46.46[a]
Wheat straw	32.43[b]	0.048[cd]	5.56[d]	35.41[c]
S.E.M.	1.132	0.004	0.137	0.877

The extent of gas production *(a+b)* and rate of gas production *(c)* are constants predicted by the exponential model proposed by Ørskov and McDonald (1979).
[a-k] Means in the same column with different superscripts are significantly different (P < 0.05).
S.E.M., standard error of mean.

Table 3: Correlation coefficient (r) between chemical composition[a] and nutritive value (ME and DOM) of *Acacia* spp.

	ME	DOM
CP	0.60***	0.39**
NDF	- 0.02[ns]	-0.01[ns]
ADF	- 0.06[ns]	-0.05 [ns]
ADL	- 0.45**	-0.4**
CT	- 0.79***	-0.75***
CT:CP	- 0.80***	-0.72***

[a]CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent fiber; CT, condensed tannins; CT:CP, condensed tannins: crude protein ratio.
[ns], not significant
** P >0.01
*** P>0.001
n= 42 for 14 species of *Acacia*.

of growth and proportion of leaves samples to be collected for analysis. Topps[40] found a high CP content of young leaves of *A. senegal* comparing with mature leaves (31.9 *vs* 21.9% of DM), moreover, shoots had lower CP content than leaves at any stage of growth. Aganga[1] found different values of CP content in *A. nilotica* among the seasons (wet *vs* dry), and the tested part of the plant (leaves *vs* pods).

Differences of NDF, ADF and ADL contents could similarly be due to species genotypic differences in factors that control fiber accumulation in the plant and stage of growth. Minson[30] reported that the fiber contents increase with advantage foliage maturity as a result of lignification. In the current study, the higher fiber fractions in *A. saligna* and *A. seyal* than that reported by Ben Salem[36,35] might be attributed to the portions of either leaves or stems in tested sample. The succulent parts were used in current study comparing with the leaves in the previous reports.

The minimal CP content of dry material for maintenance of sheep has been indicated by Milford and Haydock[28] to be 7.2 %; however, it was suggested to be at least 8.9 % CP in plant material[31]. The CP value of tested Acacia in the present study were mostly well above the recommended levels by Milford and Haydock[28] and NRC[31], suggesting that they might maintain animals. On the other hand, the CP of wheat straw was lower than the level which would sustain sheep if used as the only sources of feed. In spite of *A. cuthbertsonii, A. ingeruilatera, A. iteaphylla, A. ligulata, A. microbotrya and A. oswaldii* have a sufficient CP content than that recommended by previous reports, the OMD and ME found to be low (P <0.05) in these species comparing with wheat straw which had 3.9 % CP in DM. The low nutritive values of *A. cuthbertsonii, A. ingeruilatera, A. iteaphylla, A. ligulata, A. microbotrya and A. oswaldii* comparing with wheat straw could be attributed to the CT content of these species, which ranged from 4 to 6.5 % of DM.

Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate[6]. Gas production from protein fermentation is relatively small as compared to carbohydrates while, contribution of fat to gas production is negligible[43]. In the current study, the OMD and ME were positively correlated (P < 0.01) to CP content, however, the utilization of CP by rumen microorganisms might be limited as tannins presence in the incubation medium. Forming hydrogen bonds between the phenolic sub-units of the polymer and carbonyle group of peptides of the protein result in a tannin-protein complex which may protect protein from ruminal digestion[4]. The effect of CT from *L. corniculatus* on 11 strains of rumen bacteria was studied by Min *et al*[29]. who concluded that CT reduced rate of proteolysis and inhibited growth of proteolytic rumen microorganism, and those negative effects were correlated to the level of CT. The correlation between the OMD and ME, and CT:CP ratio are in agreement with the previous reports, showing a highly negative correlation (P < 0.001) between either ME and DOM; and CT:CP ratio in tested Acacia spp (Table 3).

Moreover, negative impacts of CT in the reduction of ME and OMD of *Acacia* spp are consistent with the *in vitro*[15,16] and *in sacco*[17]). Moreover,[20] reported that microbial gas production and *in vivo* DM disappearance decreased with increased concentration of extractable polyphenolics in browse species.. Similarly, Chiquette[9] demonstrated lower gas production from high tannin than low tannin containing variety of *Louts corniculatus*. Results of this study are in agreement with the extensively reported suppressive effects of CT on rumen degradation, and on the interference of these compounds with microbial attachment to feeds[25,23,2,15,35]. Results are also consistent with Frutos[13] who observed a negative correlation between CT and *in vitro* gas production in sheep with different leguminous shrubs. Evitayani[10] suggested that the CT not bound to protein can inhibit fermentation of structure carbohydrates in the rumen by forming indigestible complex with cell wall carbohydrates, rendering them undegradable. It can also form complex with microbial enzymes, rendering then inactive[14], which could reduce gas production. It may be difficult to attribute the reduction of either ME or OMD solely to the content of CT. In the present study the ME and DOM were negatively correlated with fiber fractions (i.e. NDF, ADF and ADL, however, this correlation was non-significant (P < 0.05) with NDF and ADF; and highly significant (P < 0.01) with ADL (Table 3). Numerous evidences[42,24,8] indicated that high cell wall constituents also set a limit to potential feed intake by physical fill effect as well as by reducing the digestibility of feeds. Nherera[32] reported that effect of polyphenolics on gas production to be complex and to vary across browse species, which made them to suggest that the fiber fraction of browse species may be more importance than tannins in limiting fermentation *in vitro*.

In the current study, factors that affecting nutritive values were summarized in equations 1 and 2 to ease nutritive values assessment of Acacia spp., by using their chemical composition (CP, NDF and CT) of Acacia spp.

Conclusions: Based on the chemical composition, all tested acacia spp. were found to have higher CP content than rice straw, however, the CP content in *A. iteaphylla* was comparable with alfalfa hay (16.7 *vs* 17.1% DM). High content of CT in Acacia spp. found to have a negative effect on its nutritive value (i.e. ME and OMD) estimated from constant parameters of gas production. The ME of tested *Acacia* spp. ranged from 4.35 to 6.69 MJ/ kg DM, which could supply the animals with the 53-84% of ME as in alfalfa hay. To ease the evaluation of nutritive value of *Acacia* spp. as ME and OMD the equations 1 and 2 could be applied.

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