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Potential nutritive value and tannin bioassay of selected *Acacia* species from Kenya

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Abstract: Six *Acacia* forage species–*A. brevispica, A. elatior, A. mellifera, A. nilotica, A. senegal* and *A. seyal* – were analysed for their chemical composition, including phenolics and rumen fermentation characteristics. *In vitro* gas production technique was used to study the rumen fermentation characteristics and the effect of tannins present in the browse forages on *in vitro* fermentation by including polyethylene glycol (PEG-6000) in the incubation. The forages had high crude protein content (145.7–270.1 g kg^{-1} DM) and low to moderate content of neutral detergent fibre (220.2–442.8 g kg^{-1} DM). The forages had variable content of total extractable phenolics ranging from 18.4 to 384.2 g kg^{-1} DM and total extractable tannins ranging from 7.1 to 364.8 g kg^{-1} DM (tannic acid equivalent). Fractionation of the condensed tannin flavonoids showed that the delphinidin/cyanidin ratio ranged from not detected:100 to 71:29. The gas production potential ranged from 28.4 to 40.8 mL gas 0.2 g^{-1} DM. The rate of gas production was highest in *A. elatior* and lowest in *A. nilotica*. Addition of PEG-6000 increased gas production significantly ($P < 0.05$) in all species except *A. mellifera*. PEG-6000 addition did not have any significant effect on *in vitro* true dry matter and organic matter degradability but decreased the partitioning factor in all the species. Gas production and degradability parameters correlated positively with the CP content and negatively with the fibre and phenolics contents. Based on chemical composition, gas production and degradability, the forages have high potential nutritive value, especially as supplements to low-quality feeds in the tropics and particularly during the dry season. However, the presence of tannins in terms of high levels and biological antinutritive activity may limit the nutritive potential of some of the forages, such as *A. seyal* and *A. nilotica*.

Keywords: *Acacia*; chemical composition; gas production; degradability; PEG

INTRODUCTION

Most areas of the tropics, including Kenya, are classified as arid or semi-arid and are always prone to prolonged drought. During such times, the common scenario is a decrease of productivity from animals and, in worst cases, death of the animals mainly due to feed shortage. To tackle the situation, there is a need to explore ways of alleviating such losses, especially through the use of locally available feed resources which may be of high nutritive value. Hence identification of nutritious feed resources is important so that farmers can be advised to harvest and keep such feeds in times of plenty (rainy season) for use during the lean periods. One such resource is the *Acacia* species.

The *Acacia* species are predominant in most of the dry zones in Africa, where they serve as an important source of feed for ruminants as well as other uses[1] such as firewood. For instance, in the semi-arid zone of northern Kenya, the foliage and fruits of *Acacia* species contribute up to 40% and 20%, respectively, of the diet of goats during the dry season.[2] While livestock farmers in such areas have developed strategies for maximizing use of such feed resources, especially during the dry season, little is known about the nutritive value of such feed resources. Previous studies in Kenya and other parts of Africa have attempted to determine the fodder potential of *Acacia* species.[3–5] According to these studies, consumable parts of Acacia species is high in crude protein (CP; 134–229 g kg^{-1} DM) and some antinutritional factors like tannins. Tannins are known to affect the nutritive value of feeds. Chemical composition analysis and *in vitro* gas production allow for rapid preliminary evaluation of the potential nutritive value of browse species, including the adverse effect of tannins present in forages. Hence, the objective of this study was to assess the chemical composition and in *vitro* gas production characteristics of *Acacia* forages and quantify the effect of tannins on the degradability of *Acacia* forages.

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MATERIALS AND METHODS

Forage samples

Browsable tree leaves from six *Acacia* species were used in this study. The species used included *A. brevispica, A. elatior, A. mellifera, A. nilotica, A. senegal* and *A. seyal*. All forages were harvested from Egerton University's Chemeron Field Station in Marigat, Baringo District, Kenya. The area is located at 0°31′S; 35°78′E and at an altitude of 1066 m above sea level, with average annual rainfall and temperature of 700 mm and 24 °C, respectively. The browse forages were harvested from at least 10 trees for each species selected at random in four locations within the study area at the end of the rainy season. The harvested samples were then pooled for each individual tree species, oven dried at 50° for 48 h to a constant weight and ground to pass through a 2.0 mm sieve. The samples were then subsampled to obtain three samples for each tree species and used for laboratory analysis. However, for the analysis of phenolics and *in vitro* gas production experiments, the forages were further ground to pass through a 1.0 mm sieve.

Chemical analysis

Organic matter (OM) and crude protein (CP) contents were measured according to the official methods of the Association of Official Analytical Chemists.[6] Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to the methods of Van Soest *et al.*[7]

Phenolics were extracted using 70% aqueous acetone. Total extractable phenolics (TEPH) were determined using Folin–Ciocalteu assay as described by Makkar.[8] The concentration of TEPH was calculated using the regression equation of tannic acid (Sigma Aldrich, Steinheim, Germany) standard. Total extractable tannins (TET) were estimated indirectly after being absorbed to insoluble polyvinylpolypyrrolidone (PVP). The concentration of TET was calculated by subtracting the TEPH remaining after PVP treatment from the TEPH. The various leucoanthocyanidin flavonoids were measured by high-performance liquid chromatography (HPLC) as described by Stewart *et al.*,[9] with slight modifications. One gram of sample was extracted with 4 mL acetone–water (70:30 v/v) in a water bath by ultrasonication (125 W, 42 kHz) for 30 min, followed by centrifugation for 15 min at $1754 \times g$. 0.5 mL of supernatant was vortexed with 0.25 mL dichloromethane and centrifuged again at $1754 \times g$ for 15 min to remove the pigments. 0.05 mL of the aqueous lower layer was combined with 3.0 mL butanol–HCl (95:5 v/v) and heated for 1 h at 95 °C. Butanol–HCl was evaporated at 50 °C under a stream of nitrogen and the residue redissolved in 0.5 mL methanol–HCl (99:1 v/v) followed by filtration through a 0.02 µm polytetrafluoroethylene membrane. Ten microlitres of the aliquot was then injected into the high-performance liquid chromatograph (Shimadzu Co., Kyoto, Japan) column (Inertsil ODS-80 A, 150 × 4.6 mm). Gradient elution rate was 0.9 mL min^{-1} using water–acetic acid (solution A, 95:5 v/v) and methanol (solution B). The gradient profile was: 80% A/20% B (0 min), 50% A/50% B (0–15 min), 0% A/100% B (15–20 min), 0% A/100% B (20–25 min) and 80%A/20%B (25–30 min). The absorbance at 525 nm was recorded. The peaks were identified using cyanidin chloride, delphinidin chloride and pelargonidin chloride, which had retention times of 9.77, 12.26 and 14.26 min, respectively. The total condensed tannins were taken as the sum of the measured flavonoids.

In vitro study

Animals

Three mature sheep fitted with a permanent rumen fistula were used in this study. The animals were fed on diet made up of 800 g DM timothy hay and 200 g DM concentrate twice daily at 09.00 and 16.00 h in equal-sized meals. The animals had free access to water and mineral licks throughout the experiment period. The animals provided the rumen liquor for the *in vitro* gas production and tannin bioassay experiments. The rumen liquor was withdrawn at 06.00 h, mixed, strained through four layers of cheesecloth and kept at 39 °C under a CO_2 atmosphere.

In vitro *gas production*

About 200 mg of sample (milled through a 1.0 mm sieve) were incubated *in vitro* with rumen fluid in calibrated glass syringes in triplicate following the procedure of Menke and Steingass.[10] The syringes were pre-warmed at 39 °C before addition of 30 ± 1 mL of rumen liquor–buffer mixture (ratio 1:2) into each syringe and incubated in a water bath maintained at 39 °C. Blanks with buffered rumen fluid only were also included. The gas production readings were recorded after 3, 6, 12, 24, 48, 72 and 96 h of incubation. The gas production characteristics were estimated by fitting the mean gas volumes to the exponential equation of Ørskov and McDonald:[11]

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

where G is gas production (mL) at time t, a is the gas production from the immediately soluble fraction (mL), b is the gas production from the insoluble but degradable fraction (mL), $a + b$ is the potential gas production (mL) and c is the rate constant of gas production (fraction h^{-1}).

Tannin bioassay

Incubation was carried out as described by Makkar *et al.*[12] About 500 mg DM of the feed samples were incubated with or without 1.0 g polyethylene glycol (PEG, molecular weight (MW) = 6000). The syringes were pre-warmed at 39 °C for 1 h before addition of 40 ± 0.5 mL rumen liquor–buffer mixture (1:3) into the syringes and incubated in triplicate in a water bath maintained at 39 °C. Blanks were also included in the incubation. The gas production readings were recorded after 2, 4, 6, 8, 12, 16 and 24 h of incubation.

In vitro *true DM and OM degradability and partitioning factor*

After termination of the incubation at 24 h, the syringe contents were quantitatively transferred into a 600 mL beaker by rinsing the syringes with about 70 mL of neutral detergent solution[13] and refluxed for 1 h. Residual DM and ash were then determined. The ratio of DM truly degraded (mg) to gas volume (mL) produced at 24 h of incubation was used as the partitioning factor PF (index of microbial protein synthesis).

Statistical analysis

In vitro gas production data were fitted to the asymptote exponential model using the Neway-Excel computer program (Macaulay Institute, Aberdeen, UK). All the data were subjected to analysis of variance (ANOVA) using the general linear model procedure[14] and significance between means tested using least significant difference (LSD). Correlations between the chemical compositions and gas production and degradability parameters were calculated.[14]

RESULTS AND DISCUSSION

The *Acacia* forages varied ($P < 0.05$) in all the chemical constituents evaluated in the study (Table 1). The OM varied ($P < 0.05$) from 852.3 g kg^{-1} DM in *A. senegal* to 944.6 g kg^{-1} DM in *A. nilotica*. Though variable, all the forages had high CP content, ranging from145.7 g kg^{-1} (*A. nilotica*) to 270.1 g kg^{-1} DM (*A. mellifera*). The NDF content was highest ($P < 0.05$) in *A. seyal* (442.8 g kg^{-1} DM), while *A. elatior* had highest ($P < 0.05$) ADF and ADL contents. The chemical compositions were within the range reported in the literature for *Acacia* forage species from Africa.[3–5,15] The high CP content of the *Acacia* forages indicates the potential of the forages as valuable feeding resources since they are intended to be used as protein supplements to the often low-CP and high-fibre basal diets such as tropical pastures and crop residues. The NDF content of *Acacia* forages was low to moderate. This means the forages have high to moderate content of cell contents which are related to high digestibility. Furthermore, El Hassan *et al.*[16] showed that the fibre of browse is more digestible than that of grasses and straws. Hence, when used as supplements, the forages may improve the digestibility of low-quality forages such as straws by providing a highly colonized fibre source to inoculate rumen bacteria into the less digestible fibre.[17] The variation in chemical constituents among species is expected as the accumulation of nutrients in plants is influenced by genotypic factors (species).[18]

Table 1. The chemical composition (g kg^{-1} DM) of *Acacia* forage species

Species	OM	CP	NDF	ADF	ADL
A. brevispica	936.5b	189.3c	382.5c	224.0c	115.0b
A. elatior	901.6d	178.3d	406.2b	264.9a	135.8a
A. mellifera	893.2e	270.1a	417.6b	251.2b	97.4c
A. nilotica	944.6a	145.7e	220.2e	130.8e	57.9d
A. senegal	852.3f	217.5b	332.5d	192.6d	57.0d
A. seyal	933.2c	181.1d	442.8a	248.2b	90.4c
SEM	9.6	11.7	22.4	13.8	8.6

SEM, standard error of the mean; means in the same column with different suffixes differ significantly ($P < 0.05$).
OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

The total composition of phenolics and tannins is presented in Table 2. TEPH ranged from 18.4 (*A. mellifera*) to 384.2 g kg^{-1} DM (*A. nilotica*). *A. nilotica* had the highest ($P < 0.05$) content of TET (364.8 g kg^{-1} DM), while *A. mellifera* had the lowest TET content (7.1 g kg^{-1} DM). *A. seyal* had the highest total condensed tannins (TCT). TCT were not detected in *A. elatior*. Fractionation of the proanthocyanidins showed that *A. seyal* had the highest delphinidin composition, while *A. nilotica* had the highest cyanidins. The delphinidin/cyanidin (D/C) ratio varied from not detected:100 in *A. nilotica* to 71:29 in *A. mellifera*.

High content of tannins present in such browse forages has been shown to be the major drawback in their use as protein supplements.[19] The high content of TET in *A. nilotica*, *A. brevispica* and *A. seyal* has been reported in other studies on *Acacia* forage from east Africa.[3,5] The D/C ratios were within the range published.[9,20,21] The nutritional implications of variations in D/C ratio are not yet clear[20] but, generally, CT with high MW interact more strongly with enzymes and other proteins than CT with low MW, and the reactivity of CT increases with increasing prodelphinidin content.[22] Hedqvist *et al.*[20] reported consistently better *in vivo* protein digestion in *Lotus corniculatus*, which had less prodelphinidins than procyanidins (D/C ratio 33:67 to 21:79) than in *L. pedunculatus*, which had more prodelphinidins than procyanidins (D/C ratio 81:18 to 77:23).

Table 2. Total phenolics, tannins, condensed tannin fractions (g kg^{-1} DM) and delphinidin/cyanidin ratio of *Acacia* forage species

Species	TEPH[a]	TET[a]	TCT	Del	Cya	Pel	D/C
A. brevispica	134.2b	105.8b	2.1b	1.1c	0.9e	0.2a	56/44
A. elatior	45.2c	32.4d	n.d.	n.d.	n.d.	n.d.	–
A. mellifera	18.4d	7.1e	0.2e	0.2b	0.1c	n.d.	71/29
A. nilotica	384.2a	364.8a	1.4c	n.d.	1.2a	0.2b	0/100
A. senegal	33.0c	17.8e	0.4d	0.2b	0.1d	n.d.	61/39
A. seyal	128.8b	73.2c	3.6a	2.5a	1.1b	0.0c	69/31
SEM	37.6	37.1	0.4	0.4	0.2	0.04	

SEM, standard error of the mean; [a] tannic acid equivalent; means in the same column with different suffixes differ significantly ($P < 0.05$).
TEPH, total extractable phenolics; TET, total extractable tannins; TCT, total condensed tannins; Del, delphinidins; Cya, cyanidins; Pel, pelargonidins; D/C, delphinidin/cyanidin ratio (expressed as %); n.d., not detected.

The mean cumulative gas production and fermentation characteristics of the forages are presented in Table 3. After 24 h of incubation, *A. mellifera* produced the highest ($P < 0.05$) gas (147.7 L kg^{-1} DM), while *A. nilotica* produced the least gas (95.9 L kg^{-1} DM). After 48 h incubation, *A. seyal* produced the least gas volume. *A. brevispica* had the highest ($P < 0.05$) potential gas production, while *A. seyal* had the lowest ($P < 0.05$) gas production potential. The rate of gas production was highest in *A. elatior* and lowest in *A. nilotica*.

The significant differences ($P < 0.05$) among the *Acacia* forage species for their *in vitro* gas production and fermentation characteristics are in agreement with previous studies on *Acacia* forage from East Africa.[5,21] Gas production results from fermentation of the feed to short-chain fatty acids (SCFAs) and CO_2 released from the buffering of the produced SCFAs by bicarbonate buffer. Therefore, the differences in gas production among the various species could be due to the amount of substrate fermented and the SCFAs produced upon substrate fermentation. The differences in the rates and extent of the fermentation of the various browse species could be related to the differences in their chemical compositions, especially the CP, fibre and tannin composition.[19] For instance, the low extent and rate of gas production in *A. nilotica* and *A. seyal* could be related to the comparatively low CP content and high fibre and tannin content of the species.

The effect of PEG-6000 addition on gas production, *in vitro* true DM degradability (IVTDMD) and *in vitro* true OM degradability (IVTOMD) are presented in Table 4. The increase in gas production was highest in *A. seyal* (102.0%). However, the effect on gas production was significant ($P < 0.05$) in all the *Acacia* species evaluated except *A. mellifera*. Both IVTDMD and IVTOMD were highest in *A. nilotica* and lowest in *A. seyal*. There was no significant ($P < 0.05$) effect of PEG-6000 addition on IVTDMD and IVTOMD. The partitioning factor (PF) ranged from 2.6 to 8.9. Addition of PEG-6000 significantly ($P < 0.05$) decreased the PF in all the species.

The *in vitro* gas method together with the use of tannin inactivating agents such as PEG-6000 has been widely used to evaluate tannin activity in browse forages.[23] PEG binds to the tannins forming inert PEG–tannin complexes, resulting in increased gas production.[12] Getachew *et al.*[24] noted that the increase in gas production upon addition of PEG correlated well with protein precipitation capacity of tannins, total phenols and tannins. Hence, the increase in gas production on addition of PEG is considered as a measure of the biological antinutritive activity of the tannins.[12,25] All the species in the current study except *A. mellifera* had a significant ($P < 0.05$) increase in gas production upon PEG-6000 addition. *A. seyal* had

Table 3. *In vitro* gas production and fermentation characteristics of *Acacia* forage species (L gas kg^{-1} DM)

	Incubation intervals (h)							Gas production parameters			
Species	3	6	12	24	48	72	96	a	b	$a + b$	c (% h^{-1})
A. brevispica	37.0bc	67.3b	100.3b	132.0b	166.3b	192.7a	204.6a	26.3ab	177.9a	204.2a	3.8c
A. elatior	36.2bcd	58.4bc	98.1b	129.9b	148.5c	158.2c	164.4c	8.1c	151.5c	159.6c	7.0a
A. mellifera	43.8ab	79.3a	110.8a	147.7a	172.4ab	183.3b	191.5b	20.5abc	165.4b	186.0b	6.4a
A. nilotica	25.0d	44.7d	69.6d	95.9d	137.9d	153.7c	160.3c	13.1bc	152.3c	165.5c	3.5c
A. senegal	51.0a	79.2a	114.7a	142.1a	174.8a	187.6ab	196.7b	33.7a	159.0bc	192.7b	5.2b
A. seyal	30.0cd	51.8cd	77.7c	106.3c	125.4e	133.5d	149.9d	15.5bc	126.4d	141.9d	5.2b
SEM	2.7	4.0	5.0	5.6	5.6	6.5	6.2	2.9	4.9	6.4	0.4

SEM, standard error of the mean; means in the same column with different suffixes differ significantly ($P < 0.05$).
a, b and c are constants in the equation $G = a + b(1 - e^{-ct})$.

Table 4. Effect of PEG-6000 addition on gas production (L kg^{-1} DM), *in vitro* true DM and OM digestibility (g kg^{-1} DM) and partitioning factor

	24 h incubation			IVTDMD		IVTOMD		PF	
Species	−PEG	+PEG	Increase[a]	−PEG	+PEG	−PEG	+PEG	−PEG	+PEG
A. brevispica	134.1b	159.2b	18.8**	767.7bc	763.9c n.s.	754.2bc	752.7c n.s.	5.3cd	4.4c**
A. elatior	115.9c	142.0d	22.6**	716.4d	729.1d n.s.	697.8de	708.8d n.s.	5.8bc	4.8b**
A. mellifera	132.6b	137.9d	3.9 n.s.	744.9cd	731.7d n.s.	729.7cd	717.2d n.s.	5.1d	4.8b**
A. nilotica	85.9e	150.8c	75.3**	824.1a	847.9a n.s.	815.0a	842.7a n.s.	8.9a	5.2a**
A. senegal	140.2a	152.3c	8.6*	809.9ab	804.1b n.s.	796.2ab	789.3b n.s.	5.2d	4.8b*
A. seyal	97.6d	197.2a	102.0***	664.8e	557.9e n.s.	654.8e	543.1e n.s.	6.2b	2.6d**
SEM	6.1	5.9		16.9	27.5	17.1	28.2	0.4	0.3

SEM, standard error of the mean; means with different suffixes in a column differ significantly ($P < 0.05$); n.s., not significant ($P > 0.05$); * significant ($P < 0.05$); ** significant ($P < 0.01$).

IVTDMD, *in vitro* true dry matter degradability; IVTOMD, *in vitro* true organic matter degradability; PEG, polyethylene glycol.

[a] % increase = (+PEG gas volume (mL) – −PEG gas volume (mL)) × 100/−PEG gas volume (mL).

Table 5. Correlation coefficient (*r*) of the relationship between the concentration of CP, NDF, ADF, TEPH, TET TCT, Del, Cya and gas production parameters, IVTDMD and IVTOMD

	24 h	$a + b$	c	IVTDMD	IVTOMD
CP	0.794***	0.309 n.s.	0.778***	0.340 n.s.	0.327 n.s.
NDF	−0.567*	−0.708**	0.311 n.s.	−0.822***	−0.817***
ADF	−0.330 n.s.	−0.523*	0.376 n.s.	−0.651**	−0.648**
TEPH	−0.794***	−0.298 n.s.	−0.743***	−0.476 n.s.	−0.461 n.s.
TET	−0.617**	−0.051 n.s.	−0.848***	−0.278 n.s.	−0.264 n.s.
TCT	−0.965***	−0.668**	−0.443 n.s.	−0.746***	−0.733***
Del	−0.987***	−0.779***	−0.309 n.s.	−0.811***	−0.799***
Cya	−0.914***	−0.525*	−0.579*	−0.650**	−0.636**

ns, not significant ($P > 0.05$); * significant ($P < 0.05$); ** significant ($P < 0.01$); *** significant ($P < 0.001$).

CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; TEPH, total extractable phenolics; TET, total extractable tannins; TCT, total condensed tannins; Del, delphinidins; Cya, cyanidins; IVTDMD, *in vitro* true dry matter degradability; IVTOMD, *in vitro* true organic matter degradability; a, b and c are constants in the equation $G = a + b(1 - e^{-ct})$.

the highest and most significant ($P < 0.001$) increase in gas production, followed by *A. nilotica*, though *A. nilotica* had higher TET content than *A. seyal*. This shows that the tannins in *A. seyal* have higher biological antinutritive activity than the tannins in *A. nilotica*. This may be due to the D/C ratio in *A. seyal*, which is higher than in *A. nilotica*, which has only cyanidins.

The IVTDMD and IVTOMD were within the range reported for similar browse forages.[26,27] Despite the large increase in gas production upon addition of PEG-6000, IVTDMD and IVTOMD in the presence of PEG-6000 was not significantly ($P < 0.05$) different from that observed in the absence of PEG-6000. Getachew *et al.*[24] and Makkar *et al.*[12] also made similar observations. This could be due mainly to the tannin–PEG complexes, which become insoluble in neutral detergent solution, thus distorting the weight of the degraded sample.[12] Addition of PEG-6000 decreased the PF in all the species, which is in agreement with other studies.[24,26,27] The higher than theoretical range of PF (2.75–4.41 mg truly degraded substrate mg^{-1} gas) suggested by Blümmel *et al.*[28] may be due to significant loss of detached tannins from fermented substrate, which do not contribute to gas production, and non-utilization of the soluble fraction due to tannin inhibition.[27]

Table 5 presents the correlation coefficients (r) between the CP, fibre and phenolics content and the gas production and degradability parameters. The CP content was positively correlated with all the parameters. However, fibre and phenolics content were negatively correlated with the gas production and degradability parameters. The negative correlation between fibre, phenolics and gas production and degradability parameters suggests the negative role of fibre and tannins on browse forage digestibility potential. The stronger negative correlation between TCT and gas production and degradability of DM and OM ($r = -0.443$ to -0.965) compared to the negative correlation between TET and the parameters ($r = -0.264$ to -0.848) supports the negative role and significance of condensed tannins on the degradability of the browse forages. Rubanza *et al.*[15] also reported a stronger and negative relationship between gas production parameters and TCT than with TET, which is consistent with the findings in this study. However, the nature and activity of the condensed tannin influence the detrimental role of the tannins in the digestibility of the forages.[29] For instance, the negative correlation was stronger between delphinidins and the parameters ($r = -0.309$ to -0.987) than between cyanidins and the parameters ($r = -0.525$ to -0.914).

CONCLUSION

On the basis of chemical composition (high CP and low fibre content) and *in vitro* gas production, the *Acacia* forage species have nutritive potential as supplements to low-quality feeds, especially during the dry season. However, high content of polyphenolics in terms of amount and biological antinutritive activity may limit the nutritive potential of some species, such as *A. seyal* and *A. nilotica*. It is also necessary to validate the nutritive value of such *Acacia* species through animal-feeding experiments.

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