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Effect of yeast supplementation on *in vitro* ruminal degradability of selected browse species from Kenya

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Abstract

Two experiments were conducted with the aim of i) evaluating the nutritive potential of seven Kenyan browse species {*Acacia brevispica* (Harms), *Acacia elatior* (Brenan), *Acacia mellifera* (Vahl) Benth, *Balanites aegyptiaca* (L.) Del, *Berchemia discolor* (Hemsley), *Grewia bicolor* (A. Juss) and *Zizyphus mucronata* (Willd)} through their chemical composition and *in vitro* gas production profiles and ii) assessing the effect of two yeast supplements (Japanese sake yeast (JSY) and bioethanol residue yeast (BRY)) on *in vitro* rumen dry matter degradability (IVRDMD). In the IVRDMD test, four treatments consisting of a control (no supplement), JSY, BRY and a positive control of soybean peptide (SP) were supplemented in the *in vitro* rumen incubation fluid and incubated with the browse foliage for 24 hours. The crude protein content ranged from 163 to 270 g kg $^{-1}$ dry matter (DM) and neutral detergent fiber from 364 to 527 g kg $^{-1}$ DM. Gas production (mL), organic matter digestibility (%) and metabolisable energy concentration (MJ/kg DM) differed significantly ($p < 0.05$) among the browse species, and were significantly influenced by the total phenolic contents ($p < 0.05$). The IVRDMD was significantly influenced by both the supplements ($p < 0.0021$) and browse foliages ($p < 0.0001$). The JSY had significantly higher IVRDMD than the other supplements ($p < 0.05$) and BRY and SP had numerically higher values than the control ($p > 0.05$). *Berchemia discolor* had significantly higher IVRDMD in response to all the supplements, while *G. bicolor* had the least response, respectively ($p < 0.05$). Difference of yeast strains (JSY *vs.* BRY) may have influenced the IVRDMD, while differences among browse foliages may have been as a result of varying levels of concentrations and chemical structure of tannins. The study shows that yeast has potential to attenuate the antinutritive effects of tannins in browse foliages on the rumen fermentation by providing microbial yeast protein and promoting growth and activity of fiber digesting microbes.

Key words: Browse foliage, nutritive value, *in vitro* digestibility, yeast supplementation.

Introduction

Presence of tannins in the tree foliage has been associated with protection of true protein from degradation in the rumen by microbes, hence increasing the supply of rumen undegradable protein to the small intestine for higher production [1]. However, tannins at high levels may reduce intake and digestibility of the foliage by ruminants [2]. Tannins depress rumen fermentation by binding and inactivating microbial cells and enzymes [3]. Supplemental polyethylene glycol (PEG), a tannin binding agent, has been used to improve the intake and digestibility of browse forage through increased protein availability [3-5]. However, cost and availability at farm level are factors to contend with in the utilization of PEG. The challenge still remains for ruminant nutritionists to find alternative, cheaper and easily available supplements that can bind to tannins or manipulate the rumen microbial ecosystem and fermentation without posing any health risks to the animal, animal products consumers and the environment [6].

Yeast is a natural feed additive generally recognised as safe and has been used as a microbial protein source in ruminant diets, a probiotic to promote growth and activity of rumen microbes and a stabilizer of rumen fermentation status, hence preventing rumen flora disorders and disturbances in ruminants [7-10]. An increase in the numbers of viable bacterial cells [10] as a result of yeast supplementation enhances ammonia utilisation by ruminal microorganisms, thus, increased microbial protein synthesis [8]. Ando *et al.* [11] reported that increased *in vitro* rumen degradability of forages was associated with ability of dried beer yeast to stimulate growth and activity of fibrolytic bacteria.

There is little information on whether yeast supplement can be utilised to attenuate the anti-nutritive effects of tannins in browse foliages and consequently increase their rumen degradability. The aim of the study was thus to asses the effect of yeast on the *in vitro* ruminal digestion of selected tannin-containing browse species from Kenya.

Materials and Methods

Browse collection: Seven browse foliage species (*Acacia brevispica, Acacia elatior, Acacia mellifera, Balanites aegyptiaca, Berchemia discolor, Grewia bicolor* and *Zizyphus mucronata*) were collected from Egerton University's Chemeron Field Station in Marigat Division, Baringo District, a semi-arid region in Kenya. The area is located at an altitude of 1066 m above

sea level with average annual rainfall and temperature of 700 mm and 24°C, respectively. The browse species are common in the area, may be collected with relative ease and have good palatability. The samples consisted of leaves plucked by hand from 10 trees for each species randomly selected at the end of the rain season. The harvested samples were pooled for each tree species, oven dried at 50°C for 48 hours to a constant weight and ground to pass through a 2 mm sieve for chemical analyses and *in vitro* ruminal digestibility (IVRDMD). The forages were further ground to pass through a 1 mm sieve for the analysis of phenolics and *in vitro* gas production test.

Chemical analyses: The dry matter (DM), crude ash (CA) and crude protein (CP) contents were determined according to AOAC [12]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the methods of Van Soest *et al.* [13]. Total extractable phenolics (TEPH), total extractable tannins (TET) and total condensed tannin (TCT) were determined using Folin-Ciocalteu method as described by Makkar [14].

In vitro gas production test: The study was conducted at the laboratory of Animal Science, Shimane University, Japan. Rumen liquor collected from three mature Japanese Corriedale sheep fitted with permanent rumen fistula was used for the study. A diet comprised of 800 g DM timothy hay and 200 g DM concentrate was allowed to the animals twice daily at 09.00 a.m and 16.00 p.m in equal sized meals. The animals had access to water and mineral lick *ad libitum*. The rumen liquor was withdrawn prior to morning feeding, mixed, strained through four layers of cheesecloth and kept at approximately 39°C in water bath and flushed with CO_2. About 200 mg of ground browse samples and blanks with buffered rumen fluid without feed sample were incubated in triplicate following the procedure of Menke and Steingass [15].

Calculation of nutritive values: The gas production characteristics were estimated by fitting the mean gas volumes to the exponential model using Neway computer programme (Macaulay Institute, Aberdeen, UK). The *in vitro* gas production data was used to estimate the metabolisable energy (ME) concentration, organic matter digestibility (OMD) and dry matter intake (DMI), based on gas production at 24 hours of incubation (Gp_{24}) using following equations:

$$Gp = a + b\,(1\text{-}e^{-c\,t})$$ [16]

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136\,Gp_{24} + 0.057\,CP$$ [17]

$$OMD\ (\%) = 14.88 + 0.889\,Gp_{24} + 0.45\,CP + 0.0651\,CA$$ [17]

$$DMI\ (kg/d) = 1.66 + 0.49\,a + 0.0297\,b - 4\,c$$ [18]

where Gp is the gas production (mL) at time t from 200 mg sample, a is the gas production from the immediately soluble fraction (mL), b is the gas production from the insoluble fraction (mL), c is the rate constant of gas production (per h), both crude protein (CP) and crude ash (CA) are expressed in % of DM.

In vitro ruminal digestion study: The study was conducted at National Agricultural Research Center for Western Region, Oda-shi, Shimane Prefecture, Japan. Japanese sake yeast (JSY) was obtained from commercial Sake Company (Gekkeikan Sake Co., Ltd., Kyoto, Japan). Bioethanol residue yeast (BRY) was an industrial by-product mainly comprised of yeast from bioethanol production from sugarcane. Soybean peptide (SP) was used as a positive control. The OM and CP contents of the supplements are shown in Table 1.

Two Japanese Black steers (4 years old), fitted with permanent rumen cannulas, were fed on a diet consisting of 5 kg Italian ryegrass hay and 1 kg commercial concentrates twice at 09:00 a.m and 17:00 p.m and had free access to water and mineral licks throughout the experiment period. Rumen fluid for *in vitro* digestion was collected before the morning feeding and filtered through four layers of cheesecloth before mixing with McDougall's buffer in the ratio of 1:4. The *in vitro* digestion procedure of Tilley and Terry [19] was used but the final digestion with pepsin was omitted. In brief, nylon bags (4 cm by 4.5 cm, 50 µm pore size) containing 200 mg of foliage samples were inserted into 50 ml test tubes containing 200 mg of supplements in quadruplicates per browse species and a control with no supplement. The rumen buffered fluid was added and the tubes sealed with rubber stoppers fitted with Bunsen valves. The contents in the tubes were incubated at 39°C and shaken periodically for 24 hours.

Statistical analyses: *In vitro* gas production and rumen DM degradability data were subjected to analysis of variance using general linear model [20] and significant means were separated by least significant difference test.

Results

The chemical composition of the browse forages are shown in Table 1. The CP content ranged from 163 to 270 g kg $^{-1}$ DM. The NDF values were in the range of 364 to 418 g kg $^{-1}$ DM, except for *G. bicolor* which had 527 g kg $^{-1}$ DM. The ADF concentrations of *B. discolor* and *Z. mucronata* were less than 200 g kg $^{-1}$ DM ,while those in the rest of the forages ranged from 224 to 262 g kg $^{-1}$ DM. The concentrations of TEPH, TET and TCT were highest in *A. brevispica* and *G. bicolor* and lowest in *A. mellifera* and *B. aegyptiaca*, respectively.

Nutritive values derived from *in vitro* gas production are presented in Table 2. *Balanites aegyptiaca* and *G. bicolor* had the highest and lowest gas production at 24 h, respectively ($p < 0.05$). *Grewia bicolor* had significantly the lowest OMD and ME, compared to other browses. The ME and OMD values were in the range of 6 to 8 MJ/kg and 433 to 555 g kg $^{-1}$ DM, respectively. The estimated DMI varied widely from 1.7 (*B. aegyptiaca*) to 5.3 (*A. brevispica*) kg/d.

Addition of supplements on IVRDMD upgrading differed between supplements ($p < 0.0021$) and within foliage species ($p < 0.0001$) (Table 3). The IVRDMD in JSY treatment was significantly higher than that of control, BRY and SP ($p < 0.05$). Despite BRY and SP not being significantly different from the control ($p > 0.05$), IVRDMD values were numerically higher. *Berchemia discolor* had significantly the highest response in IVRDMD for all the supplements ($p < 0.05$). Addition of the supplements increased the IVRDMD of all the browse forages, except for *B. aegyptiaca* ($p > 0.05$). *Acacia mellifera* also had a

Table 1. Chemical composition of browse foliages and supplements (g kg $^{-1}$ DM).

Species	OM	CP	NDF	ADF	ADL	TEPH	TET	TCT
A. brevispica	936.5	189.3	382.5	224.0	115.0	134.2	105.8	2.1
A. elatior	901.6	178.3	406.2	264.9	135.8	45.2	32.4	ND
A. mellifera	893.2	270.1	417.6	251.2	97.4	18.4	7.1	0.2
B. aegyptiaca	897.9	162.6	364.3	253.5	128.4	20.4	4.2	ND
B. discolor	913.4	210.8	381.3	164.9	61.9	72.3	33.0	1.4
G. bicolor	922.5	201.9	527.0	261.7	71.2	113.9	101.1	3.8
Z. mucronata	904.6	190.4	377.8	174.4	67.8	59.9	22.0	0.4
JSY	860.0	433.8						
BRY	716.0	344.4						
SP	897.0	859.1						

OM organic matter; CP crude protein; NDF neutral detergent fiber; ADF acid detergent fiber; ADL acid detergent lignin; TEPH total extractable phenolics; TET total extractable tannins; TCT total condensed tannins; ND not detected; JSY Japanese sake yeast; BRY bioethanol residue yeast; SP soybean peptide.

Table 2. Gas production parameters and nutritive estimates of browse foliages derived from *in vitro* gas test.

Species	24h	a†	b†	c†	OMD	ME	DMI
	(mL/200 mg DM)			(per h)	(%)	(MJ/kg DM)	(kg/d)
A. brevispica	26.4^{d}	5.3^{a}	35.6^{c}	0.008^{c}	47.3^{d}	6.9^{d}	5.3^{a}
A. elatior	26.0^{d}	1.6^{c}	30.3^{a}	0.014^{b}	46.6^{d}	6.8^{d}	3.3^{c}
A. mellifera	29.5^{c}	4.1^{ab}	33.1^{b}	0.013^{b}	54.0^{b}	7.8^{b}	4.6^{ab}
B. aegyptiaca	36.7^{a}	-2.3^{d}	42.4^{e}	0.030^{a}	55.5^{a}	8.1^{a}	1.7^{d}
B. discolor	33.0^{b}	1.9^{bc}	47.7^{f}	0.009^{c}	54.2^{ab}	7.9^{b}	4.0^{bc}
G. bicolor	21.2^{e}	0.5^{c}	32.5^{b}	0.008^{c}	43.3^{e}	6.2^{e}	2.8^{cd}
Z. mucronata	29.8^{c}	4.3^{ab}	39.2^{d}	0.008^{c}	50.5^{c}	7.3^{c}	4.9^{ab}
SEM	1.3	0.7	1.6	0.002	1.2	0.2	0.3

$^{a, b, c, d, e}$: Means in the same column with different superscripts differ ($p < 0.05$). a† gas production from soluble fraction; b† gas production from insoluble fraction; c† rate constant of gas production; OMD organic matter digestibility; ME metabolisable energy; DMI voluntary dry matter intake; DM dry matter.
† Parameters of gas production kinetics were estimated by using the exponential equation of Ørskov and McDonald [16].

Table 3. Effect of yeast addition on *in vitro* rumen dry matter degradability (IVRDMD) of browse foliages.

Browse	IVRDMD (%)				IVRDMD Increment (%) †		
	C	JSY	BRY	SP	JSY	BRY	SP
A. brevispica	53.6^{c}	56.2^{c}	54.2^{b}	54.9^{c}	5.0^{b}	1.3^{b}	2.6^{b}
A. elatior	44.9^{Bd}	55.5^{Ac}	48.2^{Bbc}	47.4^{Bd}	23.9^{Aa}	7.1^{Bab}	6.0^{Bb}
A. mellifera	59.6^{ABb}	57.8^{ABc}	61.3^{Aa}	56.3^{Bbc}	-3.0^{Bb}	2.8^{Aab}	-5.6^{Bc}
B. aegyptiaca	68.1^{a}	64.2^{b}	65.3^{a}	62.8^{ab}	-5.0^{b}	-3.0^{b}	-6.8^{c}
B. discolor	54.1^{Bbc}	71.7^{Aa}	64.0^{Aa}	67.5^{Aa}	33.4^{a}	19.8^{a}	24.8^{a}
G. bicolor	33.6^{e}	35.2^{e}	34.0^{d}	35.3^{e}	5.9^{b}	2.2^{ab}	5.1^{b}
Z. mucronata	41.8^{d}	44.9^{d}	42.9^{c}	46.0^{d}	7.7^{b}	3.0^{ab}	10.5^{ab}
SEM	2.2	2.2	2.2	2.1	2.9	2.5	2.5

$^{A, B}$: Means in the same row with different superscripts differ ($p < 0.05$). $^{a, b, c, d, e}$: Means in the same column with different superscripts differ ($p < 0.05$). C Control; JSY Japanese sake yeast; BRY bioethanol residue yeast; SP soybean peptide; SEM standard error of the mean.
† Calculated as: Increment (%) = 100 × {(JSY, BRY, SP)-C}/C.

negative response with exception of BRY supplement that had a 2.8% increase on the degradability. A correlation analysis performed between the chemical composition (Table 1) and percentage increment in IVRDMD of control and supplements (Table 3) gave low linear regression coefficients. The coefficients of determination (R^2) were low and ranged from 0.002 to 0.03.

Discussion

The chemical composition of the selected browse forages was within the range reported on similar species [5, 21]. The high CP and relatively low NDF values make the browse species suitable supplements to low quality forages and crop residues common in Kenya that are characterized by low CP (less than 8% of DM) and high NDF (more than 60% of DM) levels [22]. The browses, with exception of *A. mellifera* and *B. aegyptiaca*, had higher than 45 and 20 g kg^{-1} levels of TEPH and TET, respectively, above the critical levels recommended to avert significant adverse effects on ruminant livestock [23]. However, the CT values were below the level suggested (30-40 g kg^{-1} DM) [24] that might improve the efficiency of nitrogen digestion by protecting proteins from rumen degradation. The extent to which protein digestion is affected does not solely rely on tannin concentrations, but also depends on other factors: the source of tannins, binding strength of tannin-protein complexes and the effect of tannins on rumen bacteria activity [25]. Thus, even tannin-rich browses can on occasion yield beneficial effects comparable to those achieved with legume hay or commercial protein meals [25].

The nutritive parameters as a result of *in vitro* gas production show that the browse foliages have relatively high OMD, ME and DMI. The average ME requirement for maintenance is 424.5 kJ/kg $BW^{0.75}$ for growing goats [26]. Therefore, the average ME value (7.3 MJ/kg DM) of the browses may be deemed able to meet maintenance requirements of a 20 to 30 kg BW goat consuming 3% DM of kg body weight of browse as a sole diet [26]. The

estimated DMI values are more than sufficient to meet the required ME for maintenance and live weight gain of goats in tropical conditions. However, DMI of tanniferous forage may be limited by palatability, which is a function of the animal, plant and environmental factors [27].

Soybean peptide was used as a positive control in order to distinguish the effects on rumen microbes and fermentation of the addition of yeast supplements from potential supplemental effects of crude protein. Addition of the SP ensured that the *in vitro* rumen environment was adequately supplied with readily degradable nitrogen to provide ammonia for microbial growth. The results of the study indicate that the yeast supplements were both able to meet the nitrogen requirements from microbial yeast protein and further stimulate the growth and activity of ruminal microbes for the increased IVRDMD observed [11]. It is also postulated that soluble yeast protein acted as a readily available substrate for the tannins to bind to, forming tannin-yeast complexes, hence availing the plant fiber for ruminal digestion [28].

The ability of yeast to increase IVRDMD of browse foliage observed in the study has been reported by various authors with different roughages [8, 11, 29]. Tang *et al*. [30] reported an increase in rate of gas production and IVRDMD from yeast supplementation of low quality cereal straws that was associated with an increase in protozoa and cellulolytic bacteria populations. Increase in bacterial population and activity of rumen microbes that led to higher IVRDMD as a result of yeast supplementation may be attributed to ability of yeast to remove oxygen from the rumen environment and to effects of organic acids, essential enzymes and vitamins derived from yeast activity or yeast components themselves such as peptides and amino acids [31, 32]. Kim *et al*. [33] reported a significant positive correlation between ruminal molar proportions of branched-chain fatty acids (BCFA) and the efficiency of microbial protein synthesis. The BCFA are required for resynthesis of branched-chain amino acids for microbial protein synthesis in the rumen [34]. An *in vitro* fermentation study demonstrated that BCFA supplementation could increase microbial protein synthesis and DM digestion [35]. It is assumed that true protein supplementation via yeast could have been beneficial for BCFA production in the process of protein degradation in the rumen and consequently resulted in a greater increase in IVRDMD for JSY and BRY as compared with SP.

Both JSY and BRY supplements increased the ruminal digestion of the browse foliages and the effect of JSY appeared to be significantly higher. Differences in effect of yeast on rumen microbes and fermentation pattern are mainly associated with the strain of *Sacharomyces cereviciae* used [29, 36]. Certain strains of yeast are more effective at stimulating certain groups of bacteria and ruminal fermentation than others. Ability of yeast to influence rumen fermantation is more pronounced when live yeast cells are used as opposed to autoclaved yeast cultures [37] or yeast derivatives [29]. The BRY, a residue from bioethanol extraction from sugarcane, may have had a lower number of viable yeast cells, compared to JSY, hence the lower foliage degradability values. Ando *et al*. [29] also point out that the differences in the yeasts' metabolic functions or cell wall structures can influence their degradability of roughages.

Efficacy of yeast products on rumen fermentation and animal performance is also greatly influenced by the diet [7, 8]. In the case of browse foliage, the level of tannin may have affected efficacy of yeast as demonstrated by the relatively low response to yeast supplements by *A. brevispica* and *G. bicolor* which had the highest TET and TCT contents. However, level of tannins alone may not accurately predict responses as observed in the case between *B. discolor* and *Z. mucronata* which had similar phenolic content profile but significantly differed in dry matter degradabilities. It is postulated that factors such as the structure and biological activity of tannins and presence of other antinutritive compounds may have influenced the results observed. Further study on the effect of yeast supplementation on the nitrogen (N) degradation in the rumen and a subsequent effect on post-ruminal N digestion status are needed.

Conclusions

The study shows that Sake yeast has remarkable potential to optimise rumen fermentation of tannin-rich forages; however, effects are influenced by strain of yeast. Further studies to quantify effect of yeast supplement on the level of bypass protein from tanniferous browse forage are needed. In addition, the differences in yeast strains also warrant evaluation of diverse, cheap and easily available yeast sources at the farm level.

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