

SHORT REPORT

***In vitro* assessment of ruminal fermentation characteristics of tropical browse mixtures supplemented with yeast**

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Abstract

An *in vitro* ruminal gas production study was conducted to assess the effect of yeast supplementation on rumen fermentation of tanniniferous tropical browse mixtures prepared by mixing *Berchemia discolor* with *Acacia brevispica*, *Acacia elatior*, *Acacia mellifera*, *Balanites aegyptiaca*, *Grewia bicolor* or *Zizyphus mucronata* in a 1:1 dry weight ratio. The six browse mixtures were incubated with buffered rumen fluid alone or supplemented with dried Japanese Sake yeast (JSY) or polyethylene glycol (PEG). Ruminal gas production characteristics were evaluated using the Gompertz equation. The JSY addition increased gas production volume ($P < 0.05$) and numerically increased the maximum gas production rate constant. Time to reach the maximum gas production rate was shortened by PEG addition as compared to the JSY and control treatments. It is possible that JSY promoted the rumen microbial activity in addition to forming complexes with tannins thereby upgrading the *in vitro* ruminal digestion of tropical browse mixtures.

Introduction

Feeding of browse–foliage mixtures is an effective feeding regimen that can compensate nutrient imbalances and dilute negative nutritional effects of tannins as compared with tanniniferous browses fed as sole diets (Mueller-Harvey 2006). The nutritional interaction caused by feeding diverse browse foliages as opposed to single foliage can alter the type and number of rumen microorganisms and subsequently have an affect upon nutrient digestion. Since mixing of different browse foliages may also cause a positive associative effect on the digestibility or intake of the mixture (Niderkorn and Baumont 2009), the elicited associative effect may consequently alter production performance of small ruminants in tropical areas (Rosales and Gill 1997).

Previous research focusing on the interaction of tannins with dietary components has shown that the nutritive potential of tanniniferous browse species can be achieved by use of a tannin binding agent, polyethylene glycol (PEG).

However, the relatively high cost of PEG has limited its use in agronomic practice by farmers all over the world (Ben Salem *et al.* 2005). Few studies have explored alternative feed additives with potential to modulate rumen ecosystem in the presence of tannins.

Yeast is a microbial feed additive used to manipulate the ruminal microbial ecosystem by providing true protein as well as to enhance fiber digestion and stabilize rumen pH (Yoon and Stern 1995). Moreover, it can be expected to form complexes with foliage tannins that are known to bind proteins and polysaccharides, and lower their digestibilities in the gastro-intestinal tract (Böer *et al.* 2009). Feeding of browses as supplements to low quality basal forages is commonly practiced by small scale farmers in tropical areas. Nonetheless, feeding strategies that promote nutrient complementarity as well as explore alternative supplements to PEG are needed.

In this study, we measured *in vitro* ruminal gas production for browse foliages commonly used by Kenyan farmers

with or without addition of yeast or PEG, and estimated the gas production parameters using the Gompertz model (Lavrenčič *et al.* 1998). The aim of the study was to evaluate the viability of yeast supplementation as an alternative to PEG in upgrading the ruminal fermentation characteristics of tannin containing tree foliages.

Materials and methods

Experimental procedures

Six browse mixtures were prepared by mixing one part of *Berchemia discolor* with one part of *Acacia brevispica*, *Acacia elatior*, *Acacia mellifera*, *Balanites aegyptiaca*, *Grewia bicolor* or *Zizyphus mucronata* on dry weight basis. The leaves of the tree species were collected from Baringo District, a semiarid region in Kenya. The fodder tree species were selected based on primary data on feeds and feeding practices within the locality in Kenya. Among the seven browses, *Berchemia discolor* has been reported to show relatively greater ruminal digestibility (Wambui *et al.* 2010), palatability and live weight gain of growing goats when supplemented to rhodesgrass hay (Osuga *et al.* 2008, 2011). The leaf samples collected at the end of the rainy season were oven dried at 50°C for 48 h to a constant weight, ground to pass through a 2 mm screen for chemical analysis and a further 1 mm screen for *in vitro* gas production study.

The six browse mixtures were incubated with buffered sheep rumen fluid alone (control) or supplemented with dried Japanese Sake yeast (JSY) obtained from a commercial Sake Company (Gekkeikan Sake Co., Ltd. Kyoto, Japan) or PEG (molecular weight = 6000). The sample (alone) or supplemented with additive were incubated *in vitro* with buffered rumen fluid (30 mL) in calibrated glass syringes in triplicate by following the procedure of Menke and Steingass (1988). Syringes containing only the incubation medium, JSY or PEG supplemented medium were incubated as blanks to correct for the gas production from buffered rumen fluid, JSY and PEG, respectively. The cumulative gas production readings were recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation and gas production originating from incubated browse mixtures were calculated using values of blanks.

The gas production parameters of the tested browse mixtures were estimated by fitting the produced gas volume (mL 200 mg⁻¹ dry matter [DM]) at each incubation time to the Gompertz model as outlined by Lavrenčič *et al.* (1998):

$$G = Be^{-Ce^{-At}} \quad (1)$$

where G is cumulative gas production (mL 200 mg⁻¹ DM) at time t (hour), B is asymptote gas production volume (mL 200 mg⁻¹ DM) and C is the relative gas production rate

affected by constant A describing the decay in fermentation rate. The first derivative of Eqn 1 can be given as follows:

$$\frac{dG}{dt} = ABC e^{-At} e^{-C} e^{-At} \quad (2)$$

The second derivative of Eqn 1 with respect to t can be given as follows:

$$\frac{d^2G}{dt^2} = [A^2BC^2(e^{-At})^2 e^{-Ce^{-At}}] - [A^2BCe^{-At} e^{-Ce^{-At}}] \quad (3)$$

The inflection point of Eqn 1 can be given by setting Eqn 3 equal to zero, and solving for t . The solution of Eqn 3, $t = \ln C \cdot A^{-1}$, can be regarded as time to reach the maximum gas production rate (TMGP, h) during *in vitro* incubation. The rate constant of maximum gas production (MGPR, % h⁻¹) was calculated by substituting the TMGP for Eqn 2.

Chemical analyses

The DM, crude ash and crude protein (CP) concentration were determined according to AOAC (1984). Neutral detergent fiber (NDF), acid detergent fiber and acid detergent lignin (ADL) were determined according to the methods of Van Soest *et al.* (1991) for browse samples and JSY. Total extractable phenolics (TEPH), total extractable tannins (TET) and total condensed tannins (TCT) of the samples were determined by the procedure described by Makkar (2000).

Statistical analyses

In vitro gas production data was fitted to the Gompertz model using commercial software (Curve Expert Ver 3.1), and one-way analysis of variance was performed for the analysis of gas production variables using general linear model of StatView for Windows® (SAS institute 1999). Statistical differences of the means between the browse mixtures and treatment supplements were tested using Fisher's least significance difference at $P < 0.05$.

Results and discussion

The chemical profile of the browse foliages was within the range reported for similar browse species from Kenya (Ondiek *et al.* 2010). The concentration of CP (121–216 g kg⁻¹ DM) and NDF (288–431 g kg⁻¹ DM) of the seven browse species (Table 1) indicate that the tested browses can be used as supplementary feed to low quality diets such as natural pasture and crop residue predominant in the tropics (Wambui *et al.* 2006). The browses studied in

Table 1 Chemical composition of browse foliages and yeast (g kg⁻¹ DM)

Material	OM	CP	NDF	ADF	ADL	TEPH	TET	TCT
<i>Acacia brevispica</i>	936.5	154.7	345.7	201.2	100.3	134.2	105.8	2.1
<i>Acacia elatior</i>	901.6	138.4	341.1	230.8	123.8	45.2	32.4	ND
<i>Acacia mellifera</i>	893.2	215.8	352.1	226.7	92.9	18.4	7.1	0.2
<i>Balanites aegyptiaca</i>	897.9	121.1	309.4	222.2	117.0	20.4	4.2	ND
<i>Berchemia discolor</i>	913.4	165.4	320.5	165.9	70.9	72.3	33.0	1.4
<i>Grewia bicolor</i>	922.5	157.0	431.0	251.2	76.0	113.9	101.1	3.8
<i>Zizyphus mucronata</i>	904.6	151.2	288.0	156.2	65.4	59.9	22.0	0.4
JSY	806.0	433.8	Trace	Trace	Trace	ND	ND	ND

ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; DM, dry matter; JSY, Japanese Sake yeast; ND, not detected; NDF, neutral detergent fiber; OM, organic matter; TCT, total condensed tannins; TEPH, total extractable phenolics; TET, total extractable tannins.

the current experiment except *Acacia mellifera* and *Balanites aegyptiaca* had TEPH and TET concentrations above the levels suggested (45 and 20 g kg⁻¹ DM, respectively) not likely to produce significant adverse nutritional effects on ruminant livestock (Getachew *et al.* 2002). In addition to phenolic concentrations, variations of tannin structures also affect their binding capacity with protein and fiber fraction. The feeding value and growth performance appear to differ between the seven tested browses when fed to ruminant livestock as a basal feed (Osuga *et al.* 2008; Wambui *et al.* 2010).

Gas production technique is a useful tool in predicting feeding value of forages (Rodrigues *et al.* 2002). Lavrenčič *et al.* (1998) used the Gompertz equation for interpreting nylon bag data of grass and legume hays. Schofield *et al.* (1994) indicated that the Gompertz growth model can also be applicable to gas production data for evaluating forage fermentation characteristics in the rumen. The Gompertz

model well describes the ruminal fermentation nature of the foliages in the early phases of incubation (Lavrenčič *et al.* 1998). The gas production curves of Kenyan browse with treatment supplements fitted the Gompertz model well ($r^2 = 0.995\text{--}0.999$).

The Gompertz gas production parameters were influenced by browse species and treatment supplements (Table 2). The differences in gas production parameters among the browse mixtures indicating that there were differences in nutrients according to the substrates being fermented in the rumen. It was prominent that the JSY supplement elicited higher value of *B* for all foliages ($P < 0.05$), and also showed numerically or significantly higher values ($P < 0.05$) for MGPR than for PEG and control treatments. These results concur with Tang *et al.* (2008) who reported an increased rate of gas production and cumulative gas volume for cereal straws by addition of yeast culture. Yeast supplementation to low quality basal forages promoted the

Table 2 Gas production parameters obtained from fermentation of browse mixtures when supplemented with Japanese Sake yeast (JSY) or polyethylene glycol (PEG)

Mixture	Gas production parameters								
	<i>B</i> (mL 200 mg ⁻¹ DM)†			MGPR (% h ⁻¹)‡			TMGP (h)§		
	C	JSY	PEG	C	JSY	PEG	C	JSY	PEG
<i>Acacia brevispica</i> + <i>Berchemia discolor</i>	36.3 ^{abB}	49.3 ^{abA}	35.7 ^{abB}	1.1 ^{bB}	1.6 ^{bA}	1.2 ^{cB}	12.2 ^{bA}	11.1 ^{bB}	10.9 ^{abB}
<i>Acacia elatior</i> + <i>Berchemia discolor</i>	34.9 ^{bB}	42.5 ^{cA}	35.5 ^{abB}	1.2 ^b	1.5 ^{bc}	1.4 ^b	10.4 ^b	10.7 ^b	9.1 ^b
<i>Acacia mellifera</i> + <i>Berchemia discolor</i>	36.9 ^{abB}	47.5 ^{abA}	35.1 ^{abB}	1.2 ^b	1.6 ^b	1.2 ^c	11.5 ^b	11.2 ^b	10.8 ^a
<i>Balanites aegyptiaca</i> + <i>Berchemia discolor</i>	39.2 ^{aC}	50.7 ^{aA}	37.4 ^{abB}	1.7 ^{aB}	2.0 ^{aA}	1.8 ^{aAB}	8.6 ^{cAB}	9.1 ^{cA}	7.5 ^{cB}
<i>Grewia bicolor</i> + <i>Berchemia discolor</i>	36.0 ^{abB}	45.9 ^{bcA}	31.9 ^{bC}	0.8 ^{cB}	1.2 ^{cA}	1.2 ^{bcA}	16.0 ^{aA}	13.9 ^{ab}	9.4 ^{bC}
<i>Zizyphus mucronata</i> + <i>Berchemia discolor</i>	39.7 ^{abB}	48.9 ^{abA}	34.4 ^{abB}	1.2 ^b	1.7 ^b	1.3 ^{bc}	12.0 ^b	10.5 ^b	9.8 ^{ab}

^{a,b,c}Means with different superscripts in the same column differ ($P < 0.05$).

^{A,B,C}Means with different superscripts in the same row in the same parameter differ ($P < 0.05$).

†Asymptote gas production volume.

‡The maximum rate constant of gas production.

§Time to reach maximum gas production rate.

C, control treatment with no supplement; DM, dry matter.

growth of fibrolytic bacteria through its ability to scavenge oxygen and production of metabolites such as peptides, amino acids and branched-chain organic acids in the rumen (Fonty and Chaucheyras-Durand 2006). Although neither the binding capacity of yeast to tannin nor measurement of metabolites production was conducted in the current study, tannins are known to have a high affinity for proteins with respect to fiber fractions (Waghorn 2008). It can therefore be expected that the addition of yeast, which had 434 g CP kg⁻¹ DM, may have formed tannin-protein complexes easily. In this respect, plant CP and fiber fraction were available for fermentation by the rumen microbes hence the improvement of gas parameters observed upon JSY addition. Böer *et al.* (2009) reported that the presence of plant phenolic compounds induced tannase production from yeast and anaerobic bacteria. The enzyme catalyses the breakdown of tannins into gallic acid and glucose, hence it could be expected to mitigate negative effects of tannins on ruminal digestion when tannase was produced in the rumen. However, further studies are needed to ascertain the mode of biological actions of dried JSY supplement to browse foliages in elucidating nutritional benefits of yeast for applying as diet supplement to ruminants. On the other hand, PEG supplement did not increase the gas production parameters of browse mixtures markedly, which concurred with Rogosic *et al.* (2008). In their study, PEG supplementation did not increase the intake by goats and sheep of foliage as the diversity of shrubs provisions were increased. Rogosic *et al.* (2008) have suggested that shrub mixing facilitate interactions between plant secondary metabolites, and the resultant complexes can lower negative impacts of tannins on the rumen digestion due to altered microbial activities in the rumen, hence prominent effects of PEG supplementation could not be obtained. The TMGP differed among browse mixtures and was significantly shortened ($P < 0.05$), by the addition of JSY and PEG supplements. The TMGP gives an indication of the time taken for feedstuff to achieve extensive ruminal degradation (Lavrenčič *et al.* 1998) and residence time in the lag compartment in the rumen (Van Milgen *et al.* 1991). It was assumed that a reduction in time taken for the ingested feed particles to be hydrated and colonized by rumen microbes before degradation processes begin in conjunction with an increased degradation rate would result in an increase in ruminal degradability of nutrients (Van Milgen *et al.* 1991), and could subsequently reflect the ruminal passage kinetics of digesta particles (Lavrenčič *et al.* 1998) and voluntary feed intake (Rodrigues *et al.* 2002).

It was noteworthy that *Balanites aegyptiaca* and *Berchemia discolor* mixture showed higher values of *B*, MGPR and lower TMGP value as compared with the other mixtures in all the supplement treatments (Table 2). This is an indication of higher susceptibility to ruminal degradation and

higher feeding value of the supplement feed. The concentration, chemical structure and biological activities of tannins affect protein and fiber digestion in the rumen and *in vitro* gas production parameters (Barahona *et al.* 2003). In this study, asymptote *in vitro* gas production volume (*B*) correlated negatively with concentration of TET ($r^2 = 0.604$, $P = 0.037$) and NDF ($r^2 = 0.627$, $P = 0.031$) among the browse mixtures for control treatment (data not shown). The data shows that high tannin and fiber concentrations in browse foliages reduce ruminal fermentation parameters. *Balanites aegyptiaca* had the lowest TET concentration among the tested browses and quite low TCT concentration (Table 1), which might partly explain the reason for mixture exhibiting better ruminal fermentation characteristics than the other mixtures in the control treatment with no supplements. In addition to tannins, lignin content and lingo-cellulose formation also contribute to species differences in nutrient degradability in the rumen (Barahona *et al.* 2003; Tiemann *et al.* 2008). Although the calculated value indicating the extent of lignification of *Balanites aegyptiaca* + *Berchemia discolor* was relatively higher (9.4 g ADL kg⁻¹ DM and 29.8 g ADL kg⁻¹ NDF) than the other browse mixtures (data not shown), the mixture exhibited better ruminal fermentation status than the other mixtures in all treatment supplements.

It was concluded that browse foliage mixing would alleviate the negative effects of tannins, which are usually elicited in single species diets and may be a useful tool in designing feed regimens that increase animal productivity from rangelands. With both practical and economical perspective, yeast (brewer's residue) supplementation has a potential to improve the rumen fermentation of tanniniferous tropical browses and offers a suitable substitute to expensive PEG addition.

References

- AOAC (1984) *Official Methods of Analysis*, 14th edn. Association of Official Analytical Chemists, Arlington, VA, USA.
- Barahona R, Lascano CE, Narvaez N, Owen E, Morris P, Theodorou MK (2003) *In vitro* degradability of mature and immature leaves of tropical forage legumes differing in condensed tannins and non-starch polysaccharide content and composition. *J Sci Food Agric* 83: 1256–1266.
- Ben Salem H, Ben Salem I, Ben Said MS (2005) Effect of the level and frequency of PEG supply on intake, digestion, biochemical and clinical parameters by goats given kermes oak (*Quercus coccifera* L.)-based diets. *Small Rum Res* 56: 127–137.
- Böer E, Bode R, Mock H, Piontek M, Kunze G (2009) AtanIp – an extracellular tannase from the dimorphic yeast *Arxula adeninivorans*: molecular cloning of the *ATAN1* gene

- and characterization of the recombinant enzyme. *Yeast* 26: 323–337.
- Fonty G, Chaucheyras-Durand F (2006) Effects and modes of action of live yeasts in the rumen. *Biologia Bratislava* 61: 741–750.
- Getachew G, Makkar HPS, Becker K (2002) Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometrical relationship between short chain fatty acid and *in vitro* gas production. *J Agric Sci* 139: 341–352.
- Lavrenčič A, Mills CR, Stefanon B (1998) Application of the Gompertz model to describe the fermentation characteristics of chemical components in forages. *Anim Sci* 66: 155–161.
- Makkar HPS (2000) Quantification of Tannins in Tree Foliage: A Laboratory Manual; A Joint FAO/IAEA Working Document, Vienna, Austria.
- Menke KH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim Res Dev* 28: 7–55.
- Mueller-Harvey I (2006) Unravelling the conundrum of tannins in animal nutrition and health. *J Sci Food Agric* 86: 2010–2037.
- Niderkorn V, Baumont R (2009) Associative effects between forages on feed intake and digestion in ruminants. *Animal* 3: 951–960.
- Ondiek JO, Abdulrazak SA, Njoka EN (2010) Chemical and mineral composition, *in-vitro* gas production, *in-sacco* degradation of selected indigenous Kenyan browses. *Livest Res Rural Dev* Vol 22, Article no 25, available online: <http://www.lrrd.org/lrrd22/2/ondi22025.htm> [Cited 22 December 2011.]
- Osuga IM, Wambui CC, Abdulrazak SA, Ichinohe T, Fujihara T (2008) Evaluation of nutritive value and palatability by goats and sheep of selected browse foliages from semiarid area of Kenya. *Anim Sci J* 79: 582–589.
- Osuga IM, Abdulrazak SA, Muleke CI, Fujihara T (2011) Effect of supplementing Rhodes grass hay (*Chloris gayana*) with *Berchemia discolor* or *Zizyphus mucronata* on the performance of growing goats in Kenya. *J Anim Physiol Anim Nutr*, doi: 10.1111/j.1439-0396.2011.01189.x.
- Rodrigues MAM, Fonseca AJM, Sequeira CA, Dias-da Silva AA (2002) Digestion kinetic parameters from an *in vitro* gas production method as predictors of voluntary intake of forage by mature ewes. *Anim Feed Sci Technol* 95: 133–142.
- Rogosis J, Pfister JA, Provenza FD, Pavlicevic J (2008) The effect of polyethylene glycol on intake of Mediterranean shrubs by sheep and goats. *J Anim Sci* 86: 3491–3496.
- Rosales M, Gill M (1997) Tree mixtures within integrated farming systems. *Livest Res Rural Dev* Vol 9 No 4, available online: <http://www.lrrd.org/lrrd9/4/mauro941.htm> [Cited 22 December 2011.]
- SAS institute (1999) Statview for Windows, Version 5.0.1. SAS institute, Cary, NC.
- Schofield P, Pitt RE, Pell AN (1994) Kinetics of fiber digestion from *in vitro* gas production. *J Anim Sci* 72: 2980–2991.
- Tang SX, Tayo GO, Tan ZL, Sun ZH, Shen LX, Zhou CS, Xiao WJ, Ren GP, Han XF, Shen SB (2008) Effects of yeast culture and fibrolytic enzyme supplementation on *in vitro* fermentation characteristics of low-quality cereal straws. *J Anim Sci* 86: 1164–1172.
- Tiemann TT, Avial P, Ramirez G, Lascano CE, Kreuzer M, Hess HD (2008) *In vitro* ruminal fermentation of tanniferous tropical plants: plant-specific tannin effects and counteracting efficiency of PEG. *Anim Feed Sci Technol* 146: 222–241.
- Van Milgen J, Murphy MR, Berger LL (1991) A compartmental model to analyze ruminal digestion. *J Dairy Sci* 74: 2515–2549.
- Van Soest PJ, Robertson JB, Lewis BA (1991) Methods of fibre, neutral detergent fibre and non-starch carbohydrates in relation to animal nutrition. *J Dairy Sci* 74: 3583–3597.
- Waghorn G (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production – Progress and challenges. *Anim Feed Sci Technol* 147: 116–139.
- Wambui CC, Abdulrazak SA, Noordn Q (2006) Performance of growing goats fed urea sprayed maize stover and supplemented with graded levels of *Tithonia diversifolia*. *Asian-Aus J Anim Sci* 19: 992–996.
- Wambui CC, Awano T, Ando S, Abdulrazak SA, Ichinohe T (2010) Effect of Yeast supplementation on *In vitro* ruminal degradability of selected Browse species from Kenya. *J Food Agric Environ* 8: 553–557.
- Yoon IK, Stern MD (1995) Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: a review. *Asian-Aus J Anim Sci* 8: 533–555.