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Ms. No. ANIFEE-10-3118R5 – response to comments from Editor in Chief:

1. L105-107: Absorbance units cannot be expressed per g DM. They depend also on sample extraction volume and the path length of the cuvette. Report the particulars and merely refer AU values (unitless) in tables to the section where explanation is found. – made the suggested changes

2. Were B and Ph tested against their interaction term? If not, this should either be corrected and reported. – reanalyzed B and Ph using General Linear Models multivariate instead of univariate to make suggested corrections. (see Table 1)

3. Provide a clean revised copy with only the recent changes shown – made the suggested changes
Chemical composition, *in vitro* dry matter digestibility and *in vitro* gas production of five woody species browsed by Matebele goats (*Capra hircus* L) in a semi-arid savanna, Zimbabwe

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ABSTRACT

The chemical composition, *in vitro* dry matter digestibility (IVDMD) and *in vitro* gas production (IVGP) of five woody species: *Commiphora pyracanthoides*, *Terminalia prunioides*, *Acacia tortilis*, *Faidherbia albida* and *Dichrostachys cinerea* browsed by Matebele goats in an open semi-arid savanna was determined at three leaf phenophases. *Terminalia prunioides* had the highest IVGP volume (80.1 ml) and *C. pyracanthoides* the highest IVDMD (0.60) with *D. cinerea* having both the lowest IVDMD (0.39) and IVGP volume (43.7 ml). This was presumably due to the high condensed tannins (CT) in *D.*
cinerea (20.1 $A_{550\text{nm}}$) as compared to the low CT in T. prunioides (1.2 $A_{550\text{nm}}$) and C. pyracanthoides (2.9 $A_{550\text{nm}}$). Terminalia prunioides had lower acid (240.6 vs 354.4 g/kg DM) and neutral (351.2 vs 430.5 g/kg DM) detergent fibre than C. pyracanthoides. Crude protein (CP) and IVDMD declined while neutral detergent fibre (NDF) and acid detergent fibre (ADF) increased with leaf maturity. In vitro dry matter digestibility was not influenced by CP ($r = 0.09$, $P>0.05$) and NDF ($r = -0.29$, $P>0.05$) while ADF negatively affected IVGP ($r = -0.54$, $P<0.05$). Gas production was positively associated with IVDMD ($r = 0.54$, $P<0.05$). Condensed tannins had a greater negative effect on IVDMD ($r = -0.58$, $P<0.05$) and IVGP (-0.68, $P<0.01$) than acid ($r = -0.46$, $P>0.05$; $r = -0.54$, $P<0.05$) and neutral ($r =-0.29$, $P>0.05$; $r = -0.51$, $P>0.05$) detergent fibre. Total phenolics were positively correlated to IVGP ($r = 0.73$, $P>0.05$). We conclude that T. prunioides had good rumen fermentation attributes due to low NDF, ADF and CT contents.

**Keywords:** leaf phenophase; neutral detergent fibre; condensed tannins; total phenolics; correlation

**Abbreviations:** A, absorbance units; ADF, acid detergent fibre; AOAC, Association of Official Analytical Chemists; CP, crude protein; CT, condensed tannins; DM, dry matter; IVDMD, *in vitro* dry matter digestibility; IVGP, *in vitro* gas production; NDF, neutral detergent fibre; TP, total phenolics
1. Introduction

Goats in semi-arid south-western Zimbabwe mainly depend on browse for their nutritional requirements. The browse is available during a short wet period between November and April (Sebata and Ndlovu, 2010). The goats need to select browse of high nutritive value to build adequate body reserves for mobilization during the dry period when feed is scarce. Woody species commonly browsed by Matebele goats in south-western Zimbabwe include *Commiphora pyracanthoides* Engl. subsp. *pyracanthoides*, *Terminalia prunioides* C. Lawson, *Acacia tortilis* (Forssk.) Hayne subsp. *heteracantha* (Burch.) Brenan, *Faidherbia albida* (Delile) A. Chev. and *Dichrostachys cinerea* (L.) Wight & Arn subsp. *nyassana* (Taub.) Brenan. The availability of these woody species is related to the extent of rangeland degradation. In heavily degraded areas *A. tortilis* is predominant while in less degraded rangelands *T. prunioides* and *C. pyracanthoides* are the main browse species. *Faidherbia albida* is browsed for short periods when the goats drink water from large rivers such as the Umzingwane while *D cinerea* is occasionally encountered along drainage systems. The nutritive value of browse species needs to be determined to understand goat foraging behaviour.

Chemical composition, rumen fermentation and digestibility can be used as proxies for nutritive value of browse (Mandal, 1997). Chemical composition parameters include crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), condensed tannins (CT) and total phenolics (TP) (Kaitho et al., 1998). Digestibility and fermentation can be determined using *in vitro* dry matter digestibility (IVDMD) (Tilley and Terry, 1963) and *in vitro* gas production (IVGP) (Menke and Steingass, 1988) respectively.
Volume of gas produced during in vitro fermentation reflects the extent of browse fermentation and digestibility (Getachew et al., 1998). The IVGP technique is also efficient in determining nutritive value of woody species which contain condensed tannins (Khazaal et al., 1993). High condensed tannins (proanthocyanidins) and fibre (NDF and ADF) contents reduce digestibility (Makkar, 1989; Rubanza et al., 2007) while low crude protein affects acceptability of browse (Lundberg and Astrom, 1990; Augner, 1995).

The objective of this study was to determine the chemical composition (CP, NDF, ADF, CT and TP), IVDMD and IVGP of five woody species: F. albida, D. cinerea, T. prunioides, C. pyracanthoides and A. tortilis during the growing season (December to June) at three leaf phenophases.

2. Materials and methods

The leaf samples were collected at Kwalu Communal lands (21°25′S, 29°30′E, altitude 1150m a.s.l.), which is 415 km² in size and 87 km northwest of Beitbridge town, Zimbabwe. Mean annual rainfall recorded over a period of 30 years was 332.9 mm (range: 83-527), with most of it falling during a single rainy season between November and April. Mean monthly temperature ranges from 7.9°C to 33.6°C, with July recording the lowest and October the highest. The area is dry with a relative humidity of between 48% and 61% and a negative moisture balance.

Fresh foliage (leaves and stems < 3 mm diameter) of each browse species were harvested from five randomly selected and tagged representative plants of each species.
10 and 20 weeks post shoot and leaf sprouting, weighed and air dried under shade. The foliage was harvested from several branches situated in the part of canopy accessible to goats (between 0.5 and 1.0 m in height) and a sample from each tree was kept separately. The foliage samples were ground in a Wiley-mill to pass through a 1 mm sieve. The rumen fluid used in the IVDMD and IVGP experiments was collected before the morning feeding from 3 rumen-fistulated Matebele goats, which were fed on a diet containing the five woody species in equal amounts. Standard methods as described in AOAC (1990) were used for determination of dry matter (DM, method no. 930.15), crude protein (CP, method no. 984.13) and acid-detergent fibre (ADF, method no. 973.18). Neutral-detergent fibre was determined by the method of Van Soest et al. (1991), without the use of sodium sulphite and amylase and expressed inclusive of residual ash. Condensed tannins (CT) were determined by extracting the sample (100 mg) with 20 ml of 50% aqueous methanol (1/1 methanol/water), adding the extract (1 ml) to n-butanol/HCL (5 ml, 95/5, v/v), placing the solution in a water-bath at 100°C for one hour and reading absorbance at 550 nm (Reed et al., 1985). Condensed tannins concentration was reported as absorbance units (A$_{550nm}$). Total phenolics (Folin–Ciocalteu) contents of the browse were analyzed as described by Makkar (2003). The IVDMD was determined as described by Tilley and Terry (1963) and IVGP following the procedure by Menke and Steingass (1988). Statistical analyses of data were performed using SPSS 11.5 (SPSS, 2002), using the general linear model with 5 browse species x 3 leaf phenophases in a factorial arrangement with 5 replicates according to the model: $Y_{ijk} = \mu + Bi + Phj + (BPh)_{ij} + e_{ijk}$, where $Y_{ijk}$ is the response variable; $\mu$ is the general mean; $Bi$ the effect of browse species; $Phj$ the effect of leaf phenophase; $(BPh)_{ij}$ is the interaction effect; $e_{ijk}$ the
residual error. Browse species and leaf phenophase were used as a fixed and random factor, respectively and linear effects were assessed. The relationships between chemical composition, IVDMD and IVGP were tested by Pearson’s correlation analysis.

3. Results

The chemical composition, IVDMD and IVGP of the browse species are presented in Table 1. Crude protein and IVDMD declined while NDF and ADF increased with leaf maturity (P<0.001). Condensed tannins, TP and IVGP varied with leaf maturity (P<0.001) with no trend. *Terminalia prunioides* had the lowest NDF, ADF, and CT and the highest TP and gas volume. *Commiphora pyracanthoides* had the highest IVDMD. *Dichrostachys cinerea* had the least IVDMD and IVGP and showed a marked decline in digestibility with leaf maturity.

The correlations between chemical composition, IVDMD and IVGP of the browse species are shown in Table 2. Crude protein had no relationship with IVDMD ($r = 0.09$, P>0.05) and IVGP ($r = -0.53$, P>0.05). Neutral detergent fibre had no relationship with IVDMD ($r = -0.29$, P>0.05) and IVGP ($r = -0.51$, P>0.05), while ADF had a negative relationship with IVGP ($r = -0.54$, P<0.05). In vitro dry matter digestibility had a positive relationship with IVGP ($r = 0.54$, P<0.05). Condensed tannins had a negative relationship with IVDMD ($r = -0.58$, P<0.05) and IVGP ($r = -0.68$, P<0.01). Total phenolics had a negative relationship with CP ($r = -0.58$, P<0.05), NDF ($r = -0.71$, P<0.01) and ADF ($r = -0.68$, P<0.01) and a positive relationship with IVGP ($r = 0.73$, P<0.01).
4. Discussion

The nutritive value of browse is influenced by its rumen fermentation and digestibility which are constrained by chemical composition. A high IVGP reflects greater fermentation to support rapid rumen microbial growth (Van Soest, 1994) and is an indirect measure of the energy supply of browse (Menke and Steingass, 1988). *Terminalia prunioides* was the most fermentable, *C. pyracanthoides* the most digestible and *D. cinerea* had both the lowest rumen fermentation and digestibility. *In vitro* gas production can also be used to predict browse digestibility (Getachew et al., 1998). *In vitro* gas production was positively associated with digestibility (Khazaal et al., 1993).

The five browse species met the minimal CP requirements for optimal rumen microbial function of 80 g/kg DM (Van Soest, 1994), explaining the lack of correlation between CP and both IVDMD and IVGP. The decline in CP with leaf maturity was probably due to the build up of lignocellulosic fibre structures (Topps, 1997). The NDF (range: 336 to 581 g/kg DM) and ADF (range: 201 and 387 g/kg DM) contents were low to moderate. Low fibre content results in high digestibility (Topps, 1997). The increase in NDF and ADF with leaf maturity could be attributed to lignification (Topps, 1997). Condensed tannins had an inverse relationship with both IVDMD and IVGP. Other researchers reported similar findings between CT and digestibility (Ammar et al., 2005; Mokoboki et al., 2005) and between CT and IVGP (Getachew et al., 2000). The high IVDMD and IVGP in *T. prunioides* was presumably due to the low acid and neutral detergent fibre and CT, while in *C. pyracanthoides* it was probably due to the low CT content. In *F. albida* and *D. cinerea* the high CT contents may have accounted for the
low digestibility and fermentation. Condensed tannins interfere with woody species
digestibility (Dube et al., 2001). *Acacia tortilis* had low CT implying that its digestibility
and fermentation was influenced by acid and neutral detergent fibre contents.

We found condensed tannins to have a greater effect on the digestibility and
fermentation of the browse species than acid and neutral detergent fibre. Makkar et al.
(1993) reported the nutritive value of browse as being reduced more by CT than by fibre
content. Our findings suggest that higher goat productivity is likely going to be achieved
in less degraded rangelands dominated by *T. prunioides* and *C. pyracanthoides* as
opposed to *A. tortilis* dominated degraded rangelands. *Dichrostachys cinerea* and *F.
albida* contributions to goats browse is further constrained by their low nutritive
attributes in addition to their low density in the rangelands.

**Conclusions**

We conclude that *T. prunioides* had good rumen fermentation attributes due to low
NDF, ADF and CT contents. Condensed tannins had a greater influence on digestibility
and fermentation than acid and neutral detergent fibre.

**References**

Ammar, H., Lopez, S., Gonzalez, J.S., 2005. Assessment of the digestibility of some


SPSS, 2002. SPSS Version 11.5 for Windows. SPSS Inc., Chicago, USA.


Table 1 Chemical composition (g/kg DM), *in vitro* dry matter digestibility and *in vitro* gas production (ml/300mg DM) of five woody species at three leaf phenophases

<table>
<thead>
<tr>
<th>Phenophase (Phe)</th>
<th>Early leaf (mid-Dec)</th>
<th>Full leaf (mid-Mar)</th>
<th>Mature leaf (mid-Jun)</th>
<th>Sed</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fa</td>
<td>Dc</td>
<td>Tp</td>
<td>Cp</td>
<td>At</td>
</tr>
<tr>
<td>CP</td>
<td>230.3</td>
<td>211.2</td>
<td>166.6</td>
<td>236.6</td>
<td>264.1</td>
</tr>
<tr>
<td>NDF</td>
<td>472.2</td>
<td>390.5</td>
<td>336.2</td>
<td>400.4</td>
<td>503.2</td>
</tr>
<tr>
<td>ADF</td>
<td>332.6</td>
<td>327.8</td>
<td>201.3</td>
<td>321.0</td>
<td>313.0</td>
</tr>
<tr>
<td>CT(^a)</td>
<td>4.2</td>
<td>6.4</td>
<td>1.6</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>TP(^b)</td>
<td>15.2</td>
<td>18.8</td>
<td>44.3</td>
<td>6.5</td>
<td>10.1</td>
</tr>
<tr>
<td>IVGP (48hrs)</td>
<td>48.7</td>
<td>42.4</td>
<td>79.2</td>
<td>62.5</td>
<td>33.9</td>
</tr>
<tr>
<td>IVDMD</td>
<td>0.55</td>
<td>0.42</td>
<td>0.57</td>
<td>0.61</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Fa: *Faidherbia albida*; Dc: *Dichrostachys cinerea*; Tp: *Terminalia prunioides*; Cp: *Commiphora pyracanthoides*; At: *Acacia tortilis*

\(^a\)Expressed as absorbance units (A\(_{520nm}\)); \(^b\)Expressed as mg / g gallic acid equivalents

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; CT: condensed tannins; TP: total phenolics; IVDMD: *in vitro* dry matter digestibility; IVGP: *in vitro* gas production; Sed: standard error of the difference.
Table 2
Pearson correlation coefficient (r) matrix of chemical composition, *in vitro* dry matter digestibility and *in vitro* gas production of five woody species

<table>
<thead>
<tr>
<th></th>
<th>NDF</th>
<th>ADF</th>
<th>CT</th>
<th>TP</th>
<th>IVDMD</th>
<th>IVGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>0.418</td>
<td>0.225</td>
<td>0.204</td>
<td>-0.581*</td>
<td>0.088</td>
<td>-0.527</td>
</tr>
<tr>
<td>NDF</td>
<td>0.642*</td>
<td>0.176</td>
<td>-0.708**</td>
<td>-0.290</td>
<td>-0.514</td>
<td></td>
</tr>
<tr>
<td>ADF</td>
<td>0.517</td>
<td></td>
<td>-0.677**</td>
<td>-0.460</td>
<td>-0.541*</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td>-0.451</td>
<td></td>
<td>-0.580*</td>
<td>-0.684**</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td></td>
<td></td>
<td>0.091</td>
<td>0.730**</td>
<td></td>
</tr>
<tr>
<td>IVDMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.537*</td>
<td></td>
</tr>
</tbody>
</table>

Level of significance * < 0.05; ** < 0.01
CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; CT: condensed tannins; TP: total phenolics; IVDMD: *in vitro* dry matter digestibility; IVGP: *in vitro* gas production