

Full Length Research Paper

Effects of season, browse species and polyethylene glycol addition on *in vitro* degradability of forages in the sub-humid subtropical savannah, South Africa

N. A. D. Basha^{1, 3*}, S. F. Peter², M. A. Ahmed¹ and I. V. Nsahlai¹

¹Animal and Poultry Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa.

²Department of Agriculture, University of Zululand, Private Bag X001, Kwa Dlangenzwa 3886, South Africa.

³Department of Animal Nutrition, Faculty of Animal Production, Shambat Campus, University of Khartoum, P. O. Box 32, Postal Code 1334, Sudan.

Received 27 March 2014; Accepted 22 April, 2014

Effects of season, browse species and polyethylene glycol (PEG) on *in vitro* digestibility of five plant species from sub-humid subtropical savannah of South Africa were investigated. Leaves of *Acacia natalitia*, *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata* were harvested during dry (June/July), early wet (November/December) and late wet (February/March) seasons. An *in vitro* technique (72 h incubating time) was used in two experiments carried out with nine replicates (3 runs x 3 replicates). Experiment 1, tested the effects of season and browse species, while Experiment 2, tested the effect of condensed tannins using PEG (4000) on an *in vitro* degradability of the forages. Season and species affected the true degradability (TrDeg), short chain fatty acids (SCFA) and partitioning factor (PF). TrDeg ranged from 684 to 856 g/kg dry matter (DM). Total SCFA varied ($P < 0.05$) among seasons and browse species. Addition of PEG decreased TrDeg and PF but increased total SCFA. Results suggested these browse species have potential to be used as feed supplements.

Key words: Tannin-rich species, tannins, true degradability, short chain fatty acid.

INTRODUCTION

The production of feed in tropical areas is associated with irregular and variable rainfall that restricts seasonal biomass yield of herbaceous and browse species. Livestock in these areas must survive on limited feed of poor quality for long periods of the year (Nyamukanza et al., 2008). However, browse trees are essential in animal feeding programs because they are better supplementary feeds than most pasture vegetation and maintain their

nutrients into the dry season when feed resources are exhausted (FAO, 1997). In tropical Africa, most ruminant livestock survive on natural pastures. However, insufficient feed during the year is one of the major factors of low gain rate and low animal productivity that mainly occur in dry season (Makkar and Becker, 1998). Browse and shrub fodders may supply important protein sources to support ruminant production in tropical

*Corresponding author. E-mail: bashan@ukzn.ac.za or bashabasha2005@gmail.com, Tel: +27 33 2605474. Fax: +27 3312606094.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](#)

savannahs (Mlambo et al., 2008).

The determination of nutritive value of browse is usually estimated by chemical analysis, though this may not provide sufficient information about true nutritive value of feed. On the other hand, there is sufficient information on seasonal and species effects on chemical composition of browse in sub-tropical savannah (Scogings et al., 2004). For instance, tannins vary from $< 10 \text{ g kg}^{-1}$ to $> 100 \text{ g kg}^{-1}$ among species, even among species that are otherwise very similar while the general trends in seasonal changes indicate crude protein (CP) increasing from around 100 g kg^{-1} in dry season to as much as 150 g kg^{-1} in wet season, neutral detergent fibre (NDF) increasing from around 300 to 400 g kg^{-1} in dry season to as much as 500 to 600 g kg^{-1} at the end of wet season, and tannin being as much as 90% lower in wet season than in dry season, although in some species the opposite trend has been observed (Scogings et al., 2004, 2011). However, little work has been done in the sub-humid subtropical savannah to investigate effects of season and species on degradability with biologically based methods such as the *in vitro* gas technique.

In vitro gas technique is receiving attention for estimating the quality of ruminant feeds (Dijkstra et al., 2005). The technique has become established for evaluating forage digestion features (Theodorou et al., 1998). It has been commonly applied for determining the nutritive value of feeds (Pashaei et al., 2010). The *in vitro* gas technique is useful for estimating degradability and measuring short chain fatty acid (SCFA) yields. Results from this technique can also be used to calculate the partitioning factor and rank feedstuffs (Dijkstra et al., 2005). Additionally, calculation of the degradation efficiency factor is valuable to show the rapidity and the amount of feed degradation in order to make nutrients available (Ouda and Nsahlai, 2009). *In vitro* studies indicated tannin effects depend upon types and concentrations (Andrabi et al., 2005). High tannin concentration may exert negative effects on the nutritive value of forages by changing their palatability, or reducing intake and digestibility (Kumar and D'Mello, 1995). Polyethylene glycol (PEG) has been applied to inhibit the negative effects of tannins on feed intake and digestibility (Makkar et al., 1995b; Baba et al., 2002), thereby providing an indirect method of testing the biological role of tannins. The objective of this study was to determine the effect of season, plant species and PEG on an *in vitro* degradability of browse species in sub-humid subtropical savannahs.

MATERIALS AND METHODS

Study area and plant samples

Plant species used in this study were sampled at the Owen Sitole College of Agriculture (OSCA), Empangeni, South Africa. Owen Sitole College of Agriculture is located at $28^{\circ}57'45''$ - $28^{\circ}57'22''$ S latitude and $31^{\circ}55'31''$ - $31^{\circ}57'22''$ E longitude within the Coastal

Forest and Thornveld (Van Der Linden et al., 2005). The mean annual rainfall of OSCA is 995 to 1022 mm, with a third of it falling in the wet season (October – April) (Van Der Linden et al., 2005). Plant species used were *Acacia natalitia* (formerly part of *Acacia karroo* (Coates Palgrave, 2002)), *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata* (invasive non-native species), which were sampled during dry season (late June/early July, 2008), early wet season (late November/early December, 2008) and late wet season (late February/early March, 2009). These browse species were randomly sampled by collecting leaves 1.0 to 1.5 m above ground from three trees per species per season. Samples were kept in paper bags and air-dried prior to oven-drying at 60°C for 48 h. Leaves were ground through a 1 mm mesh sieve (Retsch GmbH & Co. KG 5657 HANN 1, Germany) and stored in plastic bottles. Gas production and chemicals analysis of those samples were measured by Basha et al. (2013).

In vitro degradability

Two experiments were carried out; first one tested the effect of season and browse species on degradability, while the second experiment tested the effect of tannins on degradability by adding PEG 4000. Only a small amount of PEG (5.6 mg) was added to each bottle. This amount of PEG was proven to produce more gas production using same browse species (Basha et al., 2013). The PEG treatment was applied to samples of early and late wet seasons only because dry season had limited samples due to some species being deciduous.

One gram dry matter was incubated in each Duran bottle. They were subjected to fermentation using an automated technique described by Pell and Schofield (1993). During a run, each sample, two controls (Lucerne (alfalfa) and *Eragrostis* hay), and blanks were represented by 3 Duran bottles. Three runs of incubations were applied to each season. A salivary buffer solution was prepared according to McDougall (1948). Solution A was made by dissolving 19.60, 7.40, 1.14, 0.94 and 0.26 g of NaHCO_3 , Na_2HPO_4 , KCl and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, respectively, in 2 L distilled water. Solution B was prepared by dissolving 5.3 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 ml distilled water. Complete salivary buffer was made by adding 2 ml of solution B to the 2 L of solution A, which was then warmed up to 39°C with continuous stirring and saturated with carbon dioxide (CO_2), immediately before starting to incubate samples. The buffer (67 ml) was added to bottles bearing sample, control or blank, and bottles were incubated (39°C) for 1 h to allow soaking of substrate before adding rumen fluid. Meanwhile rumen fluid was collected before morning feeding from three fistulated cows, which had free access to graze on kikuyu pasture, water and mineral lick, and were supplemented with 2 kg of Lucerne hay per day. Rumen fluid was filtered through four layers of cheesecloth into a pre-warmed flask (39°C) that had been flushed with CO_2 ; and transported to the laboratory. Inoculation was completed by adding 33 ml of rumen fluid to each bottle under a stream of CO_2 , the bottle lids tightened, incubated in the incubator channels for 72 h. The bottles were stirred for 12 s every 10 min.

At the end of 72 h incubation, the terminal pH was measured and bottles content were centrifuged at 18,000 g for 20 min at 4°C . The supernatant after centrifugation was prepared for short chain fatty acids (SCFA) analysis as described by Cottyn and Boucque (1968). The top part of the supernatant was poured into 50 ml flask and 10 ml of it was pipetted into a 15 ml plastic tube containing 2 ml of 25% metaphosphoric acid and stored in a refrigerator prior to analysis for SCFA.

The solid part (R) was dried in a fanned oven at 60°C for 72 h until constant weight was reached. The difference in weight between incubated material and R was the apparent degradability (ApDeg). The dried solid part was subjected to neutral detergent solution according to Van Soest et al. (1991). The difference

between the incubated material and neutral detergent fibre (NDF) weight was the true degradability (TrDeg) as described by Van Soest et al. (1991). The partitioning factor (PF) and degradation efficiency factor (DEF) for browse species were calculated as follows:

$$PF = \text{TrDeg}/GP \quad (\text{Blümmel et al., 1997})$$

$$DEF = 2PF/T_{1/2} \quad (\text{Ouda and Nsahlai, 2009})$$

where: TrDeg = True degradability (mg); GP = Total gas volume (ml); $T_{1/2}$ = time taken to produce $GP_{1/2}$ (h); $GP_{1/2}$ = half of GP (ml).

Short chain fatty acid

For analyses of SCFA, samples were thawed and centrifuged at 10,000 g for 10 min at 4°C, 2 ml was filtered through 0.45 µm filter (SUPELCO, USA) into 2 ml new ABC screw top vials that were loaded into an automatic sampler (HT280, Brescia, Italy) linked to a gas chromatograph (YL6100 GC, 600, Young Lin Instrument, Korea) fitted with SGE forte GC capillary column (25 m x 0.22 mm ID-BP21 0.25 µm). The column temperature was programmed at a temperature-cycle from 90 to 120°C rising by 5°C. The injector block temperature was maintained at 260°C with nitrogen as the carrier gas (60 ml/min). Flow rate of H₂ and air to the flame ionization detector were 60 and 300 ml/min, respectively. A 1 µl/split 1:80 was auto-injected. The obtained peak of each particular SCFA was calibrated with a standard curve of five different concentrations of pure standard (SIGMA-ALDRICH®).

Statistical analysis

The experiment design was a factorial arrangement for Experiment 1 (5 species x 3 seasons) and for Experiment 2 (5 species x 2 PEG levels) to each season. The apparent, true degradability and SCFA raw data were corrected using *Eragrostis* hay data by calculating the mean for each season per run, overall mean and then the ratio of the two (the overall mean divided by the mean of each season). The raw data of each parameter were multiplied by the ratio and then subjected to the General Linear Model procedure of SAS (2002) to determine the effects of season, plant species and PEG on *in vitro* degradability; significance was declared at $P < 0.05$. The models used were:

$$Y_{ijk} = \mu + S_i + p_j + (sp)_{ij} + \varepsilon_{ijk} \quad (\text{Experiment 1});$$

where, Y_{ijk} is the observation, μ is the population mean, S_i is the season effect ($i = 1-3$), p_j is the plant species effect ($j = 1-5$), $(sp)_{ij}$ is the interaction between season and plant species and ε_{ijk} is the residual error.

$$Y_{ijk} = \mu + p_i + t_j + (pt)_{ij} + \varepsilon_{ijk}; \quad (\text{Experiment 2})$$

where, Y_{ijk} is the observation, μ is the population mean, p_i is the plant species effect ($i = 1-5$), t_j is polyethelene glycol (PEG) effect ($j = 1-2$), $(pt)_{ij}$ is the interaction between plant species and PEG, and ε_{ijk} is the residual error.

RESULTS

Effects of season and plant species on *in vitro* degradability (Experiment 1)

Effects of season and plant species on *in vitro* pH,

ApDeg, TrDeg, MY, SCFA, PF and DEF were shown in Table 1. The interaction of season x species affected ($P < 0.001$) ApDeg, TrDeg, and SCFA, and lesser affected ($P < 0.05$) the PF but did not affect the pH and DEF. For *A. natalitia* and *A. nilotica* ApDeg decreased from dry season to early and late stages of the wet season. The ApDeg for *D. cinerea* and *S. myrtina* decreased from dry season to early wet season from where it increased in late wet season. *Chromolaena odorata* had similar ApDeg in dry and early wet seasons from where it decreased in late wet season. The TrDeg of *A. natalitia*, *D. cinerea* and *S. myrtina* did not change across the three seasons. For *A. nilotica* and *C. odorata* TrDeg decreased from dry season to wet season. The SCFA did not change throughout three seasons in *A. nilotica* and *S. myrtina*, while in *A. natalitia* and *C. odorata* they decreased while in *D. cinerea* increased from the dry to the early wet and late wet seasons.

The PF for *A. natalitia* increased from dry to early wet seasons from where it decreased in late wet season. The PF of *A. nilotica*, *D. cinerea* and *C. odorata* decreased from dry to early wet seasons then decreased in late wet season. For *S. myrtina* PF was similar during dry and early wet season and then increased in late wet seasons.

The DEF of *A. natalitia* increased from dry to early seasons from where it decreased in late wet season. For *A. nilotica*, *D. cinerea* and *C. odorata*, DEF decreased from dry to early wet seasons. Then from early to late wet seasons DEF stayed similar for *A. nilotica* or increased for *D. cinerea* and decreased for *C. odorata*. For *S. myrtina* DEF increased from dry to late wet seasons. Within five plant species and three seasons, *A. nilotica* had greatest ApDeg in dry and late wet seasons while *C. odorata* had greatest value during early wet season. The lowest ApDeg was observed with *A. natalitia* in dry and late wet seasons and *S. myrtina* in early wet season. For TrDeg, *S. myrtina* had highest value in dry and late wet seasons while *C. odorata* had highest value in early wet season and lowest value in dry season and *A. nilotica* lowest values in two wet seasons. For SCFA, *C. odorata* had greatest concentration in early wet season and *C. odorata*, *A. nilotica*, *D. cinerea*, and *S. myrtina* in early wet. Whilst the concentration of SCFA in late wet season followed this decreasing trend: *D. cinerea*, *C. odorata*, *S. myrtina*, *A. nilotica* and *A. natalitia*. For the PF and DEF, PF in *A. nilotica* and DEF in *C. odorata* had recorded highest values in the three seasons as well as PF in *S. myrtina* during late wet season and DEF in *A. nilotica* during dry season.

Effects of PEG on *in vitro* degradability (Experiment 2)

Tables 2 and 3 showed the effect of PEG on *in vitro* pH, ApDeg, TrDeg, SCFA, PF and DEF in early wet and late wet seasons, respectively. Figures 1 and 2 showed the nature of interactions (plant species x PEG) during early

Table 1. Effects of season and plant species on *in vitro* degradability parameters of plant species harvested from sub-humid subtropical savannah and fermented using rumen fluid.

Season	Species	pH	ApDeg	TrDeg	SCFA	PF	DEF
Dry	<i>A. natalitia</i>	6.88 ^{bc}	195 ^c	833 ^b	24.1 ^c	6.44 ^b	0.345 ^b
	<i>A. nilotica</i>	6.78 ^{cd}	524 ^a	844 ^{ab}	29.8 ^b	12.71 ^a	0.879 ^a
	<i>D. cinerea</i>	6.97 ^a	200 ^{bc}	805 ^b	25.2 ^c	8.08 ^b	0.427 ^b
	<i>S. myrtina</i>	6.91 ^{ab}	223 ^b	847 ^a	30.4 ^d	5.14 ^b	0.342 ^b
	<i>C. odorata</i>	6.81 ^d	520 ^a	845 ^b	48.6 ^a	6.25 ^b	0.923 ^a
Early wet	<i>A. natalitia</i>	6.92 ^{ab}	173 ^c	815 ^{ab}	18.4 ^b	7.26 ^b	0.560 ^{ab}
	<i>A. nilotica</i>	6.86 ^{abc}	407 ^b	688 ^c	33.8 ^a	10.05 ^a	0.535 ^{ab}
	<i>D. cinerea</i>	6.87 ^{bc}	166 ^c	793 ^{abc}	34.1 ^a	5.64 ^{bc}	0.337 ^b
	<i>S. myrtina</i>	6.97 ^a	171 ^c	816 ^a	33.6 ^a	-	-
	<i>C. odorata</i>	6.80 ^c	532 ^a	739 ^{bc}	32.5 ^a	5.12 ^c	0.720 ^a
Late wet	<i>A. natalitia</i>	6.86 ^{ab}	163 ^c	833 ^a	18.4 ^c	4.04 ^b	0.267 ^b
	<i>A. nilotica</i>	6.78 ^{bc}	392 ^a	684 ^c	31.2 ^b	9.08 ^a	0.530 ^{ab}
	<i>D. cinerea</i>	6.83 ^{bc}	220 ^b	782 ^{ab}	36.0 ^a	4.58 ^b	0.364 ^b
	<i>S. myrtina</i>	6.94 ^a	210 ^b	856 ^a	31.6 ^b	7.58 ^a	0.359 ^b
	<i>C. odorata</i>	6.78 ^c	369 ^a	710 ^{bc}	34.5 ^a	3.85 ^b	0.709 ^a
P <		0.001	0.001	0.001	0.001	0.001	0.001
RMSE		0.226	47.86	70.78	2.747	1.593	0.160
Sources of variation effects							
Season		ns	***	***	***	**	ns
Species		***	***	***	***	***	***
Season x Species		ns	***	***	***	*	ns

^{a,b,c,d} Different superscripts within a column indicate significant differences ($P < 0.05$) among plant species within season; ApDeg - apparent degradability (g kg^{-1} DM); TrDeg - true degradability (g kg^{-1} DM); SCFA - total of short chain fatty acids (mmol L^{-1}); PF - partitioning factor; DEF=degradation efficiency factor; RMSE - root mean square error; ns ($P > 0.05$), * ($P < 0.05$), *** ($P < 0.001$).

Table 2. Effects of PEG on *in vitro* degradability parameters of browse species samples in early wet season in Zululand Thornveld and fermented using rumen fluid.

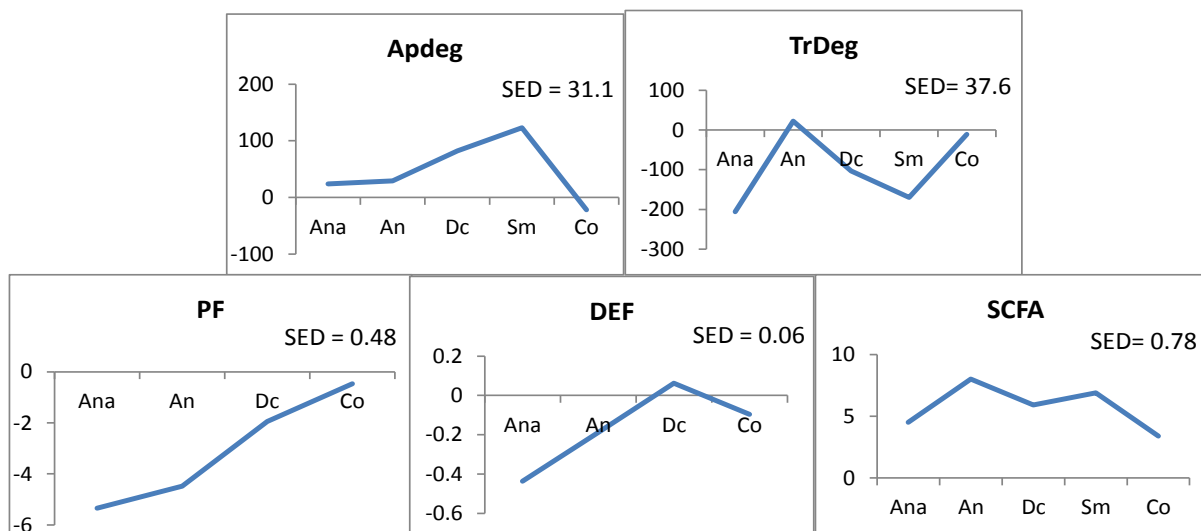
Species	PEG	pH	ApDeg	TrDeg	SCFA	PF	DEF
<i>A. natalitia</i>	-	6.92	173	815	18.4	7.83	0.693
<i>A. natalitia</i>	+	6.80	197	609	22.9	2.48	0.257
<i>A. nilotica</i>	-	6.86	407	688	30.8	9.41	0.616
<i>A. nilotica</i>	+	6.70	436	710	38.8	4.93	0.427
<i>D. cinerea</i>	-	6.87	166	793	33.1	5.30	0.330
<i>D. cinerea</i>	+	6.84	248	690	39.0	3.36	0.392
<i>S. myrtina</i>	-	6.97	171	817	32.3	-	-
<i>S. myrtina</i>	+	6.81	294	647	39.2	3.17	0.310
<i>C. odorata</i>	-	6.83	532	739	33.7	4.98	0.724
<i>C. odorata</i>	+	6.78	510	728	37.1	4.52	0.628
P <		0.014	0.001	0.001	0.001	0.001	0.001
RMSE		0.264	66.06	79.66	1.651	1.017	0.121
Sources of variation effects							
Species		*	***	ns	***	***	***
PEG		*	***	***	***	***	*
Species x PEG		ns	*	***	***	**	*

ApDeg - apparent degradability (g kg^{-1} DM); TrDeg - true degradability (g kg^{-1} DM); SCFA - total of short chain fatty acids (mmol L^{-1}); PF - partitioning factor; DEF=degradation efficiency factor; RMSE - root mean square error; ns ($P > 0.05$), * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$).

Table 3. Effects of PEG on *in vitro* degradability parameters of browse species samples in late wet season in Zululand Thornveld and fermented using rumen fluid.

Species	PEG	pH	ApDeg	TrDeg	SCFA	PF	DEF
<i>A. natalitia</i>	-	6.86	163	833	18.2	4.04	0.270
<i>A. natalitia</i>	+	6.82	231	658	26.4	3.41	0.416
<i>A. nilotica</i>	-	6.78	392	684	31.2	8.46	0.644
<i>A. nilotica</i>	+	6.72	383	644	36.7	4.95	0.458
<i>D. cinerea</i>	-	6.83	220	782	33.3	4.83	0.366
<i>D. cinerea</i>	+	6.81	280	695	39.0	3.73	0.490
<i>S. myrtina</i>	-	6.94	210	856	31.8	7.31	0.535
<i>S. myrtina</i>	+	6.78	287	696	37.5	3.32	0.301
<i>C. odorata</i>	-	6.78	369	710	35.5	3.92	0.729
<i>C. odorata</i>	+	6.76	584	725	36.3	4.84	0.733
P <		0.001	0.001	0.001	0.001	0.001	0.012
RMSE		0.073	49.60	66.13	2.183	1.068	0.157
Sources of variation effects							
Species		***	***	***	***	***	**
PEG		ns	***	***	**	***	ns
Species x PEG		***	***	***	***	**	ns

ApDeg - apparent degradability (g kg⁻¹ DM); TrDeg – true degradability (g kg⁻¹ DM); SCFA – total of short chain fatty acids (mmol L⁻¹); PF – partitioning factor; DEF=degradation efficiency factor; RMSE - root mean square error; ns (P>0.05), * (P<0.05); ** (P<0.01); *** (P<0.001).

**Figure 1.** The nature of interaction between plant species and PEG during the early wet season using the difference between –PEG and +PEG of each parameters (Table 3). Ana - *A. natalitia*; An - *A. nilotica*; Dc - *D. cinerea*; Sm - *S. myrtina*; Co - *C. odorata*; ApDeg - apparent degradability; TrDeg – true degradability; SCFA – total of short chain fatty acids; PF – partitioning factor; DEF – degradation efficiency factor.

wet and late wet seasons, respectively. This interaction affected (P<0.001) TrDeg and SCFA, and PF (P<0.01) in both seasons. The interaction species x PEG affected (P<0.001) ApDeg in early wet season and less affected (P<0.05) in late wet season, and DEF only affected (P<0.05) in early wet season.

With addition of PEG, in both seasons, ApDeg and SCFA of the five plant species increased with the exceptions of *C. odorata* and *A. nilotica* that did not change, whereas TrDeg, PF and DEF decreased with the exception of early wet season TrDeg of *A. nilotica* and DEF of *D. cinerea* that were slightly increased, and late wet season

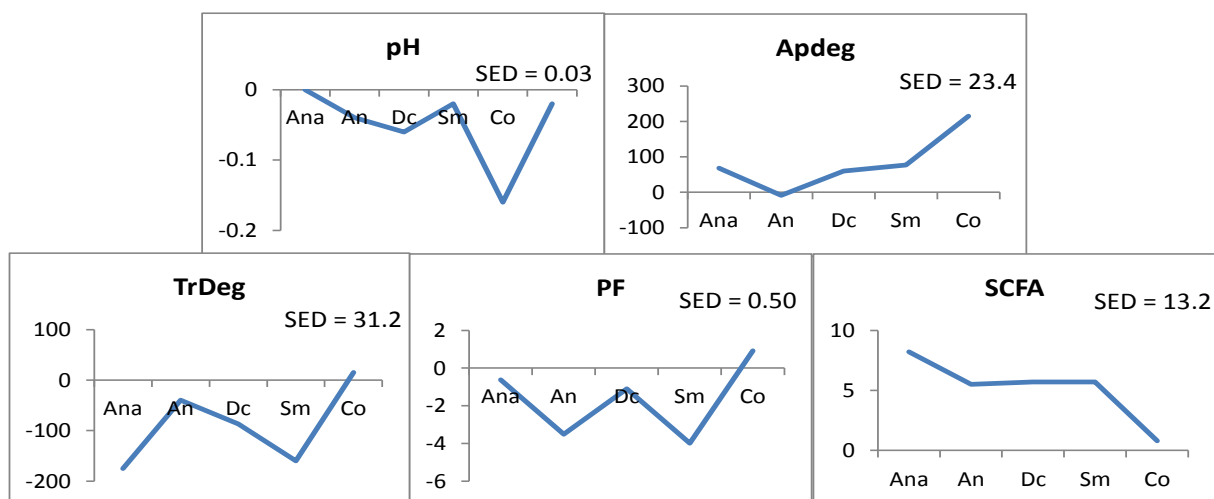


Figure 2. The nature of interaction between plant species and PEG during the late wet season using the difference between -PEG and +PEG of each parameters (Table 4). Ana - *A. natalitia*; An - *A. nilotica*; Dc - *D. cinerea*; Sm - *S. myrtina*; Co - *C. odorata*; ApDeg - apparent degradability (g kg^{-1} DM); TrDeg – true degradability (g kg^{-1} DM); SCFA – total of short chain fatty acids (mmol L^{-1}); PF – partitioning factor; DEF – degradation efficiency factor.

TrDeg and PF of *C. odorata* and DEF of *A. natalitia*, *D. cinerea* and *C. odorata* that were increased.

DISCUSSION

True degradability across the seasons agreed with studies elsewhere (Njidda and Nasiru, 2010; Sallam et al., 2010). True degradability was higher during dry season than either early or late stage of wet season, which may be related to the fact that plant leaves in early wet season had high condensed tannins and high protein, while in late wet season leaves had higher NDF level. This may explain why the TrDeg was low in the two wet stages. Reed et al. (1990) reported condensed tannins are present in fibres which bound to cell wall and protein, and appear to be responsible for decreasing digestibility. Ngwa et al. (2001) reported the decrease in digestibility of dietary fibres because of the presence of anti-nutritional factors in the feed, reduce fibrolytic micro-organisms. Seasonal differences in the *in vitro* digestibility are mainly related to chemical composition of samples, particularly their cell wall content. The cell wall fraction could have a negative effect on browse digestibility (Mertens, 1993). For CP, the observed variation in TrDeg may be due to complexes formed between tannins and proteins. These complexes mostly insoluble in the neutral detergent and could add to the un-degradable fraction (Makkar et al., 1995a, b).

However, gases are mainly the outcome of carbohydrates fermented to SCFA (Wolin, 1960). The SCFA results were varied among the three seasons could be related to natural difference among species. Beuvink and Spoelstra (1992) reported the molar

proportion of individual SCFA produced is dependent on the type of substrate. Fermentation of tannin-rich plant species produced less SCFA than low tannin plant species (*C. odorata*). Tannins reduce the production of SCFA in tannin-rich feeds compared to tannin-low feeds (Mbugua et al., 2008).

To compare wide variation of partitioning factor results to previous results (Blümmel et al., 1997; Jackson et al., 2010; Edwards et al., 2012) some explanations will take a place. In theory, the PF for tannin-free plants differs from 2.75 to 4.41 mg ml^{-1} equivalent to 10 to 32 mg of YATP (adenosine triphosphate yield) which is assumed to be microbial efficiency (Blümmel et al., 1997). Makkar et al. (1997a) assumed that a PF value higher than the theoretical maximum value (4.41 mg ml^{-1}) is not a reasonable biological value. In this study, PF values for *A. natalitia* and *C. odorata* (4.04 and 3.85 mg ml^{-1} , respectively) in late wet season are reasonable biological values while PF values for the rest were higher (4.58 - 12.71 mg ml^{-1}) than the theoretical upper limit value. Ouda and Nsahlai (2009) without correcting gas released from buffers reported PF values within the current range. Baba et al. (2002) reported PF for browses ranged from 5.05 to 11.05. Makkar et al. (1997a) found estimation of PF for many browse species may not work properly. Overestimation of degradability values due to non-degradable substrate fraction like tannin complexes in the residual. Thus, the PF level was generally low in late wet season that attributed to low values of TrDeg in this season, same time maximum gas production was varied among the three seasons (Basha et al., 2013). Since the PF was calculated as the ratio between TrDeg and gas production, increase in gas production might result in lower PF (Makkar et al., 1998).

In this study, DEF were varied among seasons. Results are in agreement with values by Ouda and Nsahlai (2009) who studied *Sericea lespedeza* rations in an *in vitro* fermentation technique. They reported increase *S. lespedeza* ratio in ration resulted in decreasing DEF, due to presents of tannin. Ouda and Nsahlai (2009) suggested is important to calculate DEF when feeds are evaluated using *in vitro* gas production technique. Since the time required to produce half the maximum gas ($T_{1/2}$) could affect DEF values, the long $T_{1/2}$ gives low DEF values which represent low nutritive value and microbial efficiency. Cone and Van Gelder (2000) found positive relationship between fermentation rates of substrate and microbial efficiency.

In respect to the pH, *S. myrtina* had the highest value while *C. odorata* had the lowest across the three seasons, attributed to high tannin concentration in *S. myrtina*. Zimmer and Cordesse (1996) recorded high pH level in tannin-rich feed compared to low-tannin feed. Variations in chemical constituents among these five browse species resulted in variable value of TrDeg, SCFA, PF and DEF (Scogings et al., 2004; Lebopa et al., 2011).

In vitro dry matter digestibility varies widely among woody tree and shrub species which ranged from 380 to 780 g kg⁻¹ DM (Skarpe and Bergström, 1986). The high value of TrDeg in *S. myrtina* overlapped this range. The low TrDeg in *A. nilotica* was within the range and higher than what Madibela et al. (2006) recorded *in vitro* for *A. nilotica*. High production of SCFA in *C. odorata* and low in *A. natalitia* may be due to high protein and lower tannin in *C. odorata* and vice versa for *A. natalitia*. Mbugua et al. (2008) reported condensed tannins could decrease the production of SCFA in tannin-rich feeds. The high PF in *A. nilotica* and low in *C. odorata* attributed to low and high TrDeg in *A. nilotica* and *C. odorata* as well as to low and high gas production (Basha et al., 2013), respectively. The PF was calculated as the ratio between TrDeg and gas production. Current results show different effects of the interaction between season and browse species on most variables, it can be due to differences of chemical composition around the year particularly fibre fractions and anti-nutritional components. These differences in chemical composition agree with Larbi et al. (1998).

Adding of PEG slightly decreased the pH in browse species suggesting PEG binds with tannins. This is consistent with findings of Zimmer and Cordesse (1996) in an *in vivo* trial. The minimum range of pH was 6.3 to 6.5 in the tannin-rich feed compared with 6.0 in the tannin-free feed. In this study, the pH values with added PEG were 6.70 to 6.84 which falls within the normal range (5.5 to 7.0) of pH in the rumen (Jones and Mangan, 1977). Makkar et al. (1995a) pointed out for the *in vitro* gas method, at the starting point the pH was 6.80 to 7.00 and at the end of 24 h incubation was 6.70 to 6.85. Moreover, different effects of the interaction (Sp x PEG) on degradability parameters might be of the CT

concentration, nature or reactivity of CT, and interaction of tannins and other feed components. Reed et al. (1990) reported it can be inferred that considerable amounts of tannins in processed browse would be in the NDF and ADF fractions, where they are bound to cell wall and protein, that appear to be involved in decreasing digestibility. Adding of PEG decreased the TrDeg for plant species except for *A. nilotica* in early wet season and *C. odorata* late wet season. Makkar et al. (1995a) reported the addition of PEG decrease true degradability due to occurrence of tannin-PEG complexes in the residues. These complexes are partly or completely insoluble in boiling water, neutral detergent solution, acid detergent solution and other organic solvents (Jones and Mangan, 1977; Makkar et al., 1995a). The increase of TrDeg in *A. nilotica* may be due to type of the tannins. Jones and Palmer (2000) reported incubation of PEG with tannin-rich diets *in vitro* may react differently with all diets because of differences in tannin chemistry in diets. The reaction of PEG and tannin depend on nature and level of tannins (Ebong, 1995). The non-significant change in the TrDeg for *C. odorata* may be due to lack or less reaction of PEG with the components or soluble complexes that form between PEG and other components.

The addition of PEG increased the availability of protein to rumen micro-organisms by releasing protein from tannin-protein complexes (Makkar et al., 1995a). Thus, reduced protein degradation in the rumen, which is one of major causes for inefficient utilization of protein in ruminants (Pathak, 2008). Results in current study support the finding of Makkar et al. (1997b) who reported addition of PEG reduced efficiency of microbial protein synthesis. Contrary to Ben Salem et al. (2000), addition of PEG reduced fermentation efficiency as confirmed by reducing partitioning factor (Mlambo et al., 2008). This finding is in agreement with results of Baba et al. (2002) who reported addition of PEG effectively depressed partitioning factors. So, decrease in PF as response to addition of PEG in this study could be ascribed to the increase in gas production. Fermentation of tannin-rich browse samples produced less SCFA than *C. odorata* (low tannin). Addition of PEG maximized SCFA production in tannin-rich browses. Similar findings *in vitro* have been reported of the effect of tannins on SCFA production (Mbugua et al., 2008). Our results confirm those of Mbugua et al. (2008) that incubation of PEG with tannin-rich samples would lead to increased SCFA production. Furthermore, *in vivo* single SCFA, particularly acetate was poor in tannin-fed sheep compared to those incubated with PEG (Waghorn et al., 1994).

Conclusions

In vitro degradability parameters varied among seasons and among different browse species harvested from sub-

humid subtropical savannah of KwaZulu-Natal Province, South Africa, during dry, early, and late wet seasons. The addition of PEG emphasizes the inhibitory effect of tannins on rumen microbial activity. Results suggest that to use these browse species efficiently, there is need to reduce the effect of condensed tannins.

ACKNOWLEDGMENT

The authors are grateful to National Research Foundation, South Africa.

REFERENCES

- Andrabi SM, Ritchie MM, Stimson C, Horadagoda A, Hyde M, McNeill DM (2005). In vivo assessment of the ability of condensed tannins to interfere with the digestibility of plant protein in sheep. *Anim. Feed Sci. Technol.* 122:13-27. <http://dx.doi.org/10.1016/j.anifeedsci.2005.04.002>
- Baba ASH, Castro FB, Ørskov ER (2002). Partitioning of energy and degradability of browse plants *in vitro* and the implications of blocking the effects of tannin by the addition of polyethylene glycol. *Anim. Feed Sci. Technol.* 95:93-104. [http://dx.doi.org/10.1016/S0377-8401\(01\)00283-8](http://dx.doi.org/10.1016/S0377-8401(01)00283-8)
- Basha NAD, Scogings PF, Nsahlai IV (2013). Effects of season, browse species and polyethylene glycol addition on gas production kinetics of forages in the sub-humid subtropical savannah, South Africa. *J. Sci. Food Agric.* 93(6):1338-1348. <http://dx.doi.org/10.1002/jsfa.5895>, PMID:23015416
- Ben Salem H, Nefzaoui A, Ben Salem L, Tisserand JL (2000). Deactivation of condensed tannins in *Acacia cyanophylla* Lindl. foliage by polyethylene glycol in feed blocks: Effect on feed intake, diet digestibility, nitrogen balance, microbial synthesis and growth by sheep. *Livestock Prod. Scie.* 64:51-60. [http://dx.doi.org/10.1016/S0301-6226\(00\)00175-5](http://dx.doi.org/10.1016/S0301-6226(00)00175-5)
- Beuvink JMW, Spoelstra SF (1992). Interactions between substrate, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms *in vitro*. *Appl. Microbiol. Biotechnol.* 37:505-509. <http://dx.doi.org/10.1007/BF00180978>
- Blümmel M, Makkar HPS, Becker K (1997). *In vitro* gas production: a technique revisited. *J. Anim. Physiol. Anim. Nutr.* 77:24-34. <http://dx.doi.org/10.1111/j.1439-0396.1997.tb00734.x>
- Coates Palgrave K (2002). *Trees of southern Africa*. Struik Publishers (Pty) Ltd, Cape Town, South Africa.
- Cone JW, Van Gelder AH (2000). *In vitro* microbial protein synthesis in rumen fluid estimated with the gas production technique, Gas Production: Fermentation Kinetics for Feed Evaluation to Assess Microbial Activity, British Society of Animal Science, Penicuik, UK, pp. 25-26.
- Cottyn BG, Boucque CV (1968). Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. *J. Agric. Food Chem.* 16:105-107. <http://dx.doi.org/10.1021/jf60155a002>
- Dijkstra J, Kebreab E, Bannink A, France J, Lopez S (2005). Application of the gas production technique to feed evaluation systems for ruminants. *Anim. Feed Sci. Technol.* 123:561-578. <http://dx.doi.org/10.1016/j.anifeedsci.2005.04.048>
- Ebong C (1995). *Acacia nilotica*, *Acacia seyal* and *Sesbania sesban* as supplements to tef (*Eragrostis tef*) straw fed to sheep and goats. *Small Rumin. Res.* 18:233-238. [http://dx.doi.org/10.1016/0921-4488\(95\)00676-6](http://dx.doi.org/10.1016/0921-4488(95)00676-6)
- Edwards A, Mlambo V, Lallo C, Garcia G, Diptee M (2012). *In vitro* ruminal fermentation parameters of tanner grass (*Brachiaria arrecta*) supplemented with leaves from three forage trees. *Livest. Res. Rural Dev.* 24: <http://www.lrrd.org/lrrd24/26/edwa24102.htm>.
- FAO (1997). Food and Agriculture Organization of the United Nations. Tree Foliage in Animal Nutrition. FAO Animal Health and Production FAO, Rome. Forest, Farm and Community Tree Network (FACT Net), 2000. Annual Publication. Little Rock, Arkansas. P. 139.
- Jackson W, Krishnamoorthy U, Robinson P, Fadel J (2010). Effect of changing partitioning factor (PF) and *in vitro* rate of gas production (k) of diets on intake and digestibility, microbial N production, as well as milk production and composition, of lactating crossbred dairy cows. *Anim. Feed Sci. Technol.* 160:128-136. <http://dx.doi.org/10.1016/j.anifeedsci.2010.07.012>
- Jones R, Palmer B (2000). *In vitro* digestion studies using 14C-labelled polyethylene glycol (PEG) 4000: Comparison of six tanniniferous shrub legumes and the grass *Panicum maximum*. *Anim. Feed Sci. Technol.* 85:215-221. [http://dx.doi.org/10.1016/S0377-8401\(00\)00134-6](http://dx.doi.org/10.1016/S0377-8401(00)00134-6)
- Jones WT, Mangan JL (1977). Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *J. Sci. Food Agric.* 28:126-136. <http://dx.doi.org/10.1002/jsfa.2740280204>
- Kumar R, D'Mello J (1995). Anti-nutritional factors in forage legumes, in: D'Mello, J., Devedra, C. (Eds.), *Tropical legumes in animal nutrition*, CAB International, USA, Wallingford, pp. 95-133.
- Larbi A, Smith JW, Kurdi IO, Adekunle IO, Raji AM, Ladipo DO (1998). Chemical composition, rumen degradation, and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in the humid tropics. *Anim. Feed Sci. Technol.* 72:81-96. [http://dx.doi.org/10.1016/S0377-8401\(97\)00170-3](http://dx.doi.org/10.1016/S0377-8401(97)00170-3)
- Lebopa C, Boomker E, Chimonyo M, Mokoboki H (2011). In sacco dry matter and crude protein degradation of woody plant species in Tswana and Boer goats. *J. Life Sci. P.* 8. http://www.lifesciencesite.com/lslife0802s/13_6242life0802s_81_90.pdf.
- Madibela O, Seitshiro O, Mochankana M (2006). Deactivation effects of polyethylene glycol (PEG) on *in vitro* dry matter digestibility of *Colophospermum mopane* (Mophane) and *Acacia* browse trees in Botswana. *Pak. J. Nutr.* 5:343-347. <http://dx.doi.org/10.3923/pjn.2006.343.347>
- Makkar H, Blümmel M, Becker K (1998). Potential and limitations of *in vitro* gas methods for studying the effects of plant defensive compounds on rumen fermentation, in: Jansman A, Huisman J, Van der Poel A (Eds.), *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed*, Wageningen Press, Wageningen, pp. 173-178.
- Makkar HPS, Becker K (1998). Do tannins in leaves of trees and shrubs from African and Himalayan regions differ in level and activity. *Agrofore. Syst.* 40:59-68. <http://dx.doi.org/10.1023/A:1006027231497>
- Makkar HPS, Blümmel M, Becker K (1995a). Formation of complexes between polyvinyl pyrrolidones or polyethylene glycol and tannins, and their implications in gas-production and true digestibility in *in-vitro* techniques. *Br. J. Nutr.* 73:897-913. <http://dx.doi.org/10.1079/BJN19950095>, PMID:7632671
- Makkar HPS, Blümmel M, Becker K (1995b). *In vitro* effects of and interactions between tannins and saponins and fate of tannins in the rumen. *J. Sci. Food Agric.* 69:481-493. <http://dx.doi.org/10.1002/jsfa.2740690413>
- Makkar HPS, Blümmel M, Becker K (1997a). Applications of an *in vitro* gas method to understand the effects of natural plant products on availability and partitioning of nutrients, in: Deaville ER, Owen E, Adegoosan AT, Rymer C, Huntington, JA, T.L., L. (Eds.), *In vitro Techniques for Measuring Nutrient Supply to Ruminants*, British Society of Animal Science, Edinburgh, UK, pp. 147-150.
- Makkar HPS, Blümmel M, Becker K (1997b). *In vitro* rumen apparent and true digestibilities of tannin-rich forages. *Anim. Feed Sci. Technol.* 67:245-251. [http://dx.doi.org/10.1016/S0377-8401\(96\)01146-7](http://dx.doi.org/10.1016/S0377-8401(96)01146-7)
- Mbugua DM, Kiruiro EM, Pell AN (2008). *In vitro* fermentation of intact and fractionated tropical herbaceous and tree legumes containing tannins and alkaloids. *Anim. Feed Sci. Technol.* 146:1-20. <http://dx.doi.org/10.1016/j.anifeedsci.2007.11.008>
- McDougall EI (1948). Studies on ruminant saliva .1. The composition and output of sheeps saliva. *J. Biochem.* 43:99-109.
- Mertens DR (1993). Kinetics of cell wall digestion and passage in ruminants, in: Jung HG, Buxton DR, Hatfield RD, Ralph J (Eds.),

- Forage cell wall structure and digestibility, USDA, Agricultural Research Service and US Dairy Forage Research Center, Madison, WI, USA, pp. 538-570.
- Mlambo V, Mould F, Sikosana J, Smith T, Owen E, Mueller-Harvey I (2008). Chemical composition and *in vitro* fermentation of tannin-rich tree fruits. *Anim. Feed Sci. Technol.* 140:402-417. <http://dx.doi.org/10.1016/j.anifeedsci.2007.03.001>
- Njidda A, Nasiru A (2010). *In vitro* gas production and dry matter digestibility of tannin-containing forages of semi-arid region of northeastern Nigeria. *Pak. J. Nutr.* 9:60-66. <http://dx.doi.org/10.3923/pjn.2010.60.66>
- Nyamukanza C, Scogings P, Kunene N (2008). Forage-cattle relationships in a communally managed semi-arid savanna in northern Zululand, South Africa. *Afr. J. Range For. Sci.* 25:131-140. <http://dx.doi.org/10.2989/AJRF.2008.25.3.5.602>
- Ouda O, Nsahlai IV (2009). Relevance and potential use of *in vitro* gas production measurements to evaluate varying ratios of roughages and protein sources for ruminants. *J. Appl. Anim. Res.* 35:9-16. <http://dx.doi.org/10.1080/09712119.2009.9706975>
- Pashaei S, Razmazar V, Mirshekar R (2010). Gas production: A proposed *in vitro* method to estimate the extent of digestion of a feedstuff in the rumen. *J. Biol. Sci.* 10:573-580. <http://dx.doi.org/10.3923/jbs.2010.573.580>
- Pathak A (2008). Various factors affecting microbial protein synthesis in the rumen. *J. Vet. World* 1(6):186-189.
- Pell A, Schofield P (1993). Computerized monitoring of gas production to measure forage digestion *in vitro*. *J. Dairy Sci.* 76:1063-1073. [http://dx.doi.org/10.3168/jds.S0022-0302\(93\)77435-4](http://dx.doi.org/10.3168/jds.S0022-0302(93)77435-4)
- Reed J, Soller H, Woodward A (1990). Fodder tree and straw diets for sheep: Intake, growth, digestibility and the effects of phenolics on nitrogen utilisation. *Anim. Feed Sci. Technol.* 30:39-50. [http://dx.doi.org/10.1016/0377-8401\(90\)90050-I](http://dx.doi.org/10.1016/0377-8401(90)90050-I)
- Sallam S, Bueno ICS, Godoy PB, Nozella EF, Vitti DMSS, Abdalla AL (2010). Ruminal fermentation and tannins bioactivity of some browses using a semi-automated gas production technique *Trop. Subtrop. Agroecosyst.* 12:1-10.
- Scogings PF, Dziba LE, Gordon IJ (2004). Leaf chemistry of woody plants in relation to season, canopy retention and goat browsing in a semiarid subtropical savanna. *Austral Ecol.* 29:278-286. <http://dx.doi.org/10.1111/j.1442-9993.2004.01347.x>
- Scogings PF, Hjältén J, Skarpe C (2011). Secondary metabolites and nutrients of woody plants in relation to browsing intensity in African savannas. *Oecologia* 121:1-11.
- Skarpe C, Bergstörn R (1986). Nutrient content and digestibility of forage plants in relation to plant phenology and rainfall in the Kalahari, Botswana. *J. Arid Environ.* 11:147-164.
- Theodorou MK, Lowman RS, Davies ZS, Cuddeford D, Owen E (1998). Principles of techniques that rely on gas measurement in ruminant nutrition, in: Deaville, E.R., Owen E, Adesogan AT, Rymer C, Huntington JA, Lawrence TLJ (Eds.), *In vitro* Techniques for Measuring Nutrient Supply to Ruminants, Occasional Publication, UK, pp. 55-63
- Van Der Linden JP, Siebert SJ, Siebert F, Ferreira DP, Breidenkamp GJ (2005). Vegetation of the Owen Sitole College of Agriculture in Zululand, KwaZulu-Natal. *Koedoe* 48:33-56. <http://dx.doi.org/10.4102/koedoe.v48i1.164>
- Van Soest P, Robertson J, Lewis B (1991). Carbohydrate methodology, metabolism and nutritional implications in dairy cattle: Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597. [http://dx.doi.org/10.3168/jds.S0022-0302\(91\)78551-2](http://dx.doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Waghorn G, Shelton I, McNabb W, McCutcheon S (1994). Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *J. Agric. Sci.* 123:109-119. <http://dx.doi.org/10.1017/S0021859600067824>, <http://dx.doi.org/10.1017/S0021859600067836>
- Wolin MJ (1960). A theoretical rumen fermentation balance. *J. Dairy Sci.* 43:1452-1459. [http://dx.doi.org/10.3168/jds.S0022-0302\(60\)90348-9](http://dx.doi.org/10.3168/jds.S0022-0302(60)90348-9)
- Zimmer N, Cordesse R (1996). Digestibility and ruminal digestion of non-nitrogenous compounds in adult sheep and goats: Effects of chestnut tannins. *Anim. Feed Sci. Technol.* 61:259-273. [http://dx.doi.org/10.1016/0377-8401\(95\)00940-X](http://dx.doi.org/10.1016/0377-8401(95)00940-X)