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Evaluation of Browse Plants Fermentability by the Estimation of Substrate Degradability and Gas Production *in-vitro*

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ABSTRACT

Browse plants and grass with varying level of secondary compounds were offered to 3-6 months old goats fed diet based on napier grass and pellet. The fermentability (gas production Menke method) was measured after 3 days of adaptation at fed napier grass and the effects on fed first was evaluated for the next 12 weeks. Napier grass average was gas production (GP), degraded in vitro and degraded *in sacco* (50.1 ml, 41.9 % and 48.7 %) respectively for control animals (Table 2), and browse plant ranged between (10.3–64.4 ml GP, 27.3–70.9 % in vitro and 29.7–90.5 % in sacco) respectively. For added PEG in browse plant increase percentase degraded between 25.7–64.2 ml GP, 34.2–75.0 % *in vitro*) respectively for treated animals. *L. leucocephala* was better fed degraded for nutrition animal, but 2nd hight degraded in sacco (79.8 %) and *A. heterophyllus* (73.0). Effect Polyetylene Glycol (PEG) in degraded fed *L. leucocephala* was increase until 50.5 %. Partitioning factor (PF) fed from browse plant *M. esculenta* can be decrease Partitioning (PF) to 92.3 % and low at *C. odorata* (0.8 % only). Treatment added Catechin and PEG to straw the result hight GP, digested and PF in the straw only and added PEG. Catechin hight level was not increase GP and digested. In these experiment browse plant higt GP and digested and low PF and grasses for the GP and digested low, increase PF. Browse plant *L.leucocephala* has Hiht tannin (TEPA 264.8 mg/g DM), but can still with higt digested and GP. In thses case effect tannin fed for animal not yet decrease quantity GP and digested.

Keywords: Browse Plants, Degradability, In Vitro, Polyethylene Glycol, Anti Nutrition

INTRODUCTION

The potential degradability and degradation rate of feeds can be estimated using gas production method (Menke *et al*, 1979). This method is based upon the measurement of gas produced, which is a result of short chain fatty acids (SCFA) and gases produced from substrate fermentation by the rumen microbes. The Menke gas production system was shown to be a good test in feed evaluation because the volume of gas produced has been well correlated with microbial protein synthesis (Krishnamoorthy *et al*, 1991) and *in vivo* digestibility (Khazaal *et al*, 1993), or potentially useful as predictor of dry matter intake (BlÜmmel and Orskov, 1993) when data obtained from *in sacco* degradability were also taken into consideration.

Newly formed microbial biomass from substrate fermentation functions as a valuable protein source for the animal. The relationship between the SCFA produced in the rumen and microbial protein available to ruminants is known to be a negative one (Hungate, 1966) and this relationship has also been demonstrated *in vivo* (Leng, 1993) and *in vitro* using the gas production method (BlÜmmel *et al*, 1997a). This implies that feed samples should not only be evaluated based on gas production since partition of energy released from degraded

substrate to large SCFA production would mean less energy are available for microbial protein synthesis (BlÜmmel *et al*, 1994). The concept of partitioning of fermentation products (partitioning factor;PF) was introduced to express the conversion of energy from truly degraded substrate (mg dry matter (DM)) required to yield 1 ml of gas (BlÜmmel *et al*, 1997c). The former is estimated by the residues remained after refluxing the fermented substrate with neutral detergent solution (BlÜmmel and Becker, 1997). PF value is unique to each plant, and is not necessarily linked to chemical composition of plants including crude protein, NDF, ADF and organic matter (Boehm, et al.1993). High PF values were shown to be peculiar to highly digestible roughage and they correlate well with dry matter intake in ruminants (BlÜmmel *et al*, 1997c). Thus the evaluation of the partitioning of fermentation end products from substrate fermented may be used to give important information on the nutritional value of ruminant feed.

Whereas studies on the assessment of PF values for conventional roughages are accumulating (BlÜmmel and Bullerdieck, 1997; Lopez et al, 1998), apparently none has been reported for browse plants. This is largely due to the difficulty in quantifying the amount of substrate degraded *in vitro*. It is important to determine the PF values for browse plants because most of these plants contain condensed tannins that may cause harmful effects on rumen microbial fermentation (Jones et al, 1994). When incubated in the presence of PEG the gas produced from the fermentation increased (Khazaal *et al*, 1994; Makkar *et al*, 1995), and this has been attributed to the binding of PEG to tannins which are released during fermentation (Khazaal *et al*, 1993). The formation of PEG-tannin complexes has been suggested (Salem *et al*, 2006) to prevent the accumulation of these compounds in the medium to a level which are inhibitory for the microorganisms metabolic activity. Therefore the degradability of browse plants in the presence of PEG is also expected to Makkar *et al*, 1995). Tannins form complexes with proteins (both from plants and microbial sources) that are insoluble in neutral detergent solution, thus forming precipitates which in turn will overestimate the undegradable fraction (Makkar *et al*, 1995increase. However the determination of true *in vitro* substrate degradability by the NDF method can be misleading in browse plants due to the presence of tannins (Atanassova, and Christova,2009). This resulted in a decrease in truly digestible dry matter, instead of an increase, which is expected to accompany an increase in accumulated gas production when plants containing tannins were incubated *in vitro* in the presence of PEG (Makkar *et al*, 1995). Attempts to separate the tannin by centrifugation before subjecting the fermented substrate to the detergent digestion was also reported to give incorrect values because the centrifugal forces appeared to enhance further the formation of tannin-protein complexes in the fiber (Makkar *et al*, 1997).

The aim of this study was to develop and validate a method suitable to estimate the degradability of browse plant incubated *in vitro* using the Menke gas production method. This new procedure would be used to investigate the changes in the degradability, gas production and PF of substrate-containing tannins fermented in the absence or presence of tannin-binding agent, polyethylene glycol (PEG).

METHODOLOGY

Plant Materials

Browses, leguminous plants and grasses were harvested by hand, air-dried in-doors for 5 days and milled in a hammer mill to pass through a 1.0 mm sieve.

Chemical analysis

The weights of substrates for incubation, feed residue, nylon bags, PEG, catechin and sintered glass crucibles for fibre analysis were determined correct to four decimal places (in gram; Sartorius

Analytic). Residual water content in the plant samples were determined at 100ºC for 24 hr. All plant samples were corrected for water content in all calculations. Neutral (NDF) and acid (ADF) detergent fibre analysis were carried out as described by Goering and Van Soest (1970). Nitrogen (N) content in samples was determined by Macro-N analysis (Foss Electric (UK) Ltd.) and N values obtained in %DM was multiplied by 6.25 to yield % crude protein (CP).

Extractable Phenolic Compounds

Analysis of phenolic compound in the plants was carried out in four replicates as described by Khazaal *et al*, (1993). Total extractable tannin (TETa; in tannic acid equivalent) was calculated by the difference between total extractable phenolics (Julkunen-Tiito, 1985) and amount of phenolic compounds remain after absorption onto polyvinylpolypyrrolidone (PPVP) (Makkar *et al*, 1992). Total extractable condensed tannins, as catechin equivalent, were determined using the vanillin assay (TECTa; Broadhurst and Jones, 1978) and in unit absorbance at 550nm (TEPAs) using the proanthocyanidins assay (Porter *et al*, 1986).

Animal Feeding And Preparation Of Incubation Medium

Sheep fitted with permanent rumen cannulae were used for *in sacco* degradation studies and also as source of rumen inoculum. Animals were fed on maintenance diets consisting of 500g/kg DM grass hay, 300g/kg DM barley, 100g/kg molases, 91g/kg DM fishmeal and 9.5g/kg mineral mix and vitamin mix. Feed was given twice a day at 0830 h and 1600h. Sample of rumen content was collected from at least two sheep prior to morning feeding and transferred to pre-warmed CO_2-filled thermo bottles. Rumen fluid was strained using three layers of cheese cloth under continuous flushing with CO_2 gas. The temperature of the rumen liquor was maintained between 37-39°C throughout the preparation of the incubation medium. Substantial changes in the medium composition suggested by BlÜmmel and Becker (1997) were adopted since the amount of substrate used in this study was increased from 200 mg to 500 mg. The volume of buffer used was increased two fold to increase the buffering capacity of the incubation medium. Equal amount of distilled water was added to match the increase in buffer volume so that the medium has the same osmolality as that used for 200mg substrate fermentation.

In Situ Degradability

Measurement of *in situ* degradability was carried out essentially as described by Ørskov and McDonald (1979) with the following changes; feed sample (1.5 g, 1.0 mm) in triplicate, was put into pre-weighed nylon bag and the bag was tied to allow substrate : nylon bag surface area ratio of 5 mg/cm^2 . The bags were incubated in the rumen for only 24 hrs and were subsequently washed in cold water for 20 minutes in a programmed washing machine (rinsing without spinning) and dried at 60-70°C for 48 hrs.

In Vitro Gas Production

The gas production method was as carried out described by Menke *et al* (1979) but the syringes were incubated in a thermostatically regulated water bath (39°C) as described by BlÜmmel and Ørskov (1993). Feed samples (500mg) in the absence or presence of 500 mg PEG 8000 (Sigma Chemical Co., U.K) were fermented in 40ml of incubation medium. All treatments were prepared in triplicate. The syringes were shaken twice during the first 2 hours and once at every reading, which were taken at 2, 4, 6, 8, 12 and 24 hr. Blanks (syringes containing only incubation media and rumen inoculum) were included in all runs. Net gas production at a given time from feed samples was calculated after the subtraction of the volume of gas produced in the blank and was corrected to a weigh-out of exactly 500mg air-dry sample. Hay, with known *in vitro* gas production and *in sacco* degradation characteristics was used as standard substrate to compare inter-incubation differences in gas production.

Only one incubation out of 14 incubations had hay gas volume differed by more than 10% and the measurement from this preparation was not used in the data analysis. The fermentation was stopped by immersing the syringe in cold water and the estimation of degradability was carried out immediately as described in the following section.

Estimation Of Degraded Substrate In Vitro

Degraded substrate was calculated as the difference between the weight of substrate incubated and the weight of undegraded substrate. Undegraded substrate after 24 hr incubation was determined using either NDF-digestion method (Van Soest and Robinson, 1985; BlÜmmel and Becker, 1997) NDS method or nylon bag washing method (incubation in Syringes followed by washing using Nylon Bag; SNB method). NDS method was used to determine true *in vitro* dry matter degradability of grass species. The content of the syringe was transferred into a beaker and the residues in the syringe were harvested by rinsing twice with NDF solution (35 ml of NDF solution dispensed through the spike of the syringe/ rinsing) and the washings were added to the beaker. The mixture was refluxed for one hour to remove microbial matter from the undegraded feed and the residue was recovered using pre-weighed sintered glass crucible No. 2 (Gallenkemp, U.K).

SNB method was used to estimate undegraded substrate incubated *in vitro* for browses and leguminous plants. The entire content of the syringe was transferred through the spike into a pre-weighed nylon bag (8cmx16cm; pore size 40-60μm). The residues in the syringe were harvested by rinsing with a total volume of 80ml of cold water which was sucked in two equal volumes through the spike. Both washings were transferred into nylon bags. The nylon bags were tied with rubber bands and then washed in a washing machine as described earlier. There was no significant difference ($P>0.05$) in the effective nylon bag surface area (30%, 50% and 75%) on the degradability estimation of P. purpureum incubated for 24 hr *in vitro* (data not shown) and 50% effective surface area (5 mg incubated sample/cm2) was used for washing of nylon bags.

Validation Of Snb Method

Effect of catechin+PEG on gas production and degradability of straw

Browse plants contain tannins that may affect microbial fermentation of substrate. The effect of condensed tannin on fermentation of straw *in vitro* was investigated using the SNB method to demonstrate changes in wheat straw degradability as a result of addition of condensed tannin in the absence or presence of PEG. Catechin was added at 5% or 10% substrate weight in the absence or presence of PEG.

Solubles And Particle Loss From Nylon Bag

The content of the syringe was transferred into a pre-weighed nylon bag which was placed in a 250 ml beaker and the syringe was rinsed twice with cold water and the washings collected as described earlier. The nylon bag was filled up to 75% capacity and the water was gently squeezed out of the nylon bag into a beaker. This process was repeated 9 times. The washings were then filtered through pre-tared Whatman filter paper. The filter paper was then rinsed with 100 ml distilled water to remove residual solubles. The filter paper and the nylon bag were dried at 70^{o}C for 48 hrs. Particles retained on the filter paper was corrected for particles contributed by the rumen liquor in the blanks. Undegraded substrate in the nylon bag and on the filter paper were compared with those remained in the nylon bag which has been washed using the washing machine.

Statistical Analysis

Measurements *in sacco* and *in vitro* were carried out in triplicate. The means of all treatments were compared using the least significant difference method (Steele and Torrie, 1980). Standard errors (s.e.) were calculated using the pooled variances of each replication of determination. Differences between treatment was

considered statistically significant at the level of P<0.05.

RESULTS AND DISCUSSION

Evaluation Nutrition browse plant

Chemical composition for 10 species sample browse plant (Table 1).

Table 1 Chemical composition of browse plants

Species	%DM	%OM	%NDF	%ADF	%CP	TETa[1]	TECT[2]	TEPA[3]
Acalipha hispida	89.7	91.2	49.9	24.6	13.3	39.0	12.6	290.2
Acacia mangium	90.2	90.7	29.4	44.4	13.2	9.9	15.7	415.2
Artocarpus heterophylus	89.6	86.8	49.6	38.4	15.9	6.1	3.2	73.2
Bixa arellana	90.2	93.7	43.7	26.1	13.5	104.6	15.9	197.4
Caliandra calothyrcus	89.8	94.8	73.7	69.8	11.0	16.7	18.5	88.5
Chromolaena odorata	88.7	89.0	36.5	35.6	20.8	25.8	7.5	34.4
Cyperus kyllinga	90.6	88.5	45.5	33.0	10.1	47.8	7.5	72.1
Leucaena leucocephala	92.1	91.0	31.8	21.5	24.3	14.8	39.8	264.8
Manihot esculanta	90.3	94.4	29.5	36.4	11.6	155.5	16.2	438.8
Euglena aromatica	90.5	95.8	48.9	36.1	7.6	89.2	11.7	130.8
Melastoma marabathricum	91.0	91.2	67.2	38.5	12.4	2.71	10.7	158.9

[1] Total extractable tannin (Tannic acid equivalent; mg/g DM)
[2] Total extractable condensed tannin (Catechin equivalent; mg/g DM)
[3] Total extractable proanthocyanidins (absorbance at 550 nm/g DM)

Analysis was carried out in duplicate and coefficient of variation was less than 5%

Dried matter (DM) browse plant for 10 species experiment estimate 88.7 – 92.1 % (*Chromolaena ordata* low DM, and *Leucaena leucocephala* hight DM). Difference for organic matter in browse plant (estimate 86.8 %, A. heterophyllus – 94.4 %, *Manihot esculentha*, Table 1). There were a wide range in the content of crude protein (7.6% low content *E. aromatica* - 24.3% hight content *L. leucocephala*), For the NDF content estimate (29.4 – 73.7 %), where *A.mangium* low content and 73.7 % hight conten NDF and ADF content estimate (21.5 % - 69.8 %) means *L.leucocephala* is low conten and *Caliandra calothyrcus* hight content. Crode protein content browse plant estimate 7.6 % (*E. aromatica*) – 24.3 % *L. leucocephala*). Anti nutritional factor (ANF) browse plant for the 10 species browse plant eq, the condensed tannins in these plants, as estimated by three different methods TETa, TECT and TEPA, ranged between 6.1% (*A. mangium*) – 155.5% (*L. leucocephala*) , 3.2 % (*A.mangium*) – 39.8% (*C. kyllinga*) and 290.2% (*A. hyspida*) – 438.8% (*L.leucocephala*), respectively. Perbezaan jenis browse plant sebagai makanan ternak, berbeda pula jenis ANF dan komposisi masing-masing.

Degradation Graminae in vitro and *in sacco*

Degradation 5 species gramiae gas production methode in vitro and insacco methode 24h in the studies estimate Table 2.

Table 2 Estimation of substrate degradation after *in vitro* incubation using NDS-digestion or nylon bag washing (SNB method[1]) and comparison with *in sacco* method.

		24 hr degradability (%)		
Species	GP24h	i*n vitro* and NDF-digestion	i*n vitro* and nylon bag washing	*in sacco*
Graminicae	68.3	54.2	51.5	52.1
Ryegrass Hay	44.5	43.9	38.3	36.6
Wheat Straw	50.1	55.5	41.9	48.7
P.purpureum Paspalum sp	51.2	62.0	62.7	67.7
Trypsacum sp	45.2	51.7	40.6	44.2
stdev	3.0	2.2	3.7	4.1

[1]SNB = syringe-nylon bag method.

Degestibility is a similar approach between the SNB between in Sacco method for all types of graminae tested. There were a wide range in the gas production between 44.5 ml (wheat straw) – 68.3 ml (Ryegrass Hay), For the Percentage digestibility in vitro and *in sacco* was 38.3% and 36.6 % (Wheat Straw) antil 62.7 % and 67.7 % (Paspalum

conjugatum), where in vitro and NDF digestion was noy difference with In vitro and Nylon bag washing.

Degradability was highest for ryegrass hay, *P.purpureum,* and *Trypsacum sp* when these were determined by NDS method and highest for *Paspalum sp*. when using the *in sacco* method. SNB method tended to give the lowest figures for degradability. When compared with the *in sacco* method it was found that SNB method gives a better correlation (y= 1.06x + 0.29; r^2= 0.98) than those of NDF method (y= 0.90x + 2.88; r^2= 0.68). The SNB method was used in subsequent studies to estimate the degradability of browse plants.

Effect of condensed tannin on wheat straw degradation

Three methods PEG and Catechin given to feed from straw having kuantity condensed tannin (Table 4).

Table 3 Influence of catechin ± PEG on gas production from *in vitro* fermentation of straw

Treatment	ml Gas/ 24h	%Substrate Degraded/ 24h	Partitioning Factor (mg DM/ ml Gas)
Straw	54.3	39.6	3.37
Straw+PEG	55.3	40.5	3.35
Straw+25mg Catechin	50.5	38.9	3.55
Straw+25mg Catechin+PEG	54.3	38.6	3.29
Straw+50mg Catechin	38.1	30.8	3.77
Straw+50mg Catechin+PEG	47.9	34.3	3.31
s.e.d.	4.5	3.7	0.24

The inhibitory effect of catechin was more pronounced on gas production (-6.9% and –29.8% respectively) than on the effect on substrate degradation (-1.7% and –22.2%), although the difference was significant (P<0.05) only when the higher amount of catechin was used. As expected, addition of PEG into the incubation suppressed the inhibitory effect of catechin (10% DM basis) on gas production and substrate degradation. The removal of the inhibitory effect of catechin by PEG however was not completed whereas PEG by itself has no significant effect (P>0.05) on straw fermentation *in vitro.* Addition of catechin increased the PF values when compared to control (3.77 and 3.37 respectively) but the effect was not significant (P>0.05).

Effect PEG to percentage degradability feed for 11 plant in the in sacco and in vitro methods in the Table 4.

Table 4: Fermentation of browse plants in vitro in the absence or presence of PEG and its effect on gas production and substrate degradation

Plant sample & Treatment	% DM degraded *in sacco*/24h	% DM degraded *in vitro*/ 24h	Volume of accumulated gas (ml)/ 24h	Partitioning Factor (mgDM/ ml Gas)
M. esculanta	*68.7*	46.7	19.2	11.04
M. esculanta+PEG		54.0(+15.6%)	44.2(+130.2%)	5.74 (-92.3%)
C. calothrcus	*29.7*	27.3	10.3	11.05
C. calothyrcus+PEG		34.2(+25.6%)	25.7(+149.5%)	5.98 (-84.8%)
A.hispida	*85.7*	55.8	27.0	8.94
A.hispida+PEG		75.0 (+34.4%)	53.6 (+98.5)	6.32 (-29.3%)
E. aromaticum	*57.3*	47.1	32.0	6.85
E. aromaticum+PEG		54.7(+16.1%)	49.7(+55.3%)	4.95 (-27.7%)
L. leucocephala	*79.8*	71.7	41.8	6.03
L. leucocephala +PEG		72.9(+1.7%)	62.9(+50.5%)	5.25 (-14.9%)
A. mangium	*53.2*	50.1	31.6	7.49
A. mangium +PEG		51.9(+3.6%)	35.4 (+12.0%)	6.84 (-8.7%)
B.arellana	*47.9*	44.4	28.2	7.09
B.arellana+PEG		47.9(+7.9%)	33.7(+19.5%)	6.52 (-8.0%)
M.marabathricum	*45.8*	48.4	45.1	5.07
M.marabathricum+PEG		51.0(+5.4%)	48.9(+8.4%)	4.77 (-6.4%)
A.heterophylus	*73.0*	54.3	64.4	5.05
A.heterophylus+PEG		71.5(+31.7%)	64.2(-0.3%)	4.74(-6.1%)
C. kyllinga	*49.7*	38.8	35.6	4.94
C. kyllinga+PEG		50.9(+31.2%)	48.8(+37.1%)	4.81(-2.7%)
C.odorata	*90.5*	70.9	52.6	6.10
C.odorata+PEG		74.7(+5.3%)	53.6(+1.9%)	6.05 (-0.8%)
s.e.d	2.4	6.1	5.2	1.24

Data are means of triplicate. Figures in brackets are % changes compared to control due to addition of polyethylene glycol 8000 (PEG).

In sacco degradability of browse plants was compared to *in vitro* DM degradability estimated using the SNB method, in the absence or presence of PEG. The degradability *in sacco* ranges between 45.8 to 90.5. The degradability *in vitro* using the SNB method was lower and range between 27.3% to 70.9%. However, upon addition of PEG degradability was positively affected, i.e. an increase between 1.7% to 34.4%. The increase in browse plant degradation due to PEG was associated with changes in gas production ranging from +1.9% to +149.5%. The largest increase was observed for *C.calothyrcus* (149.5%) followed by *M. esculanta* (130.2%), *A.hispida* (98.5%), *E. aromaticum* (55.3%) and *C.kyllinga* (37.1%). The addition of PEG resulted in the reduction of PF values which ranges between -0.8% to -92.3%. The greatest reduction in PF values due to PEG, -92.3% and -84.8%, were observed for *M. esculanta* and *C.calothyrcus* respectively which also have the highest PF values (11.04 and 11.05 respectively) in the absence of PEG.

L.leucocephala responded differently to PEG; an increase in gas production (+50.5%) was associated with very small (+1.7%) and insignificant ($P > 0.05$) increase in dry matter degradability. Two plant species, *C.odorata* and *M. marabathricum* showed no significant ($P>0.05$) response in gas and dry matter degraded upon addition of PEG. No relationship was found between the changes in substrate degradability or gas production, upon addition PEG, and TETa, TECTa or TEPA.

DISCUSSION

Estimation Of The Degradability Of Incubated Substrate In Vitro Bwith Nylon Bag

Nylon bag has long been used to estimate feed degradation characteristics in the rumen (Seradja, *et al*, 2019). The washing of nylon bags resulted in free outward-flow of small particles including degraded feed particles, tannin-protein complexes and cell solubles (Osawa, 1992). In the present studies nylon bag was also used in the estimation of substrate degraded after 24 hour incubation using the Menke gas production test. Substrate degradability for grass species as estimated by SNB method is generally lower than those estimated by NDF-digestion and this may be explained by the microbial matter extracted from undegraded feed in the latter method (BlÜmmel and Becker, 1997). Nevertheless, the estimates of degraded substrates determined by the SNB method match closely those determined in *in sacco.* Thus, apart from information on the products of substrate fermentation *in vitro*, the SNB method provides complimentary information on the degradability of the same substrate in the rumen.

Addition Of Peg And Its Effect On Substrate Degradability And Gas Production

PEG was chosen as tannin-binding agent because PEG complexes with tannin more efficiently than does PVP (Khazaal *et al*, 1993; Makkar *et al*, 1995). In addition, because of its solubility in aqueous solution, practically all PEG was excluded from the nylon bag and thus did not interfere with the determination of DM degradation (data not shown). The use of SNB method to estimate substrate degradability after 24h incubation *in vitro* is important in two respects. Firstly the inhibitory effect of tannins on microbial fermentation and the degradability of substrate may be investigated (Baptist. 2008). The SNB method developed was thus able to demonstrate the changes in the degradation of substrate as a result of addition of catechin and subsequently of PEG. Secondly, it can also be used to estimate the degradability of tannin-free or tannin-containing (provided PEG is also

added) feed in the rumen using the *in sacco* method.

PEG, by itself, has no significant effect on the degradation of wheat straw (Table 3) but its presence increased the degradability of browse plants (Table 4). Regression analysis showed that the estimation of browse plant DM degraded after incubation *in vitro* with those determined using the *in sacco* method was better when PEG was also added (y= 0.71x + 14.9; r^2 = 0.92) in the incubation medium than when PEG was absent (y= 0.59x + 15.0; r^2 = 0.72). Better correlation of degradability in the former may be explained by the fact that most of the effects of browse plants tannin *in vitro* were removed by PEG, which in a way mimmicks the effect of extensive tannin dilution when the plant samples *in sacco* were incubated in the rumen (Vadiveloo and Fadel, 1992).

It is interesting to note that the increase in browse plant degradation (ranges from +1.7 t0 +34.4%) was generally smaller than the changes in gas production (ranges from +1.9 to 140.5%). This highlight the possibility that for browse plants, the addition of PEG markedly enhance microbial utilization of plant fractions (both 'soluble fractions' and insoluble but degradable) that readily passes through the nylon bag and that the size of this fraction may be different for different types of plants.

The increase in dry matter degradability was associated with an increase in gas produced at t=8h (mostly fraction 'a') and at t=24h (fraction 'b') which suggests that PEG added into the incubation suppressed inhibitory effect of tannins on microbial fermentation of soluble and insoluble but degradable substrate respectively, probably through formation of complexes with tannin

PF Values Of Incubated Substrates

Products of fermented substrate include short chain fatty acids (SCFA), fermentative gases, and microbial cells. The more the substrate is being degraded and fermented by the rumen microbes the more energy is made available as SCFA (measured partly as accumulated gas) or to be used for the synthesis of microbial cells. Since the volume of gas produced is inversely proportional to microbial yield (BlÜmmel *et al*, 1997a) the value of calculated PF reflects variations in microbial biomass yield.

This would mean proportionally more products of substrate degraded in plant with PF value of 4.00 would be utilised for microbial biomass production (higher microbial efficiencies) than those in plant with PF value of 3.00. In the present studies the addition of PEG resulted in the reduction of PF values (PF_{+PEG}), suggesting partitioning of energy more towards SCFA production when the inhibitory effect of tannins was reduced. Makkar and co-workers (1997) demonstrated that the increase in gas production upon incubation of leaf sample (*Dichostachys cinera*) rich in tannin with PEG was associated with a decrease in the efficiency of microbial protein synthesis as measured by the incorporation of 15N into microbial mass. Findings from the current studies points out that the presence of tannins in browse plants do not only protect feed protein from extensive microbial degradation in the rumen, thus increasing protein availability post-ruminally (Wadhwa, *et al*, 2007), but also reduces the partitioning of nutrient to SCFA formation. It is important to understand the effect of tannin on substrate fermentation and the partitioning of energy.

The theoretical range for PF values for tannin-free plants was suggested (BlÜmmel *et al*, 1997) to be 2.75 to 4.41 mg truly degraded substrate/ml gas, assuming maximum Y_{ATP} was 32 for rumen microbes. PF figures greater than 4.41 is not theoretically possible (Makkar *et al*, 1997) and if this occurred would simply indicate I) significant loss of detached tannins from fermented substrate but which do not contribute to gas, ii) non-utilisation of soluble fraction due to tannins, or combination of (I) and (ii. In the present

studies calculated PF values for browse plants were much higher with values ranging from 5.05 to 11.05, and which could easily be attributed to combination of factors (I), (ii) and factor (iii) feed particles which escaped the nylon bag during washing but which did not contribute to gas, either due to inhibitory tannin effects on substrate fermentation or particles which are insoluble and undegradable. These factors stress the importance not to regard the calculated PF and PF_{+PEG} values obtained for browse plants as absolute figures.

The values, however, may be used in relative manner and appears particularly useful in the comparison of PF values for each tannin-containing plants (Table 4) in the absence or presence of PEG on gas production and substrate degraded. It can be inferred that the greater the changes in the values of PF_{+PEG} from PF values the greater the harmful effects of inhibitory tannin on microbial fermentation of substrate. This may provide some insight into why certain browse plants leaves such as Jackfruit (*A. heterophylus*) tree leaves and ipil-ipil (*L.leucocephala*) can be fed up to 50-60% (Meriksa, 2010), whereas cassava (*M.esculanta*) leaves can only be fed up to 30% of daily feed intake before signs of toxicity began to develop. It is also interesting to note that the increase in gas production in the presence of PEG was not always contributed by proportional increase in degraded substrate which suggests different effects of tannin from different browse plants on substrate degradability. Further studies using greater variety and number as well as stage of growth of browse plants are necessary to establish the relationship between browse plants characteristics (chemical composition, voluntary dry matter intake, maximum inclusion in the diet), and the values of PF and PF_{+PEG}. Equally important are the evaluations on the effect of inclusion of tannin-containing plants in the diet on the degradability of other roughage consumed.

CONCLUSION

Based on the explanation abovewe can conclude that the method developed enables the Menke gas production test to be used as a tool both to measure gas production and to estimate 24 hr browse plant degradation. Using this method changes in the PF values of browse plants in the absence or presence of PEG can be determined. Browse plants degradation *in sacco* after 24 hour in the rumen may also be estimated *in vitro* provided polyethylene glycol (PEG) is also added in the incubation medium. Selectively feed based browse plant for animal nutrition use the degradability and gas production method at 24h.

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