Effect of Tree Leaves on Rumen Fermentation, Microbial Count and Blood Urea Nitrogen of West African Dwarf Goats

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Abstract

This experiment was carried out to assess the effect of *Azadirachta indica, Newbouldia laevis* and *Spondias mombin* leaves on rumen fermentation, microbial count and blood urea nitrogen (BUN) of West African Dwarf (WAD) goats. Sixteen WAD bucks (11.6 ± 0.9 kg in body weight) were allocated to 4 treatments: 1) Control and 2) 40 g/day of *Azadirachta indica*, 3) 40 g/day of *Newbouldia laevis* and 4) 40 g/day of *Spondias mombin* leaves arranged in a completely randomised design. The ground leaves were included in concentrate diets served on dry matter basis at 2% of body weight while *Panicum maximum* was fed *ad libitum*. The control diet had no tree leaves. Data were collected on chemical composition, rumen fermentation and microbial ecology, and BUN. Saponin was highest ($P < 0.05$) in *S. mombin* (8.14%) while *A. indica* and *N. laevis* had 5.78% and 1.56%, respectively. Rumen ammonia nitrogen was least ($P < 0.05$) in goats fed *A. indica* (8.35 mg/dL) while the highest ($P < 0.05$) total volatile fatty acid (TVFA) was obtained from goats fed *S. mombin* with 125.51 mM. Goats fed *N. laevis* yielded the highest ($P < 0.05$) acetate with 70.65 mol/100 mol while propionate production was highest ($P < 0.05$) in the rumen of goats fed *S. mombin* (27.15 mol/100 mol). Viable bacteria count was lowest ($P < 0.05$) in rumen of goats fed *A. indica* (3.95×10^{12} cfu/ml) while the least ($P < 0.05$) protozoa population was obtained from the rumen of bucks fed *S. mombin* (4.18×10^{9} cfu/ml). All goats in the treatments containing tree leaves had higher ($P < 0.05$) and a rapid increase in BUN between 0 and 6 h post feeding when compared with the Control. It is concluded that feeding ground leaves of *S. mombin* to goats increases rumen total volatile fatty acid and propionate production and reduces the protozoa population.

Keywords: saponin, rumen, volatile fatty acids, microbial, goats

Introduction

The breakdown of feed in the rumen takes place through the associated processes of physical breakdown and microbial fermentation during rumination. The process of microbial degradation of plant components in the rumen is carried out by a large and diverse population of bacteria and ciliate protozoa, together with a small but metabolically important, population of anaerobic fungi (Dehority, 2003). Products of rumen fermentation, mainly volatile fatty acids (VFA) and microbial proteins, are available for absorption in the small intestine. Indeed VFA formed in the rumen can supply

the majority of the animal's energy requirement, approximately 80% (France and Siddons, 1993), while microbial protein leaving the rumen can account for much (typically 60–85%), (Ørskov, 1982) if not all of the protein entering the small intestine.

The fermentation process is critical to the nutritional survival of the ruminant animal; however, these activities of ruminal micro-organisms also result in emission of large amounts of gases (CO_2 and CH_4). The excretion of methane especially represents a loss of up to 0.15 of the digestible energy, depending on the type of diet, and enteric methane also contributes approximately 0.30-0.40 unit of total methane produced from agricultural sources (Moss et al., 2000).

In view of this importance of the rumen in the nutrition of ruminants, a great deal of effort has and is being devoted to investigating methods for manipulating this complex ecosystem (Nagaraja et al., 1997). Manipulating rumen fermentation is aimed at increasing hydrogen-consuming processes, which will make less H_2 available for methane formation, and fermentative pathways for VFA proportions is therefore proposed to shift from acetate and butyrate formation to more of propionate (Mirzaei-Aghsaghali and Maheri-Sis, 2011). The inclusion of phytochemicals (tannins and saponins especially) in diets of animals have received wide interest for rumen manipulation. Saponins for instance are known to lyse protozoa and decrease protozoal counts (Teferedegne, 2000) and affect methane production (Hess et al., 2003; Santoso et al., 2004).

The first step involved in the assessment of rumen modulators, is for their effects on rumen fermentation and how they alter the chemical products, while the second is finding natural materials which might be used to suppress the growth of rumen ciliate protozoa (Wanapat et al., 2008). Protozoa can represent almost half of the microbial biomass in the rumen (Jouany and Ushida, 1999); their proteolytic activity and the vigorous engulfment of bacteria make their presence in the rumen undesirable. The objective of this study was to evaluate the effect of the leaves of *Azadirachta indica*, *Newbouldia laevis* and *Spondias mombin* on rumen fermentation, microbial profile and blood urea nitrogen of WAD goats.

Materials and Methods

Feeding Trial

The experiment was conducted at the Small Ruminant Unit of the Teaching and Research Farm Directorate, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the derived savannah zone of the south-western part of Nigeria on latitude 7^{o} 13′ 49.46″ N and longitude 3^{o} 25′ 11.98″ E (Google Earth, 2013). The climate is humid with a mean annual rainfall of 1,037 mm and mean temperature and humidity of 31.7^{0}C and 83%, respectively.

The experimental set-up comprised of four treatment groups: 1) Control (no tree leaves) and 2) 40 g/day of *Azadirachta indica*, 3) 40 g/day of *Newbouldia laevis* and 4) 40 g/day of *Spondias mombin* leaves arranged in a completely randomised design. Leaves of the tree species were collected through random and manual harvest from different parts of the tree branching to obtain both young and mature leaves from each tree species. The leaves were sun-dried for 72 h, ground in a mill to pass through a 1-mm sieve and then stored in plastic bags.

Sixteen West African Dwarf (WAD) bucks with mean body weight of 11.6 ± 0.9 kg were used for the experiment to give four animals per treatment. The bucks were housed in individual pens with asbestos roofing material and slatted planks as raised floor. The experimental pens were properly

cleaned and disinfected, and the animals were kept intensively. Concentrate feed was served at 2% of body weight on dry matter basis while *Panicum maximum* grass and water were made available *ad libitum* intake. The animals were kept for an initial adaptation period of 14 d before application of treatments.

Dietary treatments were prepared by adding 40 g of each ground leaf to the concentrate feed served to the animals. The concentrate was composed of maize (13%), wheat offal (54%), palm kernel cake (20%), groundnut cake (7%), oyster shell (4%) and common salt (2%). Diets were fed once daily at 0700 with concentrate served first and chopped forage after 1 h in a separate feeding trough. Daily adjustments were made for about 10% of the feed leftovers. The feeding trial lasted for 70 d after which samples of blood and rumen fluid were collected from the animals.

Sample Collection

Prior to the daily morning supply, leftovers of concentrate and grass from individual pens were collected, sampled and stored to form weekly composites for animal diets. Five ml of blood sample were collected from each animal via jugular vein puncture using a hypodermic needle and a syringe before the commencement of the experiment and at the end of the experiment for the measurement of blood urea nitrogen (BUN). Rumen fluid was also collected at the beginning and at the end of the experiment, and were analysed for fermentative and microbial characteristics. At the end of the experiment, rumen fluid was collected from each buck at 0 and 6 h post-feeding via the oesophagus through the use of a suction tube. Rumen fluid was taken into aseptic bottles, and the temperature and pH were measured. The fluid samples were then filtered through 4 layers of cheesecloth, divided into 3 parts, kept in sample bottles and stored for fermentation and microbial analyses.

Chemical Analyses

Proximate composition parameters (dry matter (DM), crude protein (CP), ether extract (EE) and ash) of leaves were carried out in 3 replicates using the methods of AOAC (2000) while neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the method of Van Soest et al. (1991). Saponin content of leaves was determined according to the method described by Obadoni and Ochuko (2001) while total tannin was quantified according to the methods described by Edeoga et al. (2005).The first portion of 50 ml of the rumen sample filtrate was acidified with 1 ml of 5% (v/v) orthophosphoric acid solution and stored frozen at -20 ^{o}C in air tight bottle containers for subsequent determination of volatile fatty acid (VFA) concentrations. Total volatile fatty acids distillate concentration was determined by titration of samples with 0.1N NaOH solution and expressed as volatile fatty acid content. The procedure was a modified protocol that replaced the conventional titration with the potentiometric titration system. The concentration of NaOH solution was matched with VFA content in the samples (Siedlecka et al., 2008). The ratio of acetate (C_2) to propionate (C_3), ratio of combined concentration of acetate and butyrate to propionate were calculated. Amount of methane produced was calculated from the various proportions of the VFAs using the formula of Ørskov et al. (1968) expressed as: Methane = 0.5 (Acetate) – 0.25 (Propionate) + 0.5 (Butyrate).

Blood sample of 5 ml from immediate sampling collection was centrifuged and serum was harvested for analysis. Consequently the plasma was separated by centrifugation at 500 × *g* for 10 min and

stored at -20 °C for analysis of plasma urea nitrogen according to method of Crocker (1967).

Microbial Analysis

The second portion (10 ml) of rumen fluid was used for the determination of ammonia-nitrogen (NH_3-N) concentration as described by Lanyansunya et al. (2007). The last portion of the rumen fluid was divided into 2 parts. A part was fixed with 10% formalin solution in sterilized 0.9% saline solution. This was used for total direct count of bacteria, protozoa and fungal zoospores through the method of Galyean (1989). A second part was cultured using total viable count bacteria roll-tube technique (Hungate, 1969).

Statistical Analysis

All data were subjected to one way analysis of variance using SPSS (2007). Level of significance was tested at 5% probability, and significant means were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

S. mombin had the highest ($P < 0.05$) DM of 88.42% followed by *N. laevis* with 71.62% (Table 1). All of the leaves had high levels of CP with 12.8%, 17.7% and 14.49% for *A. indica, N. laevis* and *S. mombin,* respectively. Minson (1981) reported that the minimum range of CP in plant foliage recommended for optimum performance of tropical animals is 6.5-8.0%, which means that if these tree leaves were incorporated as feed component, will contribute towards the nutritive requirement of goats. These CP values are also above the critical limit below which foliage intake by ruminants could negatively affect microbial activities (Van Soest, 1994). Ether extract value was higher for *S. mombin* than those for the grass and concentrate signalling probable high levels of essential oils. Higher EE content in the leaves of *A. indica* and *S. mombin* is a reflection of the presence of essential oils. The NDF and ADF values of *N. laevis* were 52.0 and 36.0%, respectively, which were higher than those of *S. mombin* (30.0 and 28.0%, respectively). The comparatively low NDF and ADF contents of *Spondias mombin* are consistent with the findings of Igwe et al. (2010) who reported CF of the leaves as 10.51%. This has implication for the fermentability of foliage to be used as source of phytochemical for rumen modification because NDF and DM degradability have an inverse relationship (Bamikole et al., 2004). The tannin content of *N. laevis* leaves (12.09%) was higher ($P < 0.05$) than values obtained in *A. indica* (3.12%) and *S. mombin* (3.17%). This low tannin content observed is similar to the results obtained by Igwe et al. (2010).

Table 1: Chemical composition of tree leaves and feed consumed by goats

Parameter (%)	*A. indica*	*N. laevis*	*S. mombin*	SEM
Dry matter	69.33[b]	71.62[b]	88.42[a]	3.057
Organic matter	84.00[c]	88.00[b]	92.00[a]	1.247
Crude protein	12.8[c]	17.70[a]	14.9[b]	0.777
Ether extract	7.00[b]	3.00[c]	9.00[a]	0.913
Neutral detergent fibre	32.00[b]	52.00[a]	30.00[b]	3.547
Acid detergent fibre	30.00[b]	36.00[a]	28.00[c]	1.225
Ash	16.00[a]	12.00[b]	8.00[c]	1.190
Saponin	5.78[b]	1.56[c]	8.14[a]	0.965
Tannin	3.12[b]	12.09[a]	3.17[b]	1.491

[abc] Means on the same row having different superscripts are significantly different ($P < 0.05$)
SEM = Standard Error of Mean

The saponin contents of *S. mombin* (8.14%) and *A. indica* (5.78%) leaves imply that they can both serve as saponin source in ruminant diet, and could conveniently be used as rumen fermentation manipulator of ruminants as the saponin content was higher than that reported for *Yucca schidigera* (4.4%) (Eryavuz and Dehority, 2004) but lower than the seed of *Sapindus saponaria* (12%) (Hess et al., 2003), both plants had been used to alter rumen environment in other studies.

Rumen fermentation parameters were significantly affected by the addition of tree leaves to the diet of goats (Table 2). An increased ($P < 0.05$) level in the pH of rumen fluid suggested a shift towards alkalinity (7.24) as obtained from goats fed *S. mombin.* The values were however generally stable around the 6.0 to 7.0 range which is considered optimal for microbial digestion of fibre and protein (Hoover, 1986). Ruminal NH_3-N is a major source of N for microbial protein synthesis (Erdman et al., 1986) and increased ($P < 0.05$) concentrations of 14.75 and 13.82 mg/dL were obtained from *N. laevis* and *S. mombin*, respectively in this study. These values are higher than to those reported by Church and Santos (1981) and Wanapat and Pimpa (1999) which implies that the tree leaves used in this experiment make more nitrogen available for the manufacture of protein by rumen micro-organisms, and thereby will aid goat performance. Higher volume of NH_3-N in goats fed *N. laevis* can be directly attributed to higher CP content of the leaves. Hosoda et al. (2006) reported that urea N concentrations increased significantly (5.0 to 5.4 mg/dL) by lemongrass leaf supplementation. On the other hand in this study, the inclusion of *A. indica* however resulted in the production of lower ($P < 0.05$) amount of rumen NH_3-N. In similar results, some other phytochemicals for rumen manipulation were reported to have reduced NH_3-N production as observed with the supplementation of essential oils from peppermint herb (Ando et al., 2003). In another study, Busquet et al. (2006) noted that supplementation of high essential oil at 3,000 mg/L significantly decreased NH_3-N concentration. The addition of essential oils resulted in a reduction in the number and diversity of hyper- NH_3-producing bacteria,

resulting in decreased rate of NH_3 production from amino acids (McEwan et al., 2002). Reduction in protein degradation suggests that at least part of the effects of certain phytochemicals can be attributed to decreased proteolysis (Molero et al., 2004). Increasing rumen NH_3-N concentrations also resulted in increasing concentrations of blood urea nitrogen (Figure 1). It was observed that there was generally a sharp increase in the BUN of goats fed tree leaves from 0 to 6 h post-feeding compared to goat fed the control diet. Lewis (1975) affirmed that concentration of plasma urea N is highly correlated with the concentration of NH_3 production in the rumen.

Total VFA production was significantly ($P < 0.05$) affected by inclusion of tree leaves with goats fed *S. mombin* recording the highest level of production with 125.51 mM while the least was obtained from the control group with 108.89 mM (Table 2). Increased VFA concentration has been reported to be an indication of increased microbial activity (Woyengo et al., 2004). The range obtained here is similar to those reported by France and Siddons (1993) for normal quantities in optimal rumen fermentation, but it however differs from the results of Hosoda et al. (2006) which in a rumen manipulation study reported that supplementation of lemongrass leaves at 5% of the diet did not alter VFA concentration in the rumen in dairy steers. The concentration of VFA is regulated by its level of production, absorption across the rumen wall and utilization by rumen microorganisms (Van Soest, 1994) and it also depends on the availability of fermentable organic matter in the feed (Oosting, 1993).

Acetate production was highest ($P < 0.05$) and similar in the rumen of goats maintained with dietary inclusion of *N. laevis* (70.65 mol/100mol) and those in the control group (70.54 mol/100mol). *N. laevis* though contained high levels of tannin, did not affect VFA concentration, a result that agrees with Mirzaei-Aghsaghali and Maheri-Sis (2011) who reported that though tannins inhibited microbial activity both *in-vitro* and *in-vivo* but proportions of VFA were unchanged resulting in similar yield of hydrogen. Conversely, goats fed *S. mombin* recorded the lowest ($P < 0.05$) measurement of acetate (62.97 mol/100mol) while there were also increases ($P < 0.05$) in levels of propionate in goats fed *S. mombin* (27.15 mol/10nol) and *A. indica* (23.97 mol/100mol) when compared to the other treatments. This kind of shift in VFA proportion profile was also reflected in the acetate: propionate ratio which was lowest ($P < 0.05$) for goats fed *S. mombin* (2.35) compared to 3.59 in the control group, had been associated with the presence of saponin in the diet of ruminant animals (Makkar et al., 1995). However, this effect of saponin on ruminal propionate, which reduced acetate to propionate ratio, was found to vary with diets and phytochemical applications. Lila et al. (2003) reported that saponin had a variable effect on ruminal propionate concentration while Ngamsaeng et al. (2006) found no significant effect of feeding saponins and tannins from mangosteen peel on total VFA and individual VFA concentrations. The inclusion of tree leaves did not have any significant effect on butyrate production in this study but it was however observed that the acetic plus butyric: propionic ratio was similar to the control group and goats fed with *N. laevis* while those with inclusion of *S. mombin* had the least ($P < 0.05$) value with 2.71.

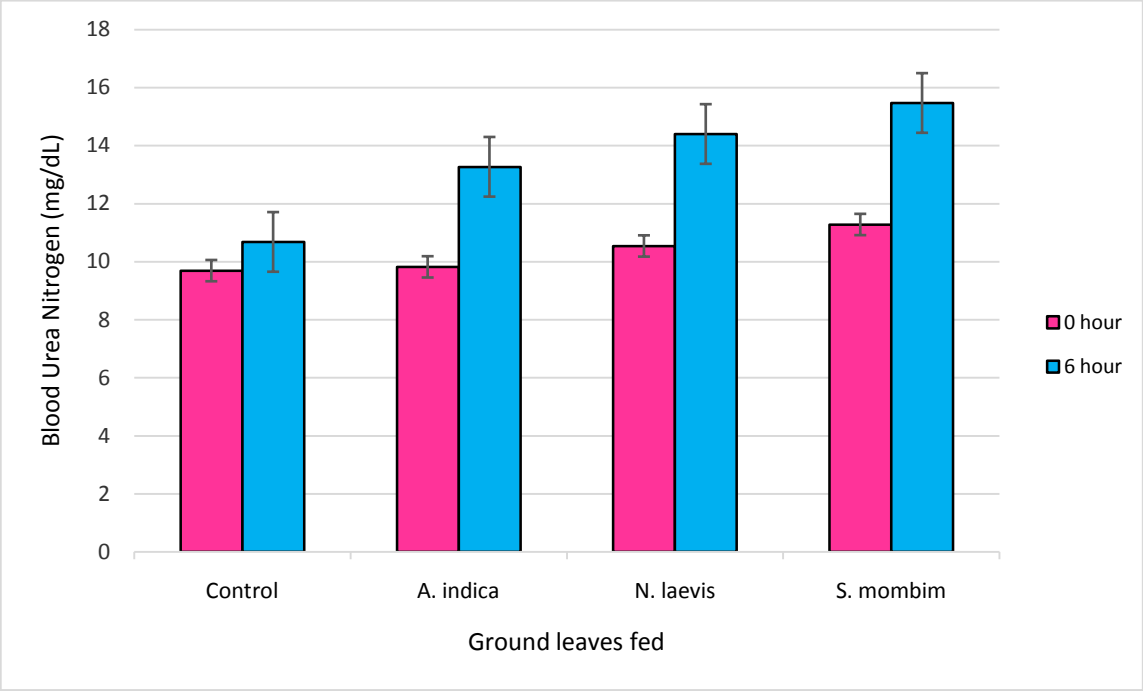

Figure 1: Chart showing the blood urea nitrogen values of bucks fed ground leaves

The control group and *N. laevis* recorded the highest ($P < 0.05$) calculated values of methane gas from VFA concentration of 35.28 mM and 35.58 mM, respectively, while a lower value was obtained for *A. indica* (32.03 mM), the lowest recorded with goats fed *S. mombin* (30.13 mM) (Table 2). These values clearly showed that the level of ruminal CH_4 production was depressed with inclusion of ground tree leaves in the diet of goats. In addition, the change in ruminal CH_4 production mirrored the changes in acetate to propionate ratio, as shown; a low ratio of acetate to propionate was obtained for all the ground leaves included with *S. mombin* being the lowest. These methane-suppressing effects of *S. mombin* and *A. indica* can be attributed to the higher level of saponin which presumably has a direct action against methanogens and protozoa which are the rumen microbes involved in methane formation (Sliwinski et al., 2002).

Table 2: Rumen NH_3-N and VFA concentration of goats fed ground tree leaves

Parameter	Control	*A. indica*	*N. laevis*	*S. mombin*	SEM
Rumen pH	6.72[b]	6.74[b]	6.74[b]	7.24[a]	0.220
Rumen ammonia - N (mg/dL)	10.16[b]	8.35[c]	14.75[a]	13.82[a]	0.258
Total VFA (mM)	108.89[c]	117.11[b]	114.49[b]	125.51[a]	1.853
Acetate (C_2) (mol/100mol)	70.54[a]	66.28[b]	70.65[a]	62.97[c]	1.000
Propionate (C_3) (mol/100mol)	19.63[c]	23.97[b]	19.23[c]	27.15[a]	0.941
Butyrate (C_4) (mol/100mol)	9.83	9.75	10.12	9.88	0.093
$C_2 : C_3$	3.59[a]	2.77[b]	3.56[a]	2.35[c]	0.165
$C_2 + C_4 : C_3$	4.06[a]	3.17[b]	4.20[a]	2.71[c]	0.181
CH_4	35.28[a]	32.03[b]	35.58[a]	30.13[c]	0.726

[abc] Means on the same row having different superscripts are significantly different ($P < 0.05$)
SEM = Standard Error of Mean

Table 3 shows that rumen ecology of goats was significantly altered after the period of application of dietary treatments. Many phytochemicals have dose-dependent effects on bacteria, protozoa, and fungi (Greathead, 2003). Lower ($P < 0.05$) counts of total viable bacteria was observed in the rumen of goats fed *A. indica* with a count of 3.95×10^{12} cfu/mL while results for *S. mombin* fed goats were similar ($P < 0.05$) to those of the control. Saponins are known to influence the composition and number of rumen bacterial species through specific selective enhancement or inhibition of the growth of individual species. The decrease in bacterial population as related to the treatments containing *A. indica* and *N. laevis* was possibly due to decreases of gram-positive bacteria which generally appear to be more susceptible to inhibition by plant compounds than did gram-negative bacteria (Davidson and Naidu, 2000). This effect has been related to the presence of an outer membrane on gram-negative organisms, which endows them with a hydrophilic surface that acts as a strong impermeable barrier (Nikaido, 1994).

Table 3: Rumen microbial counts of goats fed ground tree leaves

Rumen microbes (cells/gram)	Control	*A. indica*	*N. laevis*	*S. mombin*	SEM
Viable bacteria (cfu/ml $\times 10^{12}$)	5.39[a]	3.95[ab]	4.01[b]	5.03[a]	0.636
Protozoa, $\times 10^9$	7.86[a]	5.47[b]	4.79[b]	4.18[b]	0.484
Fungal zoospores, $\times 10^6$	4.27[a]	1.37[b]	1.35[b]	1.21[b]	0.405

[abc] Means on the same row having different superscripts are significantly different ($P < 0.05$)
SEM = Standard Error of Mean

Protozoal population was also significantly ($P < 0.05$) reduced by dietary inclusion of tree leaves, with the lowest values recorded in rumen fluid obtained from goats fed *S. mombin* with a count of 4.18×10^9 cells/g. This population reduction in comparison to the animals on the control diet was more than 40%. It has been noted that the sensitivity of protozoa towards saponins may be explained by the presence of sterols in protozoa, but not in bacterial membranes (Williams and Coleman, 1992). Thus, the sterol-binding capacity of saponins most probably causes the destruction of protozoal cell membranes. It was opined that defaunation of the rumen could be from disruption of protozoal membranes as a result of precipitation of medicagenic acid (an antimycotic agent) in saponins and sterols in the cell membrane. Lu and Jorgensen (1987) found that using saponins extracted from alfalfa by ethanol extraction and partial acid hydrolysis had a characteristic similar to defaunation. The first treatment resulted in total protozoal count in sheep fed roughage diet reduced 34 and 47% when fed 2 and 4% saponins, respectively, while observations were made in the other treatment that in sheep fed concentrate diet, total protozoal count was also by reduced 33 and 76% when fed 2 and 4% saponins, respectively. Significant reductions in fungal zoospores were also observed for goats fed various tree leaves as compared to the control goats with no inclusion of tree leaves.

Conclusion

Inclusion of tree leaves significantly altered the rumen environment of WAD goats. *S. mombin* increased ruminal production of VFA and propionate, while causing a reduction in protozoa population. *N. laevis* on the other hand did not show any effect on rumen proportions of acetate and propionate. Inclusion of tree leaves caused a sharp increase in blood urea nitrogen post-feeding.

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