







# EFFECTS OF FODDER TREE LEAVE SUPPLEMENTATION FOR BASAL RICE STRAW DIET ON RUMEN AMMONIA, pH, AND DEGRADATION CHARACTERISTICS IN SHEEP

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✉Supporting Information

**ABSTRACT:** Fodder tree leaves (FTLs) contain high levels of protein, vitamins, and minerals that play a major role in enhancing roughage intake by ruminants, thus improving low-quality roughage utilization. The study sought to measure the rumen degradation characteristics, pH, and ammonia N concentration of sheep fed rice straw (RS) and supplemented with FTLs. Four forest-type rumen-fistulated rams of an average weight of 19.0±1.2 kg were randomly assigned to one of four treatments in a 4 × 4 Latin Square design. Treatment diets consisted of urea-ammoniated straw (UAS; control), RS+100% *Leucaena leucocephala* (L), RS+100% *Samanea saman* (S), and RS+50% L+50% S (LS). Data obtained were subjected to the Glimmix procedure of SAS (2016) and significant means were separated using Tukey's test at (P<0.05). Treatments differed significantly (P<0.001) in the quantity of readily soluble materials (a), rate of degradation (c), and a potentially degradable fraction (P) with LS recording the highest a, c, and P among the treatments. Ruminal pH and ammonia concentrations differed significantly (P<0.0001) among the treatments. Overall mean rumen pH values obtained ranged from 6.44 in UAS to 6.72 in the S-supplemented diet whereas mean rumen ammonia values ranged from 4.59 mg/100 ml in sheep fed UAS diet to 9.15 mg/L in sheep fed L diet. The pH values obtained imply that the experimental diets could improve rumen fermentation and, hence, serve as good sources of feed for ruminants. The rumen DM degradation values indicated that sufficient amounts of DM would be degraded over a period of time, thus releasing substantial quantities into the small intestines for digestion to provide essential nutrients needed for better animal performance. The rumen ammonia values obtained were higher than the minimum values recommended for optimal microbial activity for animals fed lignocellulosic materials. This indicated that such FTLs could be utilized for moderate animal performance, especially during the dry seasons when natural pastures are qualitatively and quantitatively poor.

**Keywords:** Ammonia concentration, Degradation, *Leucaena leucocephala*, *Samanea saman*, Sheep.

## INTRODUCTION

Fodder tree leaves contain high levels of protein, vitamins, and minerals which play a major role in enhancing roughage intake by ruminants (Larbi et al. 1993; Idan et al., 2020), and hence improve low-quality roughage utilization (Idan et al., 2021; Adogla-Bessa et al., 2022). Sarkwa et al. (2020a) fed dried sole tree leaves to sheep and reported higher weight gain and lower methane emission than sheep fed urea-treated rice straw. However, feeding the same tree leaves in combinations resulted in higher weight gain and lower methane emission compared to feeding sole tree leaves (Sarkwa, 2020a). Improved weight gain of small ruminants as a result of feeding combinations of tree leaves has also been reported by Papachristou and Plastis (2011) and Idan et al. (2023).

The nylon-bag technique (*in sacco* degradability) is one of the powerful tools for ranking feeds according to the rate and extent of degradation of dry matter, organic matter, nitrogen, or other nutritional parameters (Osuji et al., 1993; Fonseca et al., 1998). This technique as reported by Abate and Kiflewahid (1991) is cheap, reliable, and easy to perform. Ørskov and McDonald (1979) described it as a major evaluation technique for determining the nutritive value of forages. A number of studies have demonstrated that the rumen's capacity for degrading feed provides a way of more thoroughly assessing the nutritional content of feedstuffs. For instance, Ørskov et al. (1980) indicated that because of its high degree of correlation with *in vivo* digestibility results, the *in sacco* nylon bag approach has been widely utilized for evaluating ruminal breakdown, screening feedstuffs, and estimating digestible organic matter intakes.

Ammonia concentration in the rumen fluid reflects the activities of fermentation of feed protein and protein synthesis by rumen microbes (Erdman et al., 1986; Gonzalez-Munoz et al., 2019; Tharwat et al., 2019). Ammonia production is the result of protein degradation from feed utilized by microbes as a source of protein (Gonzalez-Munoz et al., 2019; Tharwat et al., 2019). However, the minimum concentration of rumen ammonia for rumen microbes to thrive is

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5mg/100 ml (McDonald et al., 2012). Rumen ammonia level is necessary for efficient rumen microbial growth (Brooks et al., 2012) and gives an indication of dietary nitrogen adequacy. Moreover, rumen ammonia concentration is affected by the level of intake of protein, the extent of degradability, the duration of feed in the rumen, and the acidity of rumen fluid (McDonald et al., 2012). According to Gonzalez-Munoz et al. (2019) and Tharwat et al. (2019), the formation of ammonia in the rumen is a relevant factor influencing feed degradation.

Ruminal pH is an important factor influencing the rumen environment and subsequent performance of small ruminants. The degree of fiber in the diet influences rumen pH, with Castillo et al. (2006) indicating that feeding an excessively low-fiber or high-energy diet is associated with ruminal acidosis. In sheep, the normal ruminal pH ranges from 6.4 to 6.8. Previous studies have indicated that pH values of 5.5 or lower, or values of 7.0 or higher are deemed abnormal with the 5.5 or lower value resulting in subacute rumen acidosis (Beauchemin and Yang, 2005). Furthermore, continuous or occasional periods of low rumen pH are linked with animal health problems and can limit fiber digestion and hence microbial efficiency (Russell and Wilson, 1996; Faniyi et al., 2019).

Despite their relevance in predicting the nutritional value of feeds, rumen pH, ammonia, and degradation characteristics have not been extensively studied in Ghana. Therefore, the study, sought to ascertain the rumen degradation characteristics of sheep fed untreated rice straw and supplemented with sole and combined fodder tree leaves (*Leucaena leucocephala* and *Samanea saman*).

## MATERIALS AND METHODS

### Ethical considerations

The fistulated animals used for the *in Sacco* degradability trial were cared for using the guidelines of the Institutional and Federation of Animal Science Societies (FASS, 2010) for the care and use of agricultural animals in research and teaching. However, the Ethics Committee of the College of Basic and Applied Sciences, University of Ghana, Legon-Accra, granted ethical approval.

### Study site

The experiment was conducted at the experimental station of the Livestock and Poultry Research Centre (LIPREC), University of Ghana (UG). LIPREC is located in the Coastal Savanna Zone in the Accra Plains of Ghana and lies at latitude 5° 68'N, 0° 10'W. The average annual precipitation is between 508 mm and 743 mm, whereas the average temperature is between 24.3°C and 32.9°C. While the minor rainy season lasts from September to October of each year, the main rainy season lasts from April to July of each year. November through March is considered the dry season.

### Rice straw and forages

The Small-Scale Irrigation Agricultural Project in Ashaiman, in the Greater Accra Region of Ghana, provided the rice straw for the study. To ease and improve feed consumption, the rice straw was chopped into 30 mm lengths with an electric forage cutter (CeCoCo forage SFC1400, Central Commercial Company®, Osaka, Japan). Briefly, the urea-ammoniated straw (UAS) was prepared by distributing chopped RS into a concrete culvert that was lined with polythene sheets. Following the procedure outlined by Fleischer et al. (2000) and Idan et al. (2020), 1 kg of urea was dissolved in 10 liters of water and sprayed onto each layer of 16 kg of rice straw in the culvert. The mixture was then ensiled for a further two weeks. The ensiled rice straw was air-dried in a ventilated area before being fed to the sheep. From mature woodlots at the University of Ghana's LIPREC, the fodder trees replicated five times for each of the two species (*Leucaena leucocephala* and *Samanea saman*) were chosen at random and marked for harvesting during the experimental period. The leaves were then harvested by hand cutting and air-dried at room temperature in a well-ventilated room to reduce the moisture content before feeding them to the sheep.

### Animals, dietary treatments, and experimental design

Four rumen-fistulated forest-type rams with an initial weight of 19.0 ± 1.2 kg were used. The rams were fitted with rumen cannulas (Nepean Rubber Mouldings Pty Ltd. - Macam Division, Baulkham Hills, Australia) and randomly assigned to one of four dietary treatments consisting of a urea-ammoniated straw (UAS), untreated rice straw (RS) supplemented with *Leucaena*, *Samanea*, or equal amounts of both *Leucaena* and *Samanea* over four periods in a repeated 4 × 4 Latin square design. The animals on the positive control diets were fed urea-ammoniated rice straw while those on the test received untreated rice straw as basal diets respectively. Animals were rested for a week after each 21-day period and allowed to accustom to new treatment diets in the subsequent period for another week.

### Housing, management, and feeding

Individual pens measuring 2 m × 1.5 m (3 m<sup>2</sup>) and constructed of a concrete floor, iron sheet roofing, and wooden sides were used to house the fistulated animals. The lighting program provided 12 h of light and 12 h of darkness per day. Over the course of the experiment, the average temperature of the pen house hovered around 25°C. The experimental diets were given to the animals twice daily. Nevertheless, water and mineral vitamin licks were made available at all times during the research period. Before rumen pH, ammonia, and *in Sacco* DM degradation measurements were made, the animals were allowed to adapt to the experimental diets for 14 days. After each period, the animals were allowed to

rest for seven days to clear their GIT of the previous experimental diet. The sheep were fed urea-ammoniated straw during the resting period.

### Rumen pH

Rumen liquor was collected from three different parts of the rumen of the fistulated animals through the cannula by inserting a tube connected to a suction pump (Sarkwa et al., 2021). About 20 ml of rumen liquor was collected at 0, 2, 4, 6, 8, 10, 12, and 24 hours after feeding and strained through cheesecloth into a beaker, and pH was measured immediately using a pH meter (Sarkwa et al., 2021). The fluid was then stored in a freezer for ammonia determination later.

### Rumen ammonia

The stored fluid from rumen pH determination was strained through a four-layer cheesecloth and kept warm and anaerobic in a thermos. Rumen ammonia was determined by using the method described by Broderick and Kang (1980) as validated by Adjorlolo et al. (2014).

### In Sacco DM degradation characteristics

Using the Dacron sample bags (Ankom, Macedon, NY, USA; 42 µm porosity), as reported by Orskov et al. (1980), the DM disappearance in Sacco was evaluated. The untreated rice straw samples used for the study were oven-dried at 55 °C and ground to pass through a 1 mm sieve. Two grams of the oven-dried samples were weighed into nylon bags (135 mm×75 mm). Triplicate samples in the bags were manually inserted into the ventral sac of the rumen's liquid phase of the fistulated animals and incubated for 96 hours. The bags were tied to a drop line which was made up of nylon cords (200 mm × 0.7 mm) with an 11 g steel bolt tied at one end to provide the required weight. The nylon bags were soaked in water to displace the air before they were inserted into the rumen. Incubation periods for the degradation studies were 3, 6, 9, 12, 24, 36, 48, 72, and 96 hours. After the required period, the bags were withdrawn sequentially and washed by hand under tap water until the water became clear and frozen to halt microbial fermentation. Samples in the bags were thawed and placed into a forced-dried oven at 55 °C for 48 h and weighed. To determine the content of water-soluble material, bags representing 0 h degradation also underwent the same washing procedure as the incubated bags. Dried residues from 4 bags of each incubation time from each sheep were pooled together. The DM disappearance values were calculated as the difference between the weight before and after the incubation of each sample. The degradability data obtained for DM for each feed was fitted to the equation:  $P = a + b(1 - e^{-ct})$  (Orskov et al., 1980).

P = potential disappearance of DM at time "t"; a = rapidly soluble fraction; b = potentially degradable but insoluble fraction (%); t = time; c = rate of constant for degradation of "b" fraction; e = the natural logarithm.

### Degradation curve

The percent DM disappearance was plotted against the time of incubation for each sample. Percent soluble fraction "a" value (the intercept of the graph on the y-axis) and the potentially degradable fraction "b" (the difference between the highest points on the graph less "a") were determined. The maximum rate of degradation, which represents the steepest section of the curves, was identified. The percentage degradation (P) and the incubation time (t) corresponding to the mid-point of this section were read off. The rate of constant degradation "c" was calculated from the exponential equation (Orskov et al., 1980).

### Chemical analysis

Moisture and dry matter contents of the fodder tree leaves and rice straws (treated and untreated) were determined after drying the samples to constant weight in an oven at a temperature of 105 °C. By ashing dried samples for 6 hours in a muffle furnace set to 600 °C, the organic matter (OM) content was determined. The weight loss was calculated as organic matter and the residue as ash. The total nitrogen content was determined using the standard Kjeldahl method (Association of Analytical Chemists, 2016) and was converted into CP by multiplying the percentage of N content by a factor of 6.25. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to (Van Soest et al., 1991).

### Statistical analysis

The data obtained from the study involving rumen ammonia, pH, and DM degradability were all subjected to a one-way Analysis of Variance of the Glimmix procedure of SAS (2016) in a replicated 4 × 4 Latin square design according to the following statistical model.

$$Y_{ijkl} = \mu + P_i + \tau_j + A_{(k)} + \epsilon_{ij(k)}$$

Where,  $Y_{ijkl}$  = measured dependent variable;  $\mu$  = overall mean;  $P_i$  = fixed effect period  $i$  ( $i = 1, \dots, 4$ );  $\tau_j$  = fixed effect of diet  $j$  ( $j = 1, \dots, 4$ );  $A_k$  = random effect of animal;  $\epsilon_{ij(k)}$  = residual variation

The mean separation was done using Tukey's pairwise comparison test at ( $P \leq 0.05$ ).

## RESULTS

### Chemical composition of rice straws and fodder tree leaves

The chemical composition values of the experimental diets varied significantly ( $P < 0.0001$ ) between the rice straws and the fodder tree leaves (FTLs) fed to the experimental animals (Table 1). The forage DM content ranged from 965.0 to 987.0 g/kg in straws to 920.0 to 949.0 g/kg in FTLs with straws having higher ( $P < 0.0001$ ) DM values than the FTLs. Similar to the DM, the OM matter values for the straws were significantly higher ( $P < 0.0001$ ) than those for the FTLs. The fodder tree leaves and their combination had higher ( $P < 0.0001$ ) values for the CP levels compared to the straws. However, untreated rice straw had the lowest CP value (66.8 g/kg), while Leucaena had the highest value (271.1 g/kg). The NDF values ranged from 300.4 to 589.0 g/kg, whereas the ADF values varied from 175.6 to 517.0 g/kg, with the

straws having higher values than the FTLs. The ash content varied greatly, ranging from 77.7 to 202.3 g/kg, with UAS having the highest and Samanea having the lowest.

**Table 1 - Chemical composition of rice straws and fodder tree leaves.**

Item	DM	OM	CP	NDF	ADF	Ash
	g/kg	(g/kg DM)				
Forage						
RS	987.0 <sup>a</sup>	826.9 <sup>d</sup>	66.8 <sup>e</sup>	589.0 <sup>a</sup>	517.0 <sup>a</sup>	173.1 <sup>b</sup>
UAS	965.0 <sup>b</sup>	797.7 <sup>e</sup>	100.2 <sup>d</sup>	542.0 <sup>b</sup>	501.0 <sup>b</sup>	202.3 <sup>a</sup>
Leucaena (L)	920.0 <sup>e</sup>	898.4 <sup>c</sup>	271.1 <sup>a</sup>	300.4 <sup>e</sup>	175.6 <sup>e</sup>	101.6 <sup>c</sup>
Samanea (S)	949.0 <sup>c</sup>	929.3 <sup>a</sup>	240.5 <sup>c</sup>	406.2 <sup>c</sup>	313.5 <sup>c</sup>	77.7 <sup>e</sup>
<sup>1</sup> LS	937.0 <sup>d</sup>	910.0 <sup>b</sup>	256.8 <sup>b</sup>	355.2 <sup>d</sup>	261.8 <sup>d</sup>	90.0 <sup>d</sup>
SEM	0.678	0.624	0.571	0.429	0.432	0.503
P Value, <	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

<sup>1</sup>The combined foliage was derived from a mixture of an equal amount of individual fodder tree leaves and pooled together into a composite before analysis. RS = Untreated rice straw; UAS = Urea-ammoniated rice straw; DM = Dry matter; OM = Organic matter, CP = Crude protein, LS = 50% Leucaena and 50% Samanea supplement

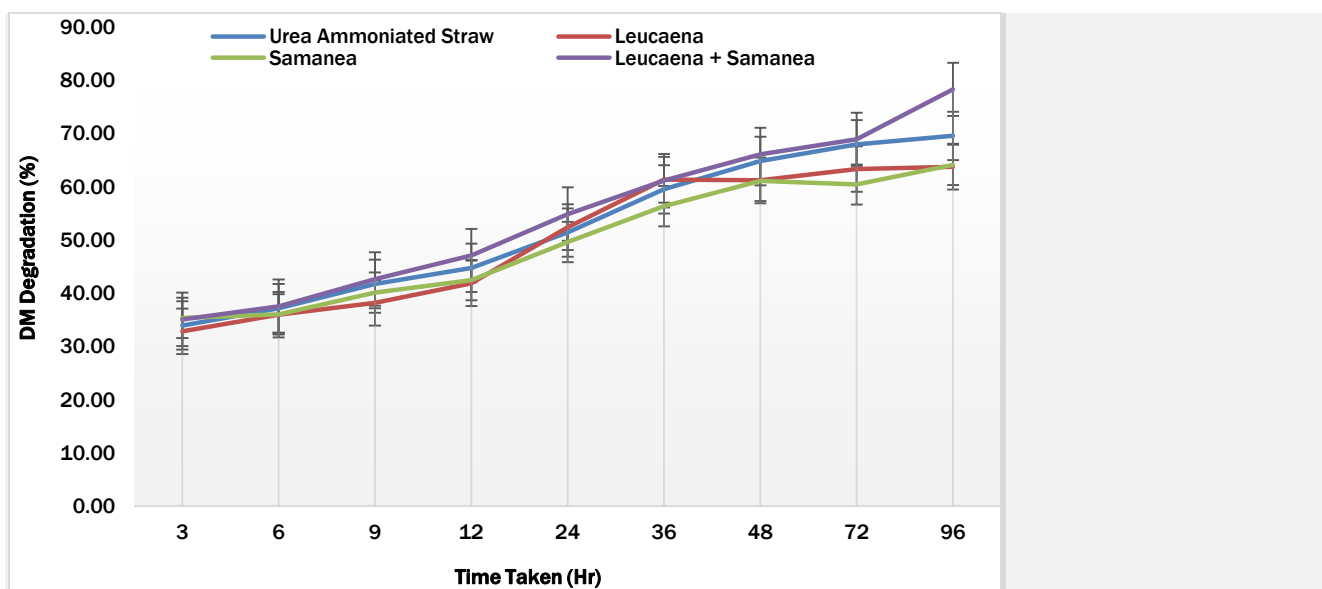
**Rumen degradation characteristics of DM**

The DM degradability in the rumen of sheep fed the sole or combined fodder tree leaves as well as urea ammoniated rice straw differed as shown in Table 2. In the current study, supplementation of rice straw with sole or combined fodder tree leaves was hypothesized to influence rumen degradability due to improvement in the rumen environment for microbial fermentation. The experimental diets differed (P<0.0001) in the amount of readily soluble material, the potentially degradable fraction, and the rate of gas production. The LS treatment had the highest percentage of soluble material (30.65%) with Leucaena recording the least fraction (25.90%). The disappearance of DM increased progressively from 3 h up to 96 h incubation time where degradability peaked for all experimental diets (Figure 1). Similar pools of LS and UAS fiber fractions represented as “b” disappeared at the same rates and were higher in terms of fiber degradation than *Leucaena* and *Samanea*. The rate of constant degradation (c) was significantly (P<0.05) lower for LS (0.038) and UAS (0.038) when compared with *Leucaena* and *Samanea*, which recorded (0.049) and (0.043) respectively. The potential disappearance of dry matter (P %) was significantly (P<0.05) lower for L and S when compared with the UAS and LS. However, those of UAS and LS were similar (P>0.05).

**Table 2 - Dry matter degradability parameters for *Leucaena*, *Samanea*, LS, and UAS**

Treatment	a (%)	b (%)	c	P (%)
UAS	28.85 <sup>c</sup>	47.33 <sup>a</sup>	0.038 <sup>c</sup>	76.15 <sup>b</sup>
<i>Leucaena</i>	25.90 <sup>d</sup>	39.20 <sup>b</sup>	0.049 <sup>a</sup>	65.35 <sup>d</sup>
<i>Samanea</i>	29.70 <sup>b</sup>	36.35 <sup>c</sup>	0.045 <sup>b</sup>	65.75 <sup>c</sup>
LS	30.65 <sup>a</sup>	47.35 <sup>a</sup>	0.038 <sup>c</sup>	78.55 <sup>a</sup>
SEM	0.063	0.105	0.001	0.050
Significance	<0.0001	<0.0001	<0.0001	<0.0001

<sup>a-d</sup> Means in the same column with different superscripts are significantly different (P<0.05) UAS= Urea ammoniated straw; LS= 50% *Leucaena* + 50% *Samanea*; SEM= Standard error of the mean; a= initially degradable fraction; b= degradable DM fraction; c= rate constant for the disappearance of b; P= potential disappearance of DM; SEM = standard error of the mean



**Figure 1 - DM Degradability of the Experimental Diets at various incubation times.**

## Rumen pH

Experimental diets significantly influenced ( $P < 0.0001$ ) the pattern of rumen pH values (Table 3). The mean rumen pH values obtained in this current study over a 24 h period ranged from 6.28 to 6.64 (UAS); 6.35 to 6.95 (*Leucaena*); 6.55 to 6.89 (*Samanea*); and 6.18 to 7.01 in LS. There was no clear trend for the pH values in the four rumen environments. However, the pH tended to decrease shortly after feeding the experimental diets and increased at 12 hours, with the exception of the sole *Samanea*-supplemented diet, which did not exhibit any clear pattern (Table 3). However, the highest and lowest pH values of 7.01 and 6.18, respectively, were recorded in animals fed untreated rice straw and supplemented with LS. Nevertheless, the overall mean rumen pH values were highest ( $P < 0.0001$ ) in the sole S-supplemented diet compared to the other treatment diets.

**Table 3 - Rumen pH for 24 hours after feeding the experiment diets**

Hours	0	2	4	6	8	10	12	24	Mean
<b>Treatment</b>	<b>pH</b>								
UAS	6.64 <sup>d</sup>	6.59 <sup>d</sup>	6.42 <sup>c</sup>	6.34 <sup>d</sup>	6.31 <sup>c</sup>	6.28 <sup>c</sup>	6.37 <sup>c</sup>	6.53 <sup>d</sup>	6.44 <sup>c</sup>
<i>Leucaena</i>	6.95 <sup>b</sup>	6.67 <sup>c</sup>	6.54 <sup>b</sup>	6.52 <sup>b</sup>	6.49 <sup>b</sup>	6.35 <sup>b</sup>	6.47 <sup>b</sup>	6.65 <sup>b</sup>	6.58 <sup>b</sup>
<i>Samanea</i>	6.89 <sup>c</sup>	6.86 <sup>a</sup>	6.82 <sup>a</sup>	6.80 <sup>a</sup>	6.71 <sup>a</sup>	6.58 <sup>a</sup>	6.54 <sup>a</sup>	6.55 <sup>c</sup>	6.72 <sup>a</sup>
LS	7.01 <sup>a</sup>	6.75 <sup>b</sup>	6.55 <sup>b</sup>	6.45 <sup>c</sup>	6.34 <sup>c</sup>	6.18 <sup>d</sup>	6.55 <sup>a</sup>	6.75 <sup>a</sup>	6.57 <sup>b</sup>
SEM	0.0071	0.0101	0.0043	0.0073	0.0069	0.0057	0.0054	0.0045	0.0058
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>a-d</sup> Means in the same column with different superscripts are significantly different ( $P < 0.05$ ); UAS = Urea ammoniated straw; LS = 50% *Leucaena* + 50% *Samanea*; SEM = Standard error of mean

## Rumen ammonia

The experimental diets significantly influenced the rumen ammonia concentrations in sheep (Table 4). The mean ammonia concentration ranged from 4.73 mg/100 ml in sheep fed UAS diet to 9.16 mg/100 ml in sheep fed *Leucaena*-supplemented diet. The highest ammonia concentration of 14.53 mg/100 ml was observed at 6 hours post-feeding in animals fed an LS-supplemented diet while the lowest value of 2.84 mg/100 ml was obtained in animals fed a UAS diet at 10 hours post-feeding. Apart from the sole *Samanea*-supplemented diet, which did not show any clear pattern for rumen ammonia concentration, the rest of the experimental diets showed a clear pattern. While the rumen  $\text{NH}_3$  concentration peaked at 2 hours post-feeding for UAS and L, that of LS increased gradually after feeding and peaked at 6 hours (Table 4).

**Table 4 - Rumen ammonia N concentration for 24 hours after feeding the experiment**

Hours	0	2	4	6	8	10	12	24	Mean
<b>Treatment</b>	<b>Ammonia N concentration (mg/100ml)</b>								
UAS	6.16 <sup>d</sup>	7.56 <sup>c</sup>	6.63 <sup>c</sup>	4.44 <sup>d</sup>	3.27 <sup>d</sup>	2.86 <sup>d</sup>	3.39 <sup>c</sup>	3.53 <sup>c</sup>	4.73 <sup>d</sup>
<i>Leucaena leucocephala</i>	8.49 <sup>a</sup>	12.61 <sup>a</sup>	12.45 <sup>a</sup>	11.04 <sup>b</sup>	9.63 <sup>b</sup>	7.68 <sup>a</sup>	6.18 <sup>a</sup>	5.17 <sup>a</sup>	9.16 <sup>a</sup>
<i>Samanea saman</i>	7.17 <sup>c</sup>	5.92 <sup>d</sup>	5.20 <sup>d</sup>	6.15 <sup>c</sup>	6.21 <sup>c</sup>	5.96 <sup>c</sup>	6.12 <sup>a</sup>	4.78 <sup>b</sup>	5.94 <sup>c</sup>
LS	7.33 <sup>b</sup>	9.84 <sup>b</sup>	12.25 <sup>b</sup>	14.55 <sup>a</sup>	9.84 <sup>a</sup>	7.44 <sup>b</sup>	5.36 <sup>b</sup>	3.48 <sup>c</sup>	8.76 <sup>b</sup>
SEM	0.008	0.013	0.008	0.0024	0.0054	0.011	0.030	0.067	0.006
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>a-d</sup> Means in the same column with different superscripts are significantly different ( $P < 0.05$ ); UAS = Urea ammoniated straw; LS = 50% *Leucaena* + 50% *Samanea*; SEM = Standard error of mean

## DISCUSSION

### Chemical composition of experimental diets

Previous research has shown that diets with CP levels of less than 6% are unlikely to provide the minimal ammonia levels necessary for the greatest possible microbial growth in the rumen (Norton, 1994). Except for the untreated rice straw, all of the experimental diets in the current study had CP levels well above 7%, indicating that they could be used as feed resources for small ruminants to achieve moderate-to-optimum growth performance.

### Effects of sole and combined fodder tree leaves as supplements on *In sacco* degradation

The disappearance parameters examined in *in sacco* degradability studies are critical because they affect rumen fill and hence feed intake (Ørskov et al., 1988). The differences in the degradability parameters (a, b, c, and P) observed were due to variations in the chemical composition of the supplements. This agreed with the findings of Melaku et al. (2003), Anele et al. (2009), and Sarkwa et al. (2021), who all reported significant variations in the DM degradation parameters of multipurpose tree leaves. The degradation differences may also be associated with the structural and non-structural protein and carbohydrate fractions of the supplements used (Whetton et al., 1997) as well as the quality and content of fiber of the experimental diets (Smith et al., 1989, Antwi et al., 2014). The potential degradability of DM (P %) obtained in



the current study (65.2–78.4 %) was lower than the values of 60.70–93.60 % reported by [Attoh-Kotoku \(2011\)](#) but comparable to those of [Sarkwa et al. \(2021\)](#) when similar browses were utilized. All the experimental diets had greater apparent DM degradability, indicating that they can be used to feed animals during the dry season when available feed is low in quantity and quality. However, the observed maximum (P%) value in LS suggested an optimal rumen environment for microbial breakdown. In addition, the (P%) value for UAS (76.40%) in the present study was higher than the value of 54.31% reported by [Sarkwa et al. \(2021\)](#). The soluble fraction (a %) for *Samanea* was consistent with results obtained by [Lowry \(1995\)](#) of (26.0 ± 4.8) but was higher than the value of (7.7 %) obtained by [Attoh-Kotoku \(2011\)](#). Reports by [Tolera et al. \(1997\)](#) and [Melaku et al. \(2003\)](#) suggested that (a %) and NDF were inversely related. This agreed with the results obtained in this study.

The potentially degradable but water-insoluble fraction (b %) of 39.30–47.8 % recorded in this study was lower than the 54.9–85.8 % reported by [Attoh-Kotoku \(2011\)](#) when similar browses were used to supplement rice straw. However, the (b %) for UAS was comparable to the results obtained by [Sarkwa et al. \(2021\)](#).

The greatest potentially degradable fraction was obtained by animals on 50% L: 50% S diets. The observed differences might be due to the variations in the chemical composition and the cell wall fractions of the various feedstuffs. [Ramírez et al. \(2000\)](#) reported that the slowly degradable fraction of plant cell walls was limited by ADL, organic matter, ash, and insoluble ash. However, several studies have reported a negative relationship between the extent of degradation of DM or CP with NDF, ADF, ADL, and CT ([Siaw et al., 1993](#); [Bonsi et al., 1995](#); [Melaku et al., 2003](#)). The findings of this study did not corroborate their observations. The variations in the (b %) and (P %) values in the rumen have also been attributed to variations in the NDF, ADF, lignin ([Van Soest, 1994](#); [Yan and Agnew, 2004](#)) and tannins ([Kamalak, 2006](#)) or maturity ([Khazaal et al., 1993](#); [Kamalak, 2006](#)). The rate of constant DM degradability of 'b' (0.038 to 0.049) observed in this study was similar to values reported by [Sarkwa et al. \(2021\)](#) but lower than those reported by [Addo-Kwafo \(1996\)](#). However, the 'c' values for *Samanea* were higher than the values obtained by [Attoh-Kotoku \(2011\)](#). The differences may be due to variations in the ecology, stage of maturity, and time of harvest of the fodder tree leaves ([Giridhar et al., 2021](#); [Navale et al., 2022](#)). The significantly higher degradation rate of DM 'c' in 100 % *Leucaena* compared to the rest reflects differences in chemical composition between the plant species. For example, *Leucaena* had the highest CP compared to the rest of the forages used in this study. The result shows that 'c' is positively correlated with CP and is consistent with the findings of [Tolera et al. \(1997\)](#) and [Kamalak \(2006\)](#).

The results obtained in this study showed that 50 % L: 50% S had the highest potential disappearance of dry matter in the rumen and the slowest degradation rate. This means that a sufficient amount of it would be degraded over a period of time thus releasing a substantial amount into the small intestines for digestion to provide essential nutrients needed for better animal performance. This is consistent with the better growth rate recorded for LS reported by [Idan et al. \(2023\)](#).

#### **Effects of sole or combined fodder trees as supplements on rumen pH and ammonia**

The mean pH values obtained in this study (6.44 to 6.72) were similar to the 6.52 to 6.65 reported by [Attoh-Kotoku \(2011\)](#) when *Samanea saman* and *Stylosanthes hamata* were used as a supplement for sheep fed a basal diet of rice straw and the 6.15–6.60 reported by [Sarkwa et al. \(2021\)](#) when sheep were offered urea-treated rice straw. However, the values recorded in this current study were slightly lower than the optimum levels of 6.7–7.2 reported by [Orskov \(1982\)](#). It is established that a low pH of 5.5 reduces ruminal microbial fermentation ([Cerrato-Sánchez et al., 2007](#)) and as a result influences the activities of fibrolytic bacteria in digesting fiber in the rumen ([Russell and Dombrowski, 1980](#)). [Russell and Dombrowski \(1980\)](#) ascribed the reduced organic matter and fiber digestion and changes in the profile of volatile fatty acids to low pH resulting in the lowered activity of the fibrolytic bacteria. The pH values at various hours post-feeding within the minimum threshold as observed in the study imply that the effects of undigested fiber within the feed, which could have reduced the access of bacteria and enzymes to the protein, were eliminated. According to [Devant et al. \(2000\)](#), low fiber digestion leads to an increase in dietary N flow while decreasing CP degradation and ammonia N concentration. This suggests that low pH inhibits bacterial N flow, which subsequently reduces microbial growth. Generally, the mean pH of 6.58 reported for all the experimental diets in this study implied that optimal pH conditions existed in the rumen for cellulolysis and improved rumen fermentation.

The rumen NH<sub>3</sub> values observed in the study were comparable to the 4.93 to 6.36 mg/100 ml reported for sheep fed on rice straws with *Samanea saman* and *Sesbania sesban* supplementation by [Attoh-Kotoku \(2011\)](#). Furthermore, several studies with sheep revealed that the concentration of ammonia in the rumen ranged between 1 and 14 mg/100 ml ([Fleischer et al., 2000](#); [Miller and Thompson, 2003](#); [Horadogado et al., 2008](#); [Lascano and Heinrichs, 2009](#); [Adjorlolo et al., 2014](#); [Gumilar et al., 2018](#)). However, [Kaur et al. \(2008\)](#) and [Sarkwa et al. \(2023\)](#) reported concentrations higher than 14 mg/100 ml. In the current study, no relationship was found between pH and rumen ammonia concentration, although [Aguerre et al. \(2009\)](#) found a negative relationship between pH and NH<sub>3</sub>-N when all measurements were considered ( $r=-0.46$ ,  $P<0.001$ ). Their result agreed with [Cajarville et al. \(2006\)](#), who reported a low but significant negative relationship between rumen pH and NH<sub>3</sub>-N concentration of cows grazing temperate pastures and supplemented with different sources of grains. The rumen NH<sub>3</sub>-N concentrations found in this study were higher than the suggested minimum values (1.65 to 3.79 mg/100 ml) for optimum microbial activity in animals fed lignocellulosic materials by [Balcells et al. \(1993\)](#). This shows that such feeds could be utilized for moderate animal performance, especially during the dry seasons.

## CONCLUSION

The chemical composition of fodder tree leaves varies greatly depending on plant species and edible plant sections. The current study demonstrates that all of the fodder tree leaves (FTLs) investigated can be used as potential protein banks to supplement grasses or fibrous crop residues during dry seasons. The mean pH values obtained from the current study were within the range of 6.2 to 7.0 required for optimal rumen function, indicating that the experimental diets could promote rumen fermentation and hence be good sources of feed for ruminants. The rumen ammonia levels measured in this study were greater than the reported minimum levels for optimum microbial activity in animals fed lignocellulosic materials. This shows that such diets might be suitable for improving animal performance, particularly during the dry seasons. The readily degradable and potentially degradable fractions and disappearance of DM were superior in the combined FTLs compared to the sole FTLs. However, the overall rumen dry matter degradation values recorded by the various diets indicate that a sufficient amount of dry matter could be degraded over time, releasing a significant amount into the small intestines for digestion to supply important nutrients required for improved animal performance. Therefore, *Leucaena leucocephala* and *Samanea saman*, or their combination, can be utilized as supplements to improve the usage of low-protein, high-lignin basal diets. Future research should use conventional feeding experiments to further understand the effects of different fodder tree leaves and their combinations on growth performance, carcass characteristics, hematological parameters, and meat quality.

## DECLARATIONS

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### Authors' contribution

F. Idan and T. Adogla-Bessa came up with the idea, planned the experiment, and wrote the manuscript; Ch. Antwi and F. O. Sarkwa worked together on the statistical analysis and took part in the manuscript review; O. Alhassan reviewed the manuscript and formatted it; Y. Abdul Aziz reviewed the manuscript; and F. Idan carried out the practical component and laboratory analysis. The final manuscript was read and approved by all authors.

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### Conflict of interests

The authors have not declared any conflict of interest.

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