Effects of supplementation of sole and combined browse leaves on *in vitro* digestibility of rice straw

E. D. Yeboah[1], T. Adogla-Bessa[2], G. N. Q. Ayorkor[3], R. Yeboah[4] and F. O. Sarkwa[5*]

[1,3] *Department of Animal Production and Health, University of Energy and Natural Resources, Dormaa Campus, P.O Box 214, Sunyani, Ghana-West Africa*

[2] *Department of Animal Science and Fisheries, Evangelical Presbyterian University College, P. O. Box HP 678, Ho, Volta Region, Ghana- West Africa*

[4,5] *Livestock and Poultry Research Centre, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Box LG 38, Legon- Accra, Ghana-West Africa*

*Corresponding Author: *ofsarkwa@ug.edu.gh*

Abstract

The utilization of browse leaves as quality livestock feeds during the late dry season can alleviate nutritional deficiencies and maintain livestock production. This study investigated the nutritional value in terms of dry matter (DM), crude protein (CP), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of both sole and combined browse leaves in Southern Ghana. Browse leaves used were *Cajanus cajan* (CC), *Spondias mombin* (SM) and *Leuceana leucocephala* (LL). Untreated Rice Straw (URS) was used as the basal diet which was harvested during the late dry season (September and October) from an irrigated rice field. The proportion of rice straw to browse leaves was 75 to 25 percent. Rumen liquor for the *in vitro* study was obtained from two forest type fistulated wethers of average weight 23 kg. The values for DM, CP, NDF, ADF and ash of the experimental diets ranges between 909.5 to 919.7 g/kg, 87.3 to 211.3 g/kg DM, 591.5 to 700.3 g/kg DM, 202.6 to 235.4 g/kg DM and 129.5 to 145.7 g/kg DM respectively. The combination of the three browse leaves (LLSMCC) had the lowest ($p<0.05$) ADF and NDF values whilst URS was the highest ($p<0.05$). All browses used improved the IVDMD of URS significantly from 388.7 g/kg DM to between 441 and 472.1 g/kg DM. The highest ($p<0.05$) IVDMD was obtained by the combination of Spondias mombin and Cajanus cajan (SMCC). Also, IVOMD of URS was improved from 346.4 g/kg DM to between 446.3 g/kg DM and 483.4 g/kg DM. Results obtained from this study showed that browse leaves were high in CP and moderately low in crude fibre and ash hence, can be recommended as supplements for ruminants most especially in the dry season when feed is scarce.

Keywords: Browse leaves, chemical composition, *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, ruminants

Introduction

Inadequate feed in the dry season is a major limiting factor to small ruminant production in West Africa where fodder is of poor nutritional value (Adogla-Bessa et al., 2012; Timpong-Jones et al., 2014). This is mainly due to soil infertility and poor rainfall pattern which is reflected in low production of grasses and fodder. During the dry season, the quality of available herbages is so low that, unless the animals have access to supplementary feeds, they lose weight resulting in fluctuation in animal performance (Adogla-Bessa et al., 2022). Most ruminant farmers in West Africa and Ghana in particular raise their animals or flocks basically on rain fed or native grassland. This is particularly true in tropical Africa where sheep contributes 75 % and goats 80 % and the rangeland is the most important source of feed (Ademosun,1998; Brown and Thorpe, 2008).

Due to the intensity and extent of the dry season and erratic rainfall recorded, the management system for livestock production in West Africa is generally nomadic and transhumant (Timpong-Jones et al., 2023). As a result, animals have to trek great distances in search of forage whose quality cannot be guaranteed to provide essential nutrients and water (Timpong-Jones et al., 2014). For both cultivated pastures (Olanite et al., 2004) and natural pastures (Adjorlolo et al., 2014) forage biomass yields have been shown to decline drastically in the dry season. Similarly, decreases in crude protein and increases in neutral detergent fibre (NDF) of some forage legumes as the season changes from wet to dry have been reported (Adjorlolo et al., 2014; Sarkwa et al., 2021).

Browses such as *Spondias mombin* and *Leaucena leucocephala* have been successfully used for small ruminant production (Adogla-Bessa et al., 2022; Idan et al., 2023). Akinfemi and Ogunwole (2012) studied the chemical composition and *in vitro* digestibility of rice straw for ruminants. Sarnklong et al. (2010) worked on improving digestibility of ruminant feed by using aqueous ammonia. Sarkwa et al, (2020) reported on *in vitro* digestibility of sole and combined browse leaves. *In vivo* studies using sole and combined browse leaves have been carried out (Papachristou et al., 2011; Sarkwa et al. 2023). Even though the studies by Papachristou et al. (2011), Samklong et al. (2010), Akinfemi and Ogunwole (2012) and Sarkwa et al. (2020) are related to this current study, the use of native combined browse leaves to improve rice straw has received less research attention. Combining browse leaves as supplement can improve digestibility because of synergies compared to sole browse leaves. This study aims at bridging this gap and throwing more light on the use of some selected native browse leaves (both sole and combined) to supplement rice straw. The objective of this study was to determine the effect of supplementation of sole and combined browse leaves on the *in vitro* digestibility of rice straw.

Materials and Methods

Study Area

The experiment was carried out at the Livestock and Poultry Research Centre- Legon (5˚68'N, 0˚10'W). Total annual rainfall is 881 mm (Adjorlolo et al., 2014). Temperature ranges between 32.22 ºC and 34.49 ºC (Sarkwa et al, 2020) while relative humidity is between 36.60 % to 73.73 % (Sarkwa et al., 2020).

Sample Collection

Fresh leaves from the three (3) selected species (*Cajanus cajan*, *Leucaena leucocephala* and *Spondias mombin*) were all collected from a fodder bank at Livestock and Poultry Research Centre, University of Ghana, Legon, in the Coastal Savannah zone from November to December 2017. Rice straw was obtained from Small Scale Irrigation Agricultural Project, Ashaiman in the Greater Accra Region. Rice straw was chopped into approximately 3 cm pieces using a forage cutter (CeCoCo forage SFC1400, Central Commercial Company,

TABLE 1
The common names and family names of the three browse leaves

Species	Common names	Family names	Codes
Cajanus cajanus	Pigeon pea	Fabaceae	CC
Leucaena leucocephala	Leadtree jumbay	Fabaceae	LL
Spondias mombin	Yellow mombin	Anacardiaceae	SM

TABLE 2
Composition of the experimental diets

Treatment	Supplement (25%)	Basal diet (75%)
1	LL	Straw
2	CC	Straw
3	SM	Straw
4	Straw	Straw
5	LLSM	Straw
6	CCSM	Straw
7	LLCC	Straw
8	LLCCSM	Straw

NB: LLCCSM was in the proportion of 8.33%: 8.33%: 8.33%

Osaka Japan) and then ground through 1mm sieve for laboratory analysis in 2017. The common names and family names of the three browse leaves can be found in Table 1. Table 2 shows the composition of the experimental diets.

Chemical analysis and in vitro digestibility determination

Chopped rice straw (untreated) and leaves from three browse leaves were shade dried for four days, oven dried at 55º C until a constant weight was obtained and ground through a 1 mm sieve before chemical analysis. Dry matter (DM), Ash (OM) and crude protein (CP) were determined using the methods of A.O.A.C. (2016). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were determined using methods described by Goering and Van Soest (1970). *In vitro* evaluation was done as described by Menke and Steingass (1988) and validated by Sarkwa et al. (2020). Rumen liquor for the *in vitro* work was obtained from two forest type fistulated wethers of average weight 23kg. The fistulation was carried out by using the Required Surgical Procedures for fistulating the gastrointestinal tract as documented by the Department of Primary Industries of New South Wales Government, Australia (NSW Government, 2012). Modified version of rumen cannulae as described by Elices et al. (2010) was used. The fistulated animals were fed fresh *Panicum maximum* (Average daily Energy intake was 21.88 MJ/kg DM) and provided water on *ad libitum* basis. There was a two week feed adjustment period before collection of rumen fluid.

The rumen content was strained through four layers of cheese cloth to obtain the rumen liquor. Half gram each of the samples were weighed in duplicates and placed into a labelled 50 ml centrifuge tube. Twenty eight millilitres (28 ml) of McDougall's solution was poured into each of the centrifuge tubes and prewarmed in water bath at 39 ºC. A buffer and ruminal fluid solution (in a ratio of 4 to one) of 7 ml was added. The tubes were flushed with carbon dioxide and the caps were placed on the tube. The tubes were inverted several times to suspend the samples and placed on a rack. Four blank tubes containing no sample and 35 ml McDougall's to rumen fluid mixture were also placed on the rack. Then, the rack was placed into a water bath at 39 ºC. The tubes were incubated for 48 hours and inverted 2, 4, 8, 20 and 28 hours after the start of incubation to suspend the samples. The tubes were removed from the water bath after 48 hours of incubation and were centrifuged for 15 minutes at 2000 xg. The liquid was suctioned off by vacuum and the samples were frozen until pepsin digestion was carried out.

Thirty five millilitres (35 ml) of pepsin solution was poured into each tube and incubated for 48 hours in a water bath at 39 ºC. The samples were shaken at 2, 4 and 6 hours after pepsin solution was added. At the end of the pepsin digestion, the samples were filtered using the modified Buchner funnel and ashless filter paper. The filter paper containing the samples were placed in an aluminium pan and dried. The dried samples (DS) were weighed and the difference between the initial weight (IW) and the DS was noted as degraded weight (DW).

IVDMD (%) was calculated as follows:

IW – DS = DW

IVDMD (%) = DW/ IW X 100 %

Organic matter digestibility (OMD) was determined as follows:

OM= DM-Ash

OMD (%) = OM/DM X 100%

OM: Organic matter

Experimental design and data analysis

There were eight treatments (Rice straw and seven browse samples) with five (5) replicates each for the *in vitro* digestibility evaluation and three (3) replicates for each sample in the chemical analysis. Data were analyzed as completely randomized design and was subjected to Analysis of variance (ANOVA) using GenStat (2009) version 12.1 according to the model as follows:

$Yij = \mu + Ti + Eij$

Yij is the response variable such as DM, Ash, CP, NDF, ADF, IVDMD and IVOMD;

μ is the overall mean;

Ti is experimental diets;

Eij is the residual error.

The Least Significant Difference (LSD) was used to separate significant means.

Results

Chemical Composition of experimental diets

The chemical composition of experimental diets is shown in Table 3. Dry matter content of the experimental diets ranged from 909.9 g/kg in untreated rice straw to 919.7 g/kg in the combinations of the three browse leaves (LLSMCC). *Cajanus cajan* had the highest ($p<0.05$) crude protein value of 211.3 g/kg DM and untreated rice straw had the lowest ($p<0.05$) value of 87.3 g/kg DM (Table 3). Urea treated rice straw (URS) had the highest

TABLE 3

Chemical Composition (g/kg DM) of sole and combined browses and untreated rice straw

Treatments	DM g/kg	CP (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	Ash (g/kg DM)
CC	912.4^{a}	211.3^{e}	220.0^{ab}	607.9^{b}	134.6^{b}
LL	912.1^{a}	118.0^{b}	220.0^{ab}	658.6^{c}	134.5^{b}
SM	919.5^{c}	118.5^{b}	207.1^{a}	692.5^{d}	135.1^{b}
LLCC	912.6^{a}	132.8^{b}	224.2^{ab}	611.7^{b}	129.5^{a}
LLSM	915.8^{b}	129.3^{b}	216.1^{ab}	607.4^{b}	132.1^{b}
SMCC	918.7^{c}	175.7^{d}	217.4^{ab}	604.1^{b}	135.3^{b}
LLSMCC	919.7^{c}	158.1^{c}	202.6^{a}	591.5^{a}	132.8^{b}
URS	909.5^{a}	87.3^{a}	235.4^{b}	700.3^{e}	145.7^{c}
P value	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscript in a column are significantly different ($p<0.05$)

CC=*Cajanus cajan* LL= *Leuceana leucocephala*; SM= *Spondias mombin*; LLCC=*Leuceana leucocephala* and *Cajanus cajan*; LLSM= *Leuceana leucocephala* and *Spondias mombin*; SMCC= *Spondias mombin* and *Cajanus cajan*; LLSMCC= *Leuceana leucocephala*, *Spondias mombin* and *Cajanus cajan*; URS=Untreated Rice Straw (p<0.05) ash value of 145.7 g/kg DM and was significantly different (p<0.05) from the browse leaves. *Leuceana leucocephala* + *Cajanus cajan* (LLCC) had the lowest (p<0.05) ash value of 129.5 g/kg DM which diferred (p<0.05) from the other browse leave samples (Table 3). With ADF content, untreated rice straw recorded the highest (p<0.05) and *Leuceana leucocephala* + *Spondias mombin* + *Cajanus cajan* (LLSMCC) had the lowest (p<0.05) value of 202.6 g/kg DM which was not different (p<0.05) from SM (Table 3). Regarding NDF levels, *Leuceana leucocephala* + *Spondias mombin* + *Cajanus cajan* (LLSMCC) gave the lowest (p<0.05) value of 591.5 g/kg DM whilst untreated rice straw had the highest (p<0.05). However, NDF value of SM was higher (p<0.05) than LL which was different (p<0.05) from the other browse leave samples which did not differ (p<0.05) from each other (Table 3).

In Vitro Dry Matter Digestibility (IVDMD) and Organic matter digestibility (IVOMD)

The IVDMD and IVOMD results is presented in Table 4. Rice straw recorded the lowest (p<0.05) IVDMD and IVOMD (Table 4). In the case of the browse leaves, *Spondias mombin* + *Cajanus cajan* (SMCC) had the highest (p<0.05) and *Spondias mombin* had the lowest (p<0.05) value of 472.1 g/kg DM and 441.0 g/kg DM respectively for IVDMD. *Spondias mombin* had the highest (p<0.05) value of 483.4 g/kg DM for IVOMD and SMCC had the lowest (p<0.05) value of 446.3 g/kg DM (Table 4).

Discussion

The values of CP reported by Chidolue (1993), Addo-Kwafo (1996), Oji et al.(1998), Annan and Tuah (1999), Barnes (1999), Ahamefule et al. (1996) and Sarkwa et al. (2020; 2021) falls within the range of CP values obtained (118.0-211.3 g/kg DM) in the case of the individual samples. The mean CP values obtained for both sole and combined browses were lower than the mean CP value of 256.8 g/kg DM reported by Idan et al. (2023) who a used

TABLE 4

In vitro digestibility of three sole and combined browse leaves and rice straw

Treatments	IVDMD (g/kg DM)	IVOMD (g/kg DM)
CC	448.3 [b]	478.2 [ab]
LL	466.5 [b]	465.8 [a]
SM	441.0 [b]	483.4 [ab]
LLCC	466.3 [b]	460.1 [a]
LLSM	446.8 [b]	478.2 [ab]
SMCC	472.1 [b]	446.3 [a]
LLSMCC	461.9 [b]	469.6 [a]
Untreated rice straw	388.7 [a]	346.4 [b]
P value	<0.001	<0.001

Means with different superscript in a column are significantly different (p<0.05)

CC=*Cajanus cajan*; LL=*Leuceana leucocephala*; SM=*Spondias mombin*; LLCC=*Leuceana leucocephala* and *Cajanus cajan*; LLSM= *Leuceana leucocephala* and *Spondias mombin*; SMCC= *Spondias mombin* and *Cajanus cajan*; LLSMCC= *Leuceana leucocephala*, *Spondias mombin* and *Cajanus cajan*: P Value: Probability Value combination of *Leucaena leucocephala* and *Samanea saman*. However, the CP obtained in the present study was higher than that reported by Aganga and Mosase (2001) with the exception of *Leucaena capassa*. The CP values obtained from this study for the browse leave samples were within the minimum level (110-120g/kg DM) considered adequate for moderate level of production for ruminants (ARC, 1980).

Hence, the better digestibility of the browse leaves over the untreated rice straw. This may be due to the fact that, forages or feedstuff with less than 70 g/kg DM CP are usually poorly digested by ruminants due to insufficient nitrogen to stimulate rumen microbial function (Ahamefule et al., 2006). The crude protein values obtained indicate that the browses could be a good source of nitrogen supplement to improve protein content of crop residues used as basal diets for ruminants. The total ash content values obtained in this study was similar to the results reported by Sottie et al. (1998), Annan and Tuah (1999), Khanal and Subba (2001) and Sarkwa et al. (2020; 2021). The results of NDF and ADF reported by Addo-Kwafo (1996), Sottie et al. (1998) and Annan and Tuah (1999) validate the range of values obtained in this study. Most of the browses had moderate to high NDF values as a result, digestibility was not very high but moderate. This is because Norton (1994) reported that, tree forages with low NDF values (200-350 g/kg DM) are usually high to moderate in digestibility. However, the NDF values reported by Sarkwa et al. (2020) and Idan et al. (2023) were lower than the values recorded in this study and this is because they used browse leaves only. The ADF recorded in this current study was within the range reported by Sarkwa et al. (2020).The chemical composition (DM, CP, Ash, ADF and NDF) indicated slight difference between sole and combined browse leaves and this corroborate earlier report by Sarkwa et al. (2020) when the researchers used sole and combined browse leaves in the Coastal Savannah zone of Ghana. The values of digestibility, CP, ash, NDF and ADF of straw obtained agree with the values reported by Sottie et al. (1998), Bruno Soares et al. (2000) and Agbagla Dohnani et al. (2001). The study of Sottie et al. (1998) was carried out in the same agroecological zone and study site as the current study but the studies of Bruno Soares et al. (2000) and Agbagla Dohnani et al. (2001) were done in different countries. However, the CP and NDF values of rice straw in the current study were higher than the reports by Idan et al. (2023) and Sarkwa et al. (2021). The ash and ADF levels reported by Idan et al. (2023) and Sarkwa et al. (2021) were higher than the ash and ADF values recorded in this study. The difference in the values recorded in this study and the reported may be due to difference in the variety of rice from which the rice straws were obtained. It was deduced that straw is characterized with high cell wall fraction. This is because of the high values recorded for NDF. Neutral Detergent Fibre (NDF) is the structural component of the plant specifically cell wall and ash contents. High NDF values could be as a result of the age (maturity) of rice straw.

The *in vitro* dry matter digestibility results obtained falls between the range of 388.7 and 472.1 g/kg DM which were similar to reports by Addo-Kwafo (1996), Sottie et al. (1998), Khanal and Subba (2001) and Kamalak (2005). However, the *in vitro* dry matter digestibility values obtained in this study were lower than the value obtained by Apori et al. (1998) who reported values of 600.0 – 875g/kg DM. *In vitro* dry matter digestibility of both sole and combined browse leaves reported by Sarkwa et al. (2020) were higher than the values recorded in this current study. The addition of rice straw to the browse leaves in the current study may have contributed to this because rice straw alone had the lowest *in vitro* digestibility. Contrary to the report by Sarkwa et al. (2020), the range of values for the sole browse leaves in this study were lower than the range of values for the combinations of browse leaves. This may be due to the positive synergies formed by combining the browse leaves.

The range of values recorded in this study was similar to the range of values reported by Sarkwa et al. (2020). The values of *in vitro* organic matter digestibility obtained for the sole browse leaves in this study were slightly higher than the values for the combinations of the browse leaves and this is not in line with earlier report by Sarkwa et al. (2020). The differences or variations in the values obtained as compared with other reported works may be due to the moisture content, location, climatic conditions, age of forage at harvest and leaf to stem ratio of the plant fraction sampled.

Conclusion

Results obtained from this study indicate an increase in the digestibility of the basal diet from 388.7 g/kg DM to 472.1 g/kg DM as a result of the inclusion or supplementation of browse leaves, which in general decreased total crude fibre and ash and improved crude protein levels.

Basically, the combined browse leaves gave a slightly improved average *in vitro* dry matter digestibility and better chemical composition as compared with sole browse leaves meaning they would be a more suitable supplement for ruminants as compared to sole browse leaves. The combination of all three browses (LLSMCC) gave the most suitable digestibility and a relatively good chemical composition hence the best supplement in this study.

It is recommended that ruminant farmers should be given regular training or education on the use of browse leaves as supplements. Also, farmers when using browse leaves as supplements should opt for combinations rather than sole browse leaves because, combined browse leaves gave an improved digestibility and better chemical composition.

Ethical Approval

Ethical clearance was sought from Noguchi Institutional Animal Care and Use Committee of University of Ghana, Legon (Protocol number 2017-02-2R).

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