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Original Article

Chemical composition and microbial loads of varying mixture of *Lablab purpureus* and *Panicum maximum*

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Abstract
A study was conducted to investigate the nutrient contents and microbial loads of different mixtures of *Lablab purpureus* and *Panicum maximum.* The five (5) experimental diets formulated were 100% *P. maximum,* 70% *L. purpureus* + 30% *P. maximum,* 50% *L. purpureus* + 50% *P. maximum,* 30% *L. purpureus* + 70% *P. maximum* and 100% *L. purpureus*. Forage samples were harvested at 8 weeks of age after sowing and arranged in a completely randomized design. Results showed significant differences (P< 0.05) among the varying inclusion levels of *L. purpureus* and *P. maximum* on CP and NDF. Highest CP (19.08%) was recorded for 100% *L. purpureus* while the least value (10.07%) was observed for 100% *P. maximum.* The NDF ranged between 40.75 and 52.54% with 100% *L. purpureus* recording the least content. Phosphorus was not affected (P>0.05) by varied inclusion levels, however, calcium content decreased with increased levels of *P. maximum.* Highest (P<0.05) saponin content (0.42%) was recorded for 100% *L. purpureus* while the varied mixtures had similar values. The 100% *P. maximum* had the least content (0.08%) of saponin. Tannin was similar (P>0.05) across the diets. 100% *L. purpureus* had the highest (P<0.05) bacterial counts (3.48 x10^5 cfu/g) while the least values (2.86 and 2.68 x 10^5cfu/g) were similar for 30% *L. purpureus* + 70% *P. maximum* and 100% *P. maximum,* respectively. Grass-legume mixtures influenced chemical compositions and microbial loads, hence 30% *L. purpureus* + 70% *P. maximum* will make a good diet and enhance the performance of ruminants, if fed.

INTRODUCTION

Adequate nutrients in feed are one of the most important factors that determine profitability of any livestock venture (Alalade, 2012). Akinlade *et al*. (2005) reported that a major problem facing ruminant animal producer is how to feed the animals adequately all year round. Ruminant animals rely on forages for their nutrition than any other feed resources (Aderinola *et al*.,2007). Forage is the cheapest and major nutritional component

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in the diets of ruminant animals particularly in rural and sub-urban areas of the tropics (Alalade *et al*.,2019).

Panicum maximum also called guinea grass and tangayika grass (FAO,2003) is a highly productive, palatable, persistent and well acceptable forages for ruminants (Aganga and Tshwenyane, 2004). It can be fed to livestock solely or with legumes. The use of forage legume such as *Lablab purpureus* as feed supplements has shown to enhance the intake of poor roughages, improve growth rates and increase production to ameliorate feed constraints during dry season, especially for cattle and other ruminants (Alalade *et al*., 2017). *Lablab purpureus* is a climbing annual or short live perennial with long stems. It is a valuable feed resource for livestock production. It is a leguminous plant that can be grazed by both large and small ruminant animals (Muhammad, 2014). Lablab forage has been reported to be a promising crop for the Northern guinea savannah (Thomas and Sumberg, 1995). The forage analysis by Alalade *et al.* (2017) showed that it had high protein content (15-30%) as well as high level of mineral contents of phosphorus (0.37%), potassium (0.89%), calcium (90.26%), magnesium (0.29%), iron (192.60mg/kg), and zinc (55.28mg/kg). *Lablab purpureus* foliage can be recommended as a reliable leguminous plant supplement for ruminant animals, thereby alleviating the crisis of poor quality feed and feeding of ruminant animal. This study was therefore conducted to investigate the nutrients and microbial loads count of varied mixtures of *L. purpureus* and *P. maximum.*

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Teaching and Research Farm of Oyo State College of Agriculture and Technology, Igbo-Ora which is located within 7°15' North and 3°30'East of the equator with an average rainfall of 1278mm and average annual temperature of 27°C (Sanusi, 2011).

Collection of Samples

Forage samples of *Lablab purpureus* and *Panicum maximum* were harvested from an experimental plot at 8weeks after planting. Samples comprising of five varied mixtures of grass-legume are designated as follows:

100% *Panicum maximum*
70% *Lablab purpureus* +30% *Panicum maximum*
50% *Lablab purpureus* +50% *Panicum maximum*
30% *Lablab purpureus* +70% *Panicum maximum*
100% *Lablab purpureus*

Chemical Analysis and Microbial Load Determinations

Collected forage samples were oven dried at 60°C for 48hours and ground. The finely ground samples were analyzed for proximate compositions according to AOAC (2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the method of Van Soest *et al*. (1991). The mineral contents (K, Ca, Mg, Fe, Zn and Cu) were determined using atomic absorption spectrophotometer while phosphorus was determined with Vonadomehydrate spectrometer method of AOAC (1995). Samples were also analyzed for microbial load using standard procedures of Taylor *et al*. (1997).

Quantitative Determination of Anti-Nutritional Factors

Oxalic acid content was estimated quantitatively by redox titration with standard potassium permanganate according to the procedure of Day and Underwood (1986). Saponin content was determined using the method similar to that of Hudson and Ei-Difrawi (1979), while tannic acid content was determined in accordance with the procedure of AOAC (1990).

Statistical Analysis

Data obtained were subjected to one-way Analysis of Variance (ANOVA) procedure and the treatment means were separated using Duncan's multiple range tests at the probability of 5% of SAS (2000).

RESULTS

The proximate compositions and fiber fraction percentages of varied mixtures of grass-legume are presented in Table 1. There were significant (P<0-05) differences among the five samples in their chemical compositions. The dry matter contents of the different mixtures of *P. maximum* and *L. purpureus* ranged from42.85 to52.35%. Highest significant (P<0.05) values were observed for 100% *L. purpureus* for crude protein (19.08%), ash (9.90%) and ether extract (2.00%).

Table1: Chemical compositions of varied mixtures of *Lablab purpureus* and *Panicum maximum*

Parameter	100%Lab	70%Lab + 30%P.max	50%Lab + 50%P.max	30%Lab + 70%P.max	100%P.max	SEM
DM	42.55^{e}	44.14^{d}	47.50^{b}	52.35^{a}	45.87^{c}	0.88
CP	19.08^{a}	16.51^{b}	15.21^{b}	13.61^{c}	10.07^{c}	0.80
Ash	9.90^{a}	9.07^{d}	9.30^{cd}	9.53^{bc}	9.78	0.49
EE	2.00	2.12	1.70	1.53	1.30	0.08
CF	14.47^{d}	17.33^{c}	20.08^{b}	22.57^{b}	25.74^{a}	1.31
NDF	40.75^{d}	45.20^{e}	49.30^{c}	50.23^{b}	52.54^{a}	1.41
ADF	33.28^{d}	31.20^{e}	33.80^{c}	35.20^{b}	40.14^{a}	1.12
ADL	10.36^{d}	10.80^{d}	11.37^{c}	12.77^{b}	14.23^{a}	0.82

$^{abcde:}$ Means along the same row with different superscripts differ significantly (P<0.05)

Table 2 shows the effect of forage mixture on the mineral compositions of the varied samples. Highest significant (P<0.05) values were observed for mineral contents of Mg (0.66%), Ca (0.26%), Fe (119.25mg/kg), Zn (38.27mg/kg) and Cu(6.14mg/kg) but least values were

Table 2: Mineral compositions of varied mixtures of *Lablab purpureus* and *Panicum maximum*

Parameter	100%Lab	70%Lab + 30%P.max	50%Lab + 50%P.max	30%Lab + 70% P.M.	100%P.max	SEM
P (%)	0.32	0.32	0.31	0.27	0.27	0.01
K (%)	0.26	0.26	0.26	0.24	0.24	0.01
Mg (%)	0.66^{a}	0.62^{ab}	0.51^{bc}	0.47^{c}	0.48^{c}	0.02
Ca (%)	0.26^{a}	0.25^{ab}	0.24^{ab}	0.22^{b}	0.22^{b}	0.00
Fe (mg/kg)	119.24^{a}	111.03^{b}	102.87^{c}	97.21^{d}	91.86^{e}	2.60
Zn (mg/kg)	38.27^{a}	36.21^{b}	30.25^{c}	26.71^{d}	24.84^{e}	1.39
Cu (mg/kg)	6.14^{a}	5.55^{b}	4.28^{c}	3.97^{d}	3.76^{e}	0.25

$^{abcde:}$ Means along the same row with different superscripts differ significantly (P<0.05) obtained in the sole *P. maximum* for all mineral contents.

Results of the anti-nutrients and microbial load counts at varied grass-legume mixtures are presented in Table3.Significant (P<0.05) differences were observed among the five treatments in terms of anti-nutrients and microbial load counts. Highest values were obtained at 100% *L. purpureus* for saponin (0.42%), tannin (0.37%), oxalate (0.70%), fungi (2.47x10^{3}cfu/g), bacteria (3.48x10^{5}cfu/g) and protozoa (2.04x10^{3}cfu/g).

good shelf-life of leaves during the drying process (Alalade *et al.,* 2019). The percentage crude protein contents, ranging from 10.07 - 19.08%, were recorded for different inclusion levels of *L. purpureus* and *P. maximum*. The CP contents of all treatments were high as they were well above the 8%CP requirement for ruminant animals which will provide ammonia requirement for the rumen microbial activity (Norton, 2003). The Lablab foliage could serve as potential protein supplement which enhances the feed intake and utilization of low quality grass and fibrous crop residues by ruminants. Since the importance of protein to animal and human health cannot be over emphasized, *L. purpureus* foliage could, therefore, be used as feed protein supplements. This agreed with the submission of Alalade *et al.* (2017) that *L. purpureus* foliage had better nutrient values due to high content of protein and minerals with relatively low levels of anti-nutrient factors which were below the lethal point in ruminant animals.

Table 3: Anti-nutritional Factors (%) and Microbial count load of varied mixtures of *Lablab purpureus* and *Panicum maximum*

Parameter	100%Lab	70%Lab + 30%P.max	50%Lab + 50%P.max	30%Lab + 70%P.max	100%P.max	SEM
Saponin	0.42^a	0.13^b	0.16^b	0.14^b	0.08^c	0.03
Tannin	0.37	0.10	0.27	0.17	0.18	0.03
Oxalate	0.70^a	0.52^b	0.52^b	0.53^b	0.42^c	0.03
Fungi(x10^{3}cfu/g)	2.47^b	2.36^b	2.23^b	2.17^c	5.23^a	0.14
Bacterial(x10^{5}cfu/g)	3.48^a	3.37^b	3.21^c	2.86^d	2.68^d	0.17
Protozoa (x10^{3}cfu/g)	2.05^a	1.92^{bc}	1.81^d	1.71^c	1.68^d	0.13

$^{abc:}$ Means along the same row with different superscripts differ significantly (P<0.05)

DISCUSSION

Proximate compositions and fiber fractions of varied mixtures of legume-grass samples

The significant high dry matter contents observed in this study was in consonance with the report of Oyewole and Aderinola (2022) for mixtures of *P. maximum* with either *Stylosanthes hamata* or *S. guianensis.* The higher dry matter values for grass-legume mixtures could be averred to higher dry matter percentage in *P. maximum*. Thus, the increase in the inclusion levels of *P. maximum* in the grass-legume mixture diets had significant influence on their dry matter. Also, high dry matter of the diets in this study suggested

The ash contents of the treatments at varied inclusion levels of *L. purpureus* and *P. maximum* ranged from 9.06- 9.90%. The values obtained were in line with range values of 9.65- 9.87% reported for different cultivars of *L. purpureus* leaf by Alalade *et al.* (2017). The highest values of 9.90% ash content in the present study was above the 5.08% recorded for *Psophocarpus tetragonolobus* leaf (Alalade *et al*., 2016) and also higher than the 3.70% reported for *Mucuna utilis* leaf (Ujowundu *et al.*, 2010). The values obtained in this present study fell within the range (3.00-9.65%) reported by Okoli *et al.* (2001). The proportion of ash content present is a reflection of mineral contents present in the feed material (Omotosho, 2006). This suggests that these feed samples could be a better source of essential valuable and useful minerals needed by ruminant, for good body development.

The ether extract value, which represented crude fat, was higher in forage samples. However, it showed that they contained adequate crude fat that will satisfy the energy requirement of ruminant animals for productive purpose. Feed stuffs having a crude fat value of 1-2% have been found sufficient to maintain good health in ruminant by reducing the risk of disease and ageing caused by its excess consumption (Sodamade *et al.*, 2013).

The crude fiber contents of the treatments ranged from 14.70 to 25.74%. The values recorded for each sample showed that the higher the percentage of *P. maximum* in the inclusion level, the higher the values recorded for crude fiber content. The fiber contents for 30% *Lablab*+70% *P. maximum* and 100% *P. maximum* were above the 15-20% recommended for improved intake and production in finishing ruminants (Buxton, 1996). The higher crude fiber content values will aid digestion and absorption of water which is in favour of ruminant animal (Ayoola and Adeyeye, 2000). The NDF concentration of the treatment sample rangedfrom40.75 and 52.54%. Increase inclusion level of *P. maximum* in the samples resulted I higher values of NDF concentration. The NDF concentrations of the different varied mixtures of legume-grass were below 60% which has been reported as the threshold level of NDF in tropical plants beyond which feed intake of ruminants is affected (Meissner *et al.,* 1991). The NDF content is the best indicator to predict fodder quality as it is related to the intake potential of the fodder. Low energy value and the low NDF contents of all treatment samples, suggested it contained potential and important available fermentable carbohydrate in ruminant feeds. ADF concentrations ranged from 31.20 to 40.14%. Similar results were observed for ADF as levels of *P. maximum* were increased, the ADF contents increased. These values agree with the values reported by Olajumoke (2003) that 30.6-40.20% was recorded for ADF on elephant grass at age of 4-8weeks. Forage with high ADF value is classified as low quality roughage (Rusdy, 2016). According to Kellems and Church (1998), roughage with less than 40% ADF is categorized as high quality and those with greater than 40% as poor quality. Based on this assertion, the varied mixtures of grass-legume and sole samples used in this study can be classified as high quality forage because their ADF values were below 40%.

Mineral composition of varying mixture of legume-grass samples

The mineral contents of all treatment samples gave idea of their possibility of being used as complete diets for ruminant animals. The phosphorus contents of all treatments ranged from0.27 and 0.33% and were higher when compared with NRC recommended values of 0.15% for phosphorus (NRC,1985). The phosphorus contents in this study for all the diets were above 0.2% recommended to satisfy livestock dietary maintenance (NRC, 1985). Therefore, all the formulated samples in this study will be adequate for ruminant production without supplementation. The values of potassium (K) in the experimental samples ranged from 0.24 and 0.26%. The levels of K in the treatments were higher than 0.18% recommended for grazing animals (Mc Dowell, 1985). The values of Magnesium (Mg) in the experimental treatments ranged from 0.47- 0.66%. Magnesium contents found in this present study were above the 0.12 – 0.20% requirement of ruminant's diet as recommended by NRC (1985). Magnesium is an important mineral element in connection with its role in circulatory disease such as ischemic heart disease and calcium metabolism (Hassan and Umar, 2006). The Calcium concentration in the treatment samples ranged from 0.22 to 0.26% and were higher than the 0.09% observed for *Canavalia ensiformis* leaves by Akinlade *et al* (2007). The Ca levels observed in this study were inadequate for optimum performance of ruminants. The Ca values across treatments did not meet the theoretical Ca requirement of 0.30% needed for all forms of production in ruminants (ARC, 1980).

All the samples analyzed had sufficient Fe levels. As the percentage of *P. maximum* increased in the sample, Fe concentration decreased. The values in this study ranged from91.86 and119.24mg/kg, although, its availability could vary due to the fact that Fe is absorbed according to the need and thus its absorption would depend on dietary factors, age of the animal and body Fe status (Khan *et al*., 2000). It is also above the critical level of Fe in animal tissue (30-50mg/kg) as reported by Mc Dowell (1985). This implies that the samples are good source of dietary iron and it is said to be an important element in the diet of pregnant animals, nursing animals and infants (Oluyemi *et al.,* 2006). The concentrations of copper in the treatment samples ranged from 3.76 and 6.14mg/kg which were below the dietary requirement of ruminant for Cu. This is because values obtained were lower than 8-14 mg/kg reported by Khan *et al.* (2006) as dietary requirement for ruminant animals. This could be linked to mineral interactions which influence their concentrations. Davis and Mertz (1987) reported that high concentrations of iron and zinc also reduce copper contents status and may increase copper requirement. Inclusion of these treatment samples which is higher in Copper serves as an essential micro nutrient for haematological and neurological system (Tan *et al*., 2006) and clinical deficiencies associated with copper such as anaemia, bone disorder and abnormal growth of hair, fur or wool, impaired body growth and reproductive system are better prevented (Gardea-Torresday *et al.,*1990). Zinc values observed in this study ranged from 24.85-38.27mg/kg. The Zn concentrations in the diets decreased as grass percentages increased. Forage Zn concentration also fell within the range of requirement for ruminants as reported by Reuter and Robinson (1997). It has been suggested that 30mg/kg Zn is a critical dietary level, although it has been recommended by NRC (1985) that concentrations of 12-20mg/kg are adequate for growing ruminants. Therefore, ruminants fed these diets will probably have access to adequate Zn concentration for enzyme production.

Anti-nutrient contents present in grass-legume mixtures and sole forages

The level of saponin obtained in this study for all treatments were lower compared with the range (0.44%-0.73%) reported for three cultivars of *Lablab purpureus* foliage in Igbo-Ora, Oyo State, Nigeria (Alalade *et al.,*2017). The variation could be averred to age at harvest (Oyewole, 2021), mixing ration for grass-legume and chemical differences based on cultivar. Cultivars have different chemical compositions. The levels of saponin reported in this work were low and may not likely pose threat to ruminant nutrition as reported by Lu and Jorgensen (1987). Saponin causes blood reduction, ulcer, decrease of growth rate, bloat (ruminant) and reduction in nutrient absorption (Alalade *et al.,* 2017).

The percentages of tannin in this present study were between 0.17 and 0.37% and were lower than 5% level which goat may reject (McLeod,1974). Feeding forage with high level of tannin to ruminants may impair its intake and apparent digestion (Bamikole *et al.,* 2004). Therefore, feeding formulated diets in this study to ruminant animals will not likely interfere with intake and protein digestion

Oxalate contents (0.42-0.70%) in this present study were low but higher than the 0.40- 0.53% reported for Lablab leaf by Alalade *et al.* (2017). The oxalate concentrations in this present study were lower than the reported values of 1.49-5.79% of some browse plants relished by ruminants in Nigeria (Fadiyimu *et al.,* 2011). The differences between this report and other studies could be associated with age at harvest and mixing ration. It was reported that ruminant can consume certain quantities of feeds within a high level of oxalate without any deleterious effects (Oke, 1969). Hence, the concentrations of the oxalate inherent in these treatments should not pose any negative effects on the animals that may consume them.

Microbial load counts of varied mixture of Lablab with Panicum maximum

Several studies (Gordon and Phillips, 1985; Soetanto, 1985; Akin, 1989) have indicated that a greater fungal population is associated with a greater degradability of the fibrous fraction of the diet. Higher fungi count recorded for 100%*P. maximum* sample negated the observation of the stated authors. The 100%*P. maximum* had significant highest fibre content and fibre fractions. However, their assertions could be supported by lowest concentration of saponinin100%*P. maximum*. Saponin inhibits microbial fermentation and synthesis in the rumen (Shimelis, 2013). Significant lower saponin concentration in 100%*P. maximum* could have increased its fungi counts. Akin *et al.* (1983) stated that fungi appear to be the first organisms to invade and commence digesting the structural plant components, beginning from the inside which reduces the tensile strength of the particles and thus increases particle breakdown in rumination for bacteria colonization of the cell material. Therefore, diets with mixture of *P. maximum* will likely degrade efficiently in the rumen. As diet samples increased in the leguminous forage, bacteria count values also increased. This implied that increase in inclusion levels of legume consequently proliferate bacteria populations. Higher nutrient, energy and protein promote bacterial growth (Adebayo *et al.,* 2017). This could be responsible for highest concentration of bacteria counts recorded for 100% *Lablab purpureus*. Also, protozoa value increase as leguminous plant inclusion level increases, this might be due to low level of saponin reported in this study. The level of saponin in the forage legume may have contributed to reduced protozoa numbers in the rumen fluid (Navas *et al.*, 1993).

CONCLUSION

The 100% *L. purpureus* had highest crude protein, saponin, bacterial counts but least neutral detergent fibre (NDF). The phosphorus concentration was not influenced by varied mixture levels. Calcium contents decreased with increasing level of *P. maximum.* The 100% *P. maximum* had the least content of saponin. The least bacterial count was similar for 30% *L. purpureus* + 70% *P. maximum* and 100% *P. maximum*. Therefore, grass-legume mixtures influenced chemical concentrations and bacterial counts; hence mixture of 30% *L. purpureus* + 70% *P. maximum* will make a good diet and enhance the performance of ruminants, if fed.

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