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Growth performance, blood profile and serum metabolites of West African dwarf growing rams fed guinea grass supplemented with differently processed pigeon pea leaves

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Target Audience: *Smallholder Ruminant Farmers, Modern Intensive Ruminant Animal Producers and Animal Scientists*

Abstract

A 56-day trial was conducted to investigate the performance and blood profile of West African Dwarf growing rams fed Panicum maximum with differently processed Pigeon pea leaves, twigs, and petioles (PPL). A total of sixteen (16) rams were randomly allotted to four treatments (T_1 - 100% P. maximum solely), T_2 - 70% P. maximum and 30% fresh PPL), T_3 - 70% P. maximum and 30% wilted PPL and T_4 - 70% P. maximum and 30% dried PPL) with four animals per treatment in a completely randomized design (CRD). Growth performance and blood analysis parameters such as initial and final weight, feed intake and leftover, packed cell volume, hemoglobin, total protein, albumin, glucose were evaluated. There were significant variation ($P<0.05$) in all the evaluated parameters. Fresh PPL recorded the highest crude protein (CP) (22.31%), followed by wilted (21.20 %), and the least (5.30 %) was obtained in P. maximum. The phytate contents of fresh, wilted and dried PPL were 1.29%, 1.28% and 1.26% respectively while tannin contents were 1.00, 0.85 and 0.79mg/100gm in fresh, wilted and dried PPL respectively. The feed intake (5.22kg) of each ram fed the T_1 diet was higher ($P<0.05$) than those of T_2 (3.50kg), T_3 (5.05kg) and T_4 (4.61kg). Average, daily and weekly weight gains varied ($P<0.05$) in the diets in $T_3>T_4>T_2>T_1$ order, while feed conversion ratio ranged between 3.48 (T_3) to 7.35 (T_1). The packed cell volume (PCV) and haemoglobin (Hb) ranged from 36.00% in T_4 to 38.00% in T_1 and 12.50g/dl in T_4 to 12.40g/dl in T_1 respectively. T_3 diet was observed to have the highest total protein (7.00 g/dl), albumin (3.30 g/dl) and globulin (4.40 g/dl) values. From the results of a significant number of evaluated parameters, T_3 proved to be the best diet of this study. However, it was concluded that the supplementation of P. maximum with either fresh, wilted or dried PPL would increase the productivity of growing rams.

Keywords: WAD rams; *Cajanus cajan*; *Panicum maximum*; haematology; serum metabolites.

Description of the Problem

Ruminants constitute a very important part of the livestock sub-sector of the Nigerian agricultural economy with its potential in alleviating the low animal protein intake has been reported (1). In Nigeria, sheep and goat rearing serve as a source of income generation for the farmers, sacrifice, festival ceremonies, household consumption and security against crop failure (2). However, the inability of ruminant farmers to feed their animals with high-quality forages throughout the year

remains one of the major challenges facing their productivity. Nigeria as a tropical country, the main feed resources for livestock are natural pastures consisting of grasses, legumes and browse tree species. Although, grasses such as guinea grass (*Panicum maximum*) can yield as much as/or more than fodder trees and shrubs during the rainy season, and their productivity and nutritive value decline sharply during the dry season (3). Alternatively, introduction and successive utilization of improved browse plants with adaptable, high yielding ability and better nutritional values than the forages has solved the feed shortage and increased livestock production and profitability (4). Tropical browse plants, such as pigeon pea (*Cajanus cajan*), are multipurpose nitrogen fixing crop (5). Pigeon pea hay, either dried or wilted, is an effective substitute for more expensive industrial concentrates for ruminants (6). The significance of determining haematological and serum biochemical indices of domesticated animals have been well documented (7). Nutrition, breed, sex, age, reproductive status, environmental factors, and stress are known to affect both haematological and serum biochemical parameters (8). Though, (9) reported that processed feed can have an effect on farm animals'blood parameters, but, there is little or no information on differently processed pigeon pea leaves as it affects growth performance and blood profile of growing ram. Hence, this study was designed to evaluate the effect of differently processed pigeon pea leaves on growth performance and blood profile of growing WAD rams fed guinea grass basal diets.

Materials and Methods

Experimental site

The experiment was carried out at the Sheep and Goat Unit, Teaching and Research Farm, Oyo State College of Agriculture and Technology, Igbo-Ora, Oyo State, Nigeria. The site lies on latitude 7˚ 434' North and longitude 3˚ 285' East with an annual average rainfall of 1278mm and an average temperature of 27˚C (10).

Experimental animals

A total of 16 growing West African Dwarf rams of 6-9months old, with an average weight of 7.00 – 12.00kg were separated from the other animals in the Sheep and Goat Unit of the Research Farm for the experiment purpose. The animals were housed intensively in a well-ventilated and disinfected experimental pen. The animals were acclimatized for two weeks prior to the commencement of the feeding trial, they were treated against ectoparasites and endoparasites with ivermectin solution (1mL/5kg body weight) injected subcutaneously.

Harvesting and processing of experimental diets, and feeding

Fresh *P. maximum* was daily cut (zero grazings) with a cutlass at 15cm above ground level and subsequently chopped manually into 3-5cm length before feeding. Leaves, twigs, and petioles of five-month-old flowering *Cajanus cajan* (termed as Pigeon pea leaves-PPL) were harvested at a height of 30 cm and classified into three forms through processing into fresh, wilted and dried forms. Fresh forms were usually harvested daily and served immediately to the rams. Wilted PPL was usually harvested between 1500-1600 hours and kept in a wooden shed till the following morning (700 hour), that is, the feeding day. The dried PPL was prepared through cutting and sun-drying for 4-5days by spreading on the concrete floor with periodic turning to facilitated uniform drying until it could be hand-crushed, packed in sacks, and stored in the air-ventilated room for safe storage before feeding. Each group of animals was assigned to an experimental diet, with each diet amounting to 1kg depending on the proportion

in the treatment while freshwater was supplied *ad libitum*. The experiment lasted for 56 days after two weeks of acclimatization.

Chemical analyses of the experimental diets

Samples were taken from the prepared experimental diets and analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), and ash according to the procedure of (11). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were determined using the method of (12). Oxalate, saponin and tannin were determined using the methods described by (13), while phytate and trypsin inhibitor were determined using the method of (14) and (15) respectively.

Experimental layout and design

The animals were randomly allotted by weight into four treatments with four rams as four replicates per treatment arranged in a completely randomized design (CRD). The experimental diets were: T_1 (100% *P. maximum* solely), T_2 (70% *P. maximum* and 30% fresh PPL), T_3 (70% *P. maximum* and 30% wilted PPL) and T_4 (70% *P. maximum* and 30% dried PPL).

Data collection and analyses

At the beginning of the experiment, the rams were initially weighed and subsequently on a weekly basis prior to feeding in the morning. The initial live weight was subtracted from the final live weight to determine the weight gained by the animals.

The weight of feed offered minus the weight of remnants were daily noted to determine the daily feed intake of the animals, while Feed Conversion Ratio (FCR) was estimated as feed intake divided by body weight gain.

At the end of the 56-day trial (on 57^{th} day), 5ml of the blood sample was drawn from three randomly selected rams per treatment via the jugular vein using 10ml sterile disposable needle and syringe. 2.5ml of the blood sample collection was released into a sample bottle containing anticoagulant and it was rocked gently to ensure easy mixing of the blood with the anticoagulant. Thereafter, haematological parameters were determined in the laboratory according to the method described by (16). The remaining 2.5ml blood sample was poured into a plain sample bottle without anticoagulant and allowed to clot at room temperature within 3hours of collection for serum harvest. The total serum protein, albumin, and creatinine were determined with the methods of (17), (18) and (19) respectively, while serum enzymes (alanine aminotransferase and aspartate aminotransferase) were evaluated using the procedure of (20). Globulin was calculated by subtraction of albumin from total serum protein.

Statistical analysis

Data obtained were subjected to one-way analysis of variance using SAS software (21), while mean value variance showing significant ($p<0.05$) difference were separated using Duncan's Multiple Range Test (22). The statistical model was:

$$\boldsymbol{Y_{ij} = \mu + T_i + e_{ij}}$$

Where: Y_{ij}= Variable of measurement; μ = overall mean; T_i = effect of i^{th} treatment diet; e_{ij} = random residual error.

Results and Discussion

Chemical composition of diets

Table 1 presents the chemical composition of the experimental diets. The dry matter (DM) ranged from 32.80% in *P. maximum* to 91.48% in dried PPL. Higher DM values observed in dried and wilted PPL compared to low DM in fresh *C. cajan* and *P. maximum* suggest processing before feeding or storage could enhance forage DM. Fresh PPL recorded the highest CP (22.31%) while the

least (5.30%) was obtained in *P. maximum*. The study *P. maximum* CP was lesser than 8.5% reported for the same forage crop by (23), however, the disparity might be attributed to the difference in the growth stage, the maturity of the plants as well as the soil types and nutrients. The CP contents of the fresh and processed PPL were beyond the 8% required to satisfy ruminants' maintenance requirement (24), and the minimum level necessary to provide sufficient nitrogen required by rumen micro-organisms to support optimum rumen activity (25). The higher CP of the PPL irrespective of the processing forms compared with *P.* maximum corroborates the submission of (26) which stated that browse forages are higher in CP than tropical grasses and roughages such as hay, straw and stover. CP of the fresh (22.31%), wilted (21.20%), but not dried (19.00%) PPL of this experiment were higher than the 19.8% reported by (29).

Panicum maximum has the least CF (22.20%) while dried PPL contains 27.12%. According to (27), a high fibre content in forages is the primary factor limiting feed intake. The CF content of the PPL was in agreement with the earlier report of (28, 29) and could be due to the presence of twigs, petioles and petiolules as harvested.

Ash content ranged from 3.30% - 9.45% with the least and highest value observed in *P. maximum* and dried PPL respectively.

Table 1: Chemical composition (%DM) of fresh, wilted, and dried pigeon pea leaves and *Panicum maximum*

Parameters	Fresh PPL	Wilted PPL	Dried PPL	*P. maximum*
DM	44.50	86.60	91.48	32.80
CP	22.31	21.20	19.00	5.30
CF	25.26	26.86	27.12	32.20
EE	4.79	4.64	4.50	2.90
ASH	9.12	9.32	9.45	3.30
NDF	33.20	33.52	33.83	51.00
ADF	29.50	29.47	29.40	31.00
Lignin	10.12	11.01	12.40	13.47

PPL= Pigeon pea leaves, twigs and petioles, DM=Dry matter, CP=Crude protein, CF=Crude fibre, EE=Ether extract, NDF= Neutral detergent fibre, ADF=Acid detergent fibre, NFE=Nitrogen free extract and ME= Metabolizable energy.

Anti-nutrients in the pigeon pea leaves

Table 2 shows the anti-nutrients in fresh and processed pigeon pea leaves (PPL). Anti-nutritional factors reduced animal productivity and caused toxicity during forage scarcity as well as in confinement when the feed rich in these substances is consumed by animals in large quantities (30). The phytate contents of fresh, wilted and dried PPL were 1.29%, 1.28% and 1.26% respectively. High level of phytate recorded for the fresh PPL (1.29%) is lower than the recommended 2.5% for small ruminants (31). As phytate in feed binds essential mineral nutrients in the digestive tract and can result in mineral deficiencies (32), these results suggest the PPL as a safe feed for the rams.

The tannin content observed were 1.00, 0.85, and 0.79mg/100gm in fresh, wilted and dried PPL respectively. A high tannin value above 3% do leads to reduced appetite, bind dietary protein and digestive enzymes to form complexes that are not readily digestible (33), reduced palatability and growth rate (34), decrease in degradation rate of degradable matters in the rumen, depressive effect on the

intestinal activity of pancreatic enzymes and hindered amino acid absorption from the intestine (35). The threshold values obtained in this study further uphold these diets as being safe for ruminants.

Table 2: Anti-nutrients of fresh, wilted, and dried pigeon pea leaves

PPL	% Phytate	% Saponin	% Oxalate	Tannin (mg/100g)	% Trypsin
Fresh	1.29	0.34	0.59	1.00	3.17
Wilted	1.28	0.33	0.56	0.85	3.01
Dried	1.26	0.29	0.48	0.79	2.92

PPL= Pigeon pea leaves, twigs and petioles

Growth performance of the West African Dwarf rams

Table 3 indicates the performance characteristics of growing West African Dwarf (WAD) rams fed *P. maximum* supplemented with differently processed pigeon pea leaves. The feed intake (5.22kg) of each ram fed the basal diet only (T_1) was higher ($P<0.05$) than that fed T_2 (3.50kg), T_3 (5.05kg) and T_4 (4.61kg) PPL. Animals fed T_1 diet consumed more as they sought to meet their requirement through the intake of relatively more grass than the other treatments, which had an alternative source of feed, legume supplements. Supplementation of guinea grass with wilted PPL increased voluntary feed intake of the rams in T_3 as compared with T_2 and T_4 diets, thus suggesting a better palatability and nutritive value of the diet which enhanced its acceptability and consumption. The improved feed intake for wilted PPL supplemented diet could have resulted due to faster rumen outflow and the provision of more degradable organic matter (36). The low feed intake in rams on T_2 could be attributed to the higher anti-nutritional factors in the fresh PPL supplemented diet.

Average, daily and weekly weight gains significantly varied ($P<0.05$) in the diets in $T_3>T_4>T_2>T_1$ order. The observed variation could be that T_3 and T_4 contain a balanced nutrient which efficiency interact to give a better daily weight gain. The utilization efficiency of a diet is largely determined by the relative balances of glycogenic energy, long-chain fatty acids and essential amino acids absorbed by the animals (37).

The observed feed conversion ratio values were better than the values (8.01 – 65.13) reported by (38) when corn bran diets supplemented with protein were fed to West African Dwarf sheep. There was an improvement in feed utilization of other treatments compared with that of control diet (T_1). The result showed that T_3 had the best feed utilization of 3.48, while the poorest value (7.35) was observed in T_1.

Table 3: Growth performance of West African Dwarf growing rams fed *Panicum maximum* supplemented with differently processed pigeon pea leaves

Growth indices	T_1	T_2	T_3	T_4	SEM±
Initial weight (kg)	9.79^{bc}	9.80^{bc}	11.15^{a}	10.30^{ab}	0.27
Final weight (kg)	10.50^{bc}	10.80^{bc}	12.60^{a}	11.40^{b}	0.40
Average weight gain (kg/day)	0.71^{cd}	1.00^{c}	1.45^{a}	1.10^{b}	0.13
Daily weight gain (g/day)	12.68^{d}	17.86^{c}	25.89^{a}	19.64^{b}	2.36
Weekly weight gain (g/wk)	101.42^{d}	142.86^{c}	207.14^{a}	157.14^{b}	18.8
Dry matter intake	5.22^{a}	3.50^{d}	5.05^{ab}	4.61^{bc}	0.34
Feed Conversion Ratio	7.35^{a}	3.50^{bc}	3.48^{bc}	4.19^{b}	0.79

^{abc}: means on the same row with different superscripts are significantly different ($p<0.05$); T_1= 100% P. maximum solely; T_2 = 70% Panicum maximum + 30% fresh pigeon pea leaves (PPL); T_3 = 70% Panicum maximum + 30% wilted pigeon pea leaves (PPL); T_4 = 70% Panicum maximum + 30% dried pigeon pea leaves (PPL).

Haematological profile of the West African Dwarf rams

Table 4 shows the haematological parameters of WAD growing rams fed *P. maximum* supplemented with differently processed pigeon pea leaves. There were significant variations ($P<0.05$) in the parameters measured. The packed cell volume (PCV) ranged from 36.00% in T_4 to 38.00% in T_1, and haemoglobin (Hb) values ranged from 12.50g/dl in T_4 to 12.40g/dl in T_1.

The values recorded for PCV and Hb were within the ranges of 24-45% and 8-16g/dl respectively reported for growing sheep by (39), thus, indicated that the diets had no adverse effects on the cells. Haemoglobin function as a carrier of oxygen to target organs by forming oxy-haemoglobin, hence, animals on all the treatment diets were well nourished (40).

The observed red blood cell (RBC) values were less than the 8.23 - 9.42×10^6/mL reported for similar animals (41), but, within the normal range of 5.00 -11.00×10^6/mL for sheep (39). This suggested that animals fed fresh and processed *Cajanus cajan* leaves supplemented diets could maintain an active immune system that defend their body against infection, allergic reactions, and parasites.

Table 4: Haematological parameters of West African Dwarf growing rams fed *Panicum maximum* supplemented with differently processed pigeon pea leaves

Parameters	T_1	T_2	T_3	T_4	SEM±
PCV (%)	38.00^{a}	37.00^{b}	37.00^{b}	36.00^{c}	3.04
Hb (g/dl)	12.50^{a}	12.30^{c}	12.40^{b}	12.20^{d}	0.06
RBC ($\times10^6$/mL)	6.24^{a}	6.21^{b}	6.19^{c}	6.15^{d}	1.06
WBC ($\times10^3$/mL)	6.55^{b}	6.20^{c}	5.15^{d}	7.40^{a}	2.03
Lymphocyte (%)	65.00^{c}	68.00^{b}	64.00^{d}	71.00^{a}	1.37
Neutrophils (%)	31.00^{a}	26.00^{c}	29.00^{b}	21.00^{d}	1.88

^{abcd}: Means on the same row with different superscripts are significantly different ($p<0.05$); T_1= 100% P. maximum solely; T_2 = 70% Panicum maximum + 30% fresh pigeon pea leaves (PPL); T_3 = 70% Panicum maximum + 30% wilted pigeon pea leaves (PPL); T_4 = 70% Panicum maximum + 30% dried pigeon pea leaves (PPL).

Serum metabolites of the West African Dwarf rams

Serum biochemical analysis is used to determine liver damage as well as to evaluate protein quality and amino acid requirement in animals as reported by (42). Serum biochemistry of WAD growing rams fed experimental diet were presented in Table 5. There were significant differences (P<0.05) in the serum parameters values across the dietary treatments. The observed total protein values agrees with the report of (43) which stated that the total protein in an animal is a reflection of the level of dietary protein and the state of health of the animals.

The range of values (2.70-3.30 g/dL) obtained for albumin were similar to those reported by (40). The higher the value of albumin, the higher the clotting ability of blood, hence, prevents haemorrhage (44). The observed globulin values of rams fed T_2 and T_4 (3.90 g/dL) and T_3 (4.40 g/dL) diets were within the range of values (3.40-5.57 g/dL) reported for healthy goats (45), thus, suggests that the diets didn't pose any adverse health challenge to the rams.

Serum glucose is an indicator of carbohydrate metabolism in high energy diets, and when lower or higher than the normal range results in hypoglycemia and hyperglycemia respectively (46). The observed glucose values were within the range of normal blood glucose content for small ruminants, and indicated that enough energy was made available to the rams. However, the observed variation in the experimental diets' serum glucose could inferred that efficiency of carbohydrate metabolism in these rams was not affected by the supplementation of different forms of PPL with *P. maximum*.

Creatinine significantly (P<0.05) ranged from 1.60g/dL in T_1 to 2.30g/dL in T_3. The creatinine level in serum has a positive correlation with muscle mass and kidney function in animals (47), thus, suggesting the significant weight gain in the experimental rams.

The observed values for ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) were significantly different (P<0.05) among animals in all treatments. The highest AST mean value of 42.00µ/L was observed in rams fed with T_1 and T_3, and the lowest mean value (35.00µL) in rams on T_4 diet. It can, therefore, be inferred that drying method affected PPL in T_4 diet, which in turn lowered the AST without any liver impairment. However, rams fed T_2 diet were observed to have the highest ALT mean values of 58.00µL while those fed T_4 diets had the lowest mean values of 33.00µL

Table 5: Serum metabolites of West African Dwarf growing rams fed *Panicum maximum* supplemented with differently processed pigeon pea leaves

Parameters	T_1	T_2	T_3	T_4	SEM±
Total protein (g/dL)	6.50^{d}	7.00^{b}	7.70^{a}	6.80^{c}	2.21
Albumin (g/dL)	2.70^{d}	3.10^{b}	3.30^{a}	2.90^{c}	1.12
Globulin (g/dL)	3.80^{c}	3.90^{b}	4.40^{a}	3.90^{b}	1.17
Glucose (mg/dL)	132.00^{a}	127.00^{c}	130.00^{b}	109.00^{d}	4.56
Creatinine (mg/dL)	1.60^{d}	2.10^{b}	2.30^{a}	1.80^{c}	1.35
AST (µL)	42.00^{a}	40.00^{b}	42.00^{a}	35.00^{c}	1.43
ALT (µL)	50.00^{b}	58.00^{a}	40.00^{c}	33.00^{d}	4.76

^{abcd}: Means on the same rows with different superscript are significantly different (p< 0.05); T_1= 100% P. maximum solely; T_2 = 70% Panicum maximum + 30% fresh pigeon pea leaves (PPL); T_3 = 70% Panicum maximum + 30% wilted pigeon pea leaves (PPL); T_4 = 70% Panicum maximum + 30% dried pigeon pea leaves (PPL); ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

Conclusion and Application

1. The anti-nutrients (especially tannin, saponin, oxalate and phytate) were at tolerable levels, and the animals did not show any sign of ill-health throughout the study.
2. Reduction in the anti-nutrients of the PPL, through different processing methods, increased palatability, digestion, and growth performance and results in significant weight gain.
3. The experimental diets did not have any adverse effect on both heamatological and serum biochemical of the rams.
4. The inclusion of fresh and processed (especially 30% wilted) PPL positively influenced the health status of growing rams, boost their immune system and no mortality was recorded.
5. The fresh or processed PPL proved to be rich supplements for *P. maximum* and other low-quality forages.

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