ISSN 1819-1878

Asian Journal of Animal Sciences

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OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2022.73.83

Research Article
Effects of *Andropogon gayanus* and Centrosema Pubescens Leaf Meal Diets on Crossbred Rabbits

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Abstract
Background and Objective: The soaring food prices have triggered and increased hunger worldwide followed by the acute shortage of protein especially that of animal origin. This study was conducted to evaluate the effect of feeding graded levels of *Andropogon gayanus* (Ag) and *Centrosema pubescens* (Cp) leaf meal diets on the growth performance, volatile fatty acid concentration and caeca microbial population of crossbred (New Zealand white ♀ X Chinchilla black ♂) rabbits. **Materials and Methods:** Twenty four crossbred weaned rabbits were used for 63 day feeding trial. The rabbits were randomly assigned to four dietary treatments in a Completely Randomized Design (CRD). There were six rabbits per treatment. Each treatment was replicated 3 times with 2 rabbits per replicate. T_1 (control) contained 0% Ag and Cp leaf meals. The T_2, T_3 and T_4 contained 5, 10 and 15% each of Ag and Cp leaf meals, respectively. Data such as feed intake, weight gain, feed conversion ratio, protein intake and protein efficiency ratio, caeca volatile fatty acid concentration and caeca microbial population were generated. The feed cost-benefit analysis was performed on the following: Feed cost/kg weight gain, the total cost of production and net returns. Data generated were subjected to statistical analysis using One-way Analysis of Variance (ANOVA). Mean values were separated using least significant difference (LSD). **Results:** Results showed no significant ($p>0.05$) differences in all the growth performance parameters except the final body weight. The result of the feed cost analysis showed that feed cost per kg weight gain decreased as the inclusion levels of Ag and Cp leaf meals increased. The total revenue and net returns increased with increasing levels of dietary inclusion of Ag and Cp leaf meals. **Conclusion:** It was observed that diet can affect the bacterial population of the hind gut of rabbits. The inclusion of a 15% level each of Ag and Cp leaf meals is adequate for grower rabbits without any adverse effect.

Key words: Andropogon, Centrosema, volatile fatty acid, microbial population, rabbits

Citation: Ekwe, O.O., O.C. Onwudike and C.C. Nwali, 2022. Effects of *Andropogon gayanus* and *Centrosema pubescens* leaf meal diets on crossbred rabbits. Asian J. Anim. Sci., 16: 73-83.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The high costs of conventional vegetable protein and energy ingredients such as groundnut cake, soybean meal and maize, sorghum and millet have largely contributed to the existing high prices of animal feeds and products in Nigeria. Rabbits though often neglected are one of the sources of animal protein in Nigeria. To improve its production and consumption in Nigeria where per caput animal protein intake is much below recommended levels, there is a need to source readily available, high-quality alternative vegetable protein and energy sources that are cheaper and capable of reducing the production cost of feeds. The solution may be found in using the known but neglected tropical grass and legume species such as gamba grass (*Andropogon gayanus*) and centro (*Centrosema pubescens*). These are under-utilized but may possess as much agronomic and nutritional potential as the conventionally used protein and energy sources[1]. These grass and legume species are not consumed by humans and do not have direct competition with industries in Nigeria also they are all-season tropical forage plants. They are hardy, drought and disease resistant and blossom more in the rainy season. The grass is called 'Egbe Oku'' while, the legume is called Onori in one of the Local Igbo dialects. Animal protein intake of many households in Nigeria is very low due to the high cost of animal feed leading to the very high cost of finished animal products. The shortage of protein, particularly those of animal origin is prevalent in most parts of Africa including Nigeria where it is estimated that a varying average of between 6.5 and 8.5 g of animal protein is consumed per person per day compared to a recommended daily intake of 53.8 g[2]. The ratio went down to below 6.5 g or 14 % of a total of 46.7 g total protein intake by 1980. The resulting high cost of feed, especially feed for monogastric animal species has brought about a low supply of animal products such as meat, milk, eggs and their by-products. The short supply of livestock products had been attributed to many factors including the problem of competition between man, industries and other livestock for the available feedstuffs. This competition has brought about a rising cost of essential feed ingredients in our market thereby putting animal farmers at a great disadvantage as they can hardly make ends meet in their business[3]. This situation has led to the closing down of so many the livestock farms as well as the production of poor quality feed by the feed mill operators in their attempt to make a profit. Based on this precarious economic condition of low animal protein intake, animal nutritionists/researchers are calling for an urgent need for ways of ameliorating the food insecurity through the use of non-conventional sources such as *Andropogon gayanus* and *Centrosema pubescens* in place of maize and the rabbit which can use these products to increase the animal protein intake of Nigerians. The search for new and alternative feedstuffs for livestock rations aims at reducing the cost of production of compounded feed by incorporating into such feeds cheap sources of nutrients that preferably do not qualify for direct consumption by man. Grass and legume species should fit logically as grain replacers in livestock feeds under their high caloric values with several agronomic advantages. The nutritional value of some of these plants had already been reported[4]. Rabbits are known to perform better when fed with a mixture of forages and concentrated diets[5] among others. Given the abundance of *Andropogon gayanus* and *Centrosema pubescens* in our environment, their effective use in rabbit production will contribute greatly to improving the animal protein intake of Nigerians[6]. This research aimed to determine the growth performance, caeca volatile fatty acid concentration and caeca microbial population and economic efficiency of growing rabbits fed 15% *Andropogon gayanus* and *Centrosema pubescens* leaf meal diets in place of maize-based diets as a means of increasing the animal protein intake of Nigerians.

MATERIALS AND METHODS

Experimental site: The studies were conducted at the Rabbitry Unit of the Department of Animal Science Teaching and Research Farm between July 5th and October 19th, 2017. Ebony State University Abakaliki is partly located in the rainforest and derived savanna regions of Nigeria. It has a mean annual rainfall between 1500 and 1800 mm and a mean temperature of 30°C during very hot weather (February to April) and 21°C during the coldest period of the year-December to January[7].

Source of rabbits and forage plants: The rabbits that were used for this study were purchased from a rabbit Farm in Akwa Ibom State while, Gamba grass and Centrosema (*Andropogon gayanus* and *Centrosema pubescens*) were harvested from the Teaching and Research Farm of the Faculty of Agriculture and Natural Resource Management, Ebony State University.

Experiment I: Chemical composition of Gamba grass (*Andropogon gayanus*) and Centro (*Centrosema pubescens*) leaf meal.

Forage processing:

Washing and drying: *A. gayanus* and *C. pubescens* were harvested and washed separately in a trough using clean water to remove dirt. The leaves were washed once again with 1% saltwater and rinsed with clean water. The forages were kept on top of a platform to drain off water and after were cut into shorter lengths (2-5 cm) and spread on cellophane for drying. They were air-dried for 4 days and thereafter milled and bagged.

Milling and bagging: The forages were milled separately using a hammer mill with a screen size of 2.0 mm. This screen size was used to allow for proper milling because *Andropogon gayanus* and *Centrosema pubescens* leaf meals strongly attract moisture and can reabsorb moisture during and after milling, the leaf meals were stored in separate air-tight plastic containers.

Chemical analysis: A fraction of each of the two milled samples was collected and used for chemical analysis to determine the proximate composition, acid detergent fibre (ADF), neutral detergent fibre (NDF), energy values, anti-nutritional factors (tannin, trypsin inhibitor, saponin, oxalates) and mineral composition:

- **Proximate and energy composition:** Dried and ground samples of each of the diets were analysed for proximate composition (crude protein, ether extract, ash, fibre and total nitrogen-free extract) and energy content all of which were carried out in triplicates[8]. The metabolizable energy values of the diets were calculated using the at water factors 4, 9 and 4 for protein, fat and carbohydrate, respectively. The metabolizable energy (ME) values of *A. gayanus* and *C. pubescens* leaf meal diets were derived
- **Anti-nutritional factors/phytochemical composition determination:** Ground samples of feed and forages were sent to the laboratory in polythene bags for proximate and phytochemical analysis. The samples were screened for tannin, cyanide and saponin. Quantitative determination of phenols and trypsin inhibitors was carried out in triplicates using Horwitz and Albert[7] and spectrophotometric methods[9], respectively

- **Fibre analysis:**
  - **Neutral detergent fiber (NDF):** The plant cell wall of cellulose, lignin silica and hemicelluloses were separated by the use of neutral detergent[10]. The method of Gidenne[11] was used. The weight of the filter paper was subtracted from the total weight to obtain the weight of the residue (NDF)

Neutral detergent solution

Reagent used: The 19.61 of disodium EDTA and 6.86 g disodium tetraborate were discovered by heating ($Na_2B_2O_7{\cdot}10H_2O$) in 150 cm^3 distilled water. 30.8 g sodium lauryl sulphate and 10 cm^3 alpha ethoxy ethanol in 700 cm^3 hot water were added to the 1st solution. The 4.56 g sodium hydrogen orthophosphate anhydrous ($NaHPO_4$) was dissolved in 150 cm^3 hot water and was added to the 1st solution. The pH was also adjusted to 6.9-7.1 with orthophosphate acid (H_3PO_4).

Procedure: The 0.5 g sample 1 mm size was reflux for 60 min with 50 cm of neutral detergent solution, 0.15 g sodium sulphate and 2-3 drops of anti-foam reagent. The digested sample were filtered through the weighed porosity sintered glass crucible and was washed thoroughly with distilled water and then with ether. The fraction remaining (NDF) was dried in an oven at 65°C, cooled and the crucible reweighed.

Calculation:

$$\text{NDF} = \frac{W_2 - W_1}{W_0} \times 100 \tag{1}$$

Where:

W_0 = Weight of the sample used
W_1 = Weight of filter paper
W_2 = Weight of filter paper+residue after drying

- **Acid detergent fiber (ADF):** The method of Saura-Calixto *et al.*[12] was used. The acid detergent fraction was taken as the difference in weight:

$$\text{ADF} = \frac{W_2 - W_1}{W_0} \times \frac{100}{1} \tag{2}$$

- **Acid detergent lignin:** The method of Horwitz and Albert[7] was used. The acid detergent lignin was taken as the difference in weight.

Procedure: The 1 g of dried sample of Gamba grass (*A. gayanus*) and Centro (*Centrosema pubescens*) leaf meal each was weighed into a soxhlet flask 100 cm^3 of a cell solution of cetyl trimethyl ammonium bromide (CTAB) (1% m/v) in 0.5 m H_2SO_4. This was fitted into a reflux condenser and boiled gently for 60 min (1 hr). The content of the flask was filtered with gentle suction through a sintered glass crucible using a Buchner funnel and flask. The fibre was washed in the crucible with water, then acetone and finally sucked dry. The crucible and the content were dried at 95°C and reweighed.

Calculation:

$$\text{Lignin} = W_2 - W_1 \times 100 \qquad (3)$$

- **Cellulose:** The method of Silva *et al.*[13] was used. The cellulose was estimated from the difference between ADF and lignin:

$$\text{Cellulose} = \text{Eq. 2} - \text{Eq. 3} \times 100$$

- **Hemicellulose:** The difference between the NDF and ADF was estimated as hemicelluloses[13]:

$$\text{NDF} = \text{Eq. 1} - \text{Eq. 2} \times 100$$

Where:
NDF = Neutral detergent fibre
ADF = Acid detergent fiber

- **Tannin:** This was determined by the Folin-Dennis spectrophotometric method described by Ashburn *et al.*[14]. The tannin content was calculated as follows:

$$\text{Tannin (\%)} = \frac{\text{An}}{\text{As}} \times \text{C} \times \frac{100}{\text{W}} \times 5$$

Where:
An = Absorbance of the test sample
As = Absorbance of standard solution
C = Concentration
W = Weight of sample used
Vf = Total volume of extract
Va = Volume of extract analyzed

- **Oxalate:** This was determined using the method of Horwitz and Albert[7]. The calcium oxalate content was calculated as:

$$\frac{\text{T} \times (\text{Vme})(\text{Df}) \times 10^5}{(\text{Me}) \times \text{Mf}} \left(\frac{\text{mg}}{100\ \text{g}}\right)$$

Where:
T = Titre of $KMnO_4$ (mL)
Vme = Volume-mass equivalent (i.e., 1 cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid)
Df = Dilution of factor Vt/A (2.4 where Vt is the total volume of titrating (300 mL) and A is the aliquot used (125 mL)
ME = Molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction)
Ms = Mass of sample used

- **Trypsin inhibitor activity:** The trypsin inhibitor activity was measured using the spectrophotometric method described by Chang and Nickerson[9]. The trypsin inhibitor activity was expressed as the number of trypsin units inhibited (TU) per unit weight (g) of the sample analyzed:

$$\text{TU1 mg}^{-1} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 0.01\text{F}$$

$$\text{TU1 mg}^{-1} \frac{\text{b} - \text{a}}{0.01} \times \text{F}$$

Where:
b = Absorbance of test sample solution
A = Absorbance of the blank (control)
F = Experimental factor, given by

$$\text{F} = \frac{1}{\text{W}} \times \frac{\text{Vf}}{\text{Va}} \times \text{D}$$

Where:
W = Weight of the sample
Vf = Total volume of extract
Va = Volume of extract used in the assay
D = Dilution factor

- **Minerals:** Macro minerals such as calcium, phosphorus, sodium, chlorine, magnesium and some micro minerals (zinc, iron, manganese, iodine, selenium and chromium) of the leaf meals and experimental diets were determined using atomic absorption spectrophotometer[9].

Experiment II: Effect of gamba grass (*Andropogon gayanus*) and Centro (*Centrosema pubescens*) leaf meals diets on the performance of weaned rabbits.

Experimental rabbits/design: Twenty four crossbred grower rabbits of mixed sexes aged between 8 and 10 weeks were used for experiment 2. They were weighed and randomly assigned to four treatment diets containing 0, 5, 10 and 15% (*A. gayanus* and *C. pubescens*) leaf meals for diets 1 (control), 2, 3 and 4, respectively, in a Completely Randomized Design (CRD). Each treatment group has 6 animals replicated 3 times with 2 animals per replicate. The study lasted for 63 days.

Experimental diets: For experimental grower diets were formulated using graded levels of *Andropogon gayanus* and *Centrosema pubescens* leaf meals in Table 1. The four consist of a control diet (0%) in which no leaf meal was included and three other diets in which the two forages meals were included each at the levels of 5, 10 and 15%, respectively. As the leaf meals increased in the diets, the maize components of the diets decreased proportionately. The energy and protein levels of the diets were kept within the National Research Council (NRC) nutrient requirement recommendation for grower rabbits. Other feed ingredients included in the diets were soybean meal, wheat offal, brewers spent grain, bone meal and salt and vitamin/mineral premix. The composition of the experimental diets and proximate compositions of the two forages used in the experiment are shown in Table 1, 2 and 3, respectively.

Data collection: Data were collected on the following parameters: Feed intake, weight gain, feed conversion ratio, protein intake, protein efficiency ratio, caeca volatile fatty acids concentration and microbial population.

Housing and management: In experiment 2, 24 rabbits were housed in 3 single-tier wood/wire meshed cages with 8 cells (pens) each. Each cell of the cages measured 61×45×40 cm in all the partitions. Each cage had a feed trough and water trough attached for the feed and water supply respectively. The cages were kept in well-ventilated asbestos roofed

Table 1: Percentage composition of experimental feed 2 (grower diets)

	Graded levels of *A. gayanus* and *C. pubescens* leaf meal			
Ingredients	D_1 (0%)	D_2 (5%)	D_3 (10%)	D_4 (15%)
Maize	38.00	28.00	18.00	8.00
A. gayanus (leaf meal)	0.00	5.00	10.00	15.00
C. pubescens (leaf meal)	0.00	5.00	10.00	15.00
Soybean meal	10.00	10.00	10.00	10.00
Wheat offal	23.50	23.50	23.50	23.50
Brewers spent grain	25.50	25.00	25.00	25.00
Bone meal	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25
*Vit/min premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Nutrient composition				
Energy (ME kcal kg^{-1})-calculated	2216.39	2081.54	2074.69	2061.84
Crude protein (%)	22.89	23.02	25.85	28.75
Crude fiber (%)	9.25	11.85	16.55	18.75
Ether extract (%)	5.06	5.94	4.81	4.69
Nitrogen free extract	51.29	49.48	40.37	59.80
Phosphorus (%)-calculated	1.70	1.75	1.79	1.85
Calcium (%)-calculated	3.19	3.24	3.29	3.34

*Vitamin/mineral premix: Content per kilogram ration, vit. A. 1251 IU, vit. D_3 2750 IU, vit. E 151 IU, vit. K 0.002 g, vit. B_2 0.006 g, nicotinic acid 0.035 g, calcium D-pantothenate 0.01 mg, vit. B_6 0.0035 g, vit. B_{12} 0.02 g, folic acid 0.001 g, biotin 0.0005 g, vit. C 0.025 g, choline chloride 0.39 g, zinc bacitracin 0.02 g, methionine 0.2 g, avatec (lasalocid) 0.09 g, manganese 0.1 g, iron 0.05 g, zinc 0.04 g, copper 0.002 g, iodine 0./00153 g, cobalt 0.000225 g and selenium 0.0001 g

Table 2: Proximate composition of *A. gayanus* and *C. pubescens* (Experiment II)

Samples	DM	CP	CF	EE	ASH	NFE	GE (kcal kg^{-1})
A. gayanus	90.7	13.83	32.75	0.88	19.2	30.45	4911.03
C. pubescens	99.9	25.03	13.10	1.02	14.75	49.48	3090.33

Table 3: Minerals composition of the forages and the experimental feed 2 (%) (Experiment I)

Samples ID	Ca	Mg	Na	Fe	Zn	Mn
C. pubescens	0.36	2.55	2.05	0.22	0.0005	0.0115
A. gayanus	0.24	1.61	1.34	0.22	NIL	0.01
D_1	1.03	1.26	1.36	0.17	NIL	0.01
D_2	1.15	1.61	1.41	0.50	NIL	0.01
D_3	1.21	1.65	1.59	0.52	NIL	0.01
D_4	1.27	1.68	1.91	0.56	NIL	0.005

building with a dwarf wall and wire mesh traversing the whole length of the wall. This is to facilitate proper ventilation and proper dissipation of heat as fast as possible. The floor of the house is made of concrete, this is to ensure easy and proper cleaning. In all, standard routine management practices were administered throughout the experiment.

Volatile fatty acid (VFA) determination: Caeca content sample was collected from one rabbit per replicate in each treatment group given a total of 12 samples. Twelve rabbits were used for this study. The rabbits were slaughtered with a scalpel blade and the caeca content was collected by cutting through the caeca and scooping out a sample each into a sterilized tube and labelled for identification. The collected caeca contents were analysed for microbial and volatile fatty acids concentrations. The caeca volatile fatty acids (VFA) were determined using gas chromatography.

Caecal content sample collection: Caecal content sample was collected from one 1 rabbit per replicate in each treatment group given a total of 12 replicate samples. Twelve rabbits were used for this study. The rabbits were slaughtered with a scalpel blade and the caeca content was collected by cutting through the caeca and scooping out a sample each into a sterilized tube and labelled for easy identification. The collected caecal content was analysed for microbial and volatile fatty acids concentrations.

Method of sample preparation: Determination of VFA by GC method included a few important steps: Acidification of sample with nitric acid, extraction of VFA into methyl esters and finally chromatographic analysis of the obtained derivatives. For quantitative analysis, the external standard methodology (ESM) was used. Quantitative calibration proceeded for three acids (acetic, propionic and butyric) among others in concentrations ranging from 5 to 1000 mg L^{-1}. A series of VFA calibration standards were prepared in the same manner as for the standard solutions of VFA methyl esters (500 mg mL^{-1} in water. The GC chromatogram of methyl esters obtained from standard solutions of three fatty acids (500 mg mL^{-1}). The concentration of VFA was expressed as mg L^{-1} of CH_3COOH.

Economics analysis: The cost per kg of diet was calculated by multiplying the percentage composition of the feedstuffs by the price per kg and summing all. Total feed intake x cost per kg feed gave the total feed cost. Feed cost per Kg weight gain was calculated as FCR x cost per kg diet. Total feed intake (TFI) is the total feed consumed in each treatment for the duration of the experiment. Total weight gain (TWG) is the total weight gained in each treatment for the duration of the experiment. Total variable cost (TVC) is the cost of inputs during the experiment. Total revenue (TR) is the amount realized from the sales of the carcass after slaughter. Gross margin (GM) is the difference between total revenue (TR) and the total variable cost (TVC).

Statistical analysis: Data generated were subjected to statistical analysis using One-way Analysis of Variance (ANOVA). Means where, applicable were separated using[15] analytical method.

RESULTS AND DISCUSSION

Experiment I: The results of the proximate composition and mineral contents of the two forages (*A. gayanus* and *C. pubescens* leaf meals) were presented in Table 2 and 3 while, the phytochemical analysis of both forages were presented in Table 4 and 5, respectively.

Proximate composition: The proximate composition of *A. gayanus* and *C. pubescens* gave the following results- 13.83% crude protein, 32.75 crude fibre, 0.88% ether extract 19.20% ash, 30.45% nitrogen-free extract while, for *C. pubescens* the results, were- 25.03% crude protein, 13.10% crude fibre, 1.0% ether extract, 14.75% ash and

Table 4: Qualitative analysis of the forages and the experimental feed 2 (Experiment I)

Samples ID	Solvent	Tannin	Saponin
C. pubescens	N-Hexane	+++	-
	H_2O	++	+
A. gayanus	N-Hexane	++	+
	H_2O	++	-
D_1 (0%)	N-Hexane	+	++
	H_2O	-	++
D_2 (5%)	N-Hexane	++	+
	H_2O	+	++
D_3 (10%)	N-Hexane	++	++
	H_2O	++	+
D_4 (15%)	N-Hexane	++	++
	H_2O	++	+

Table 5: Quantitative analysis of the forages and the experimental diets (Experiment I)

Samples ID	Saponin (%)	Tannin (%)
C. pubescens	0.36	2.5
A. gayanus	0.64	8.2
D_1 (0%)	0.1	4.63
D_2 (5%)	0.34	4.82
D_3 (10%)	0.52	4.92
D_4 (15%)	0.64	4.98

49.48% nitrogen-free extract, respectively. The result of this study agrees with the earlier reports of other study[4,5], which indicated that most tropical legumes have an appreciable amount of crude protein and that legumes have superior protein content when compared to grasses. The higher crude fibre content of different forages assists rabbits in normal digestion transit Osakwe and Ekwe[15], Udeh *et al.*[16]. Forages generally contain an appreciable amount of protein, fibre, fat and minerals and these can support growth and production[16]. The analysis of the two forages was presented in Table 1 and 2 with the mineral contents of the two forages as 0.36% Ca, 2.55% Mg, 2.05% Na, 0.22% Fe, 0.0005% Zn and 0.00115% Mn values for *Centrosema pubescens* and 0.24% Ca, 1.61% Mg, 1.34% Na, 0.22% Fe, 0.01% Mn for *Andropogon gayanus*, respectively. However, these values were not in agreement with the previous report[12,13]. The values reported in this report were also lower than results attained[4] but agreed with the previous reports[16], respectively. These variations may have been due to several factors among which could be the soil type, the plant parts and the period of the year as well as the processing method among others. One thing very clear from the result obtained in this study is generally the higher mineral content of the legume when compared with those of the grass species. Generally, minerals are very important in the diets of animals as they play important roles in the body functions of animals such as rabbits[16]. From the result of the analysis of the diets, it is clear that the use of the forages made substantial contributions to the mineral requirements of the rabbits[4,5].

Phytochemical analysis: The results of the phytochemical analysis in both the qualitative and the quantitative fractions were shown in Table 4 and 5. The tannin contents of *A. gayanus* and *C. pubescens* were 8.2 and 2.5% were higher than those observed[1]. The saponin and tannin contents of 0.64 and 0.36%, respectively also were higher than 0.29-0.30% observed[1]. It was clear from this result that *A. gayanus* is higher in both tannin and saponin content than *C. pubescens* as observed by Onyeonagu *et al.*[1]. The contributions of tannins and saponin from the two forages also increased the tannin and the saponin contents of the diets that contained forage samples.

Growth performance of grower rabbits: The growth performance of grower rabbits fed diets containing *A. gayanus* and *C. pubescens* leaf meal diets were shown in Table 6.

Weight gain: Rabbits fed diet 2 (5% inclusion rate) showed a higher value in total weight gain (517.17 g) than rabbits fed diets 1 (0%-control) (447.50), 3 (10%) (455.67) and 4 (15%) (334.17). Also, rabbits fed diet 3 (10%) had a better total weight gain over those that were fed diets 1 (0%) and 4 (15%)[1,16]. The level of crude fibre usually recommended for an all-purpose rabbit ration is 12-14% as fed. This level is a practical compromise between a high fibre diet which is difficult to pellet and usually supports poor performance because of its low energy content (diet 4) and lower fibre diet (diets 1 and 2), respectively which was associated with digestive troubles, mortality and low intake during the first 2 weeks of the experiment. These explained the relatively high fibre needs in the diets of young and growing rabbits. The nutrient contribution of fibre is small because digestibility is low but more precise identification of the constituents of the traditional crude fibre fraction may help future definition of the rabbit's requirements. In all the work relating to fibre, the requirements are expressed as a proportion (usually %) of the ratio. Digestive disorders accompany the feeding of diets containing 5% or less crude fibre. The performance of young rabbits may be satisfactory on such diets but mortality is often higher than normal. Some possible physical dietary requirements for the functioning of the digestive tract may not be satisfied by low fibre levels. High fibre diets had demonstrated that the best performance in terms of health, growth and feed conversion in 6-12 weeks old rabbits was obtained on a diet containing 8-9% crude fibre, raising the

Table 6: Growth performance of grower rabbits fed experimental diets (63 days) ((Experiment II)

	T_1 (0%)	T_2 (5%)	T_3 (10%)	T_4 (15%)	SEM
Initial body weight (g)	629.83	630.00	663.83	651.00	0.11
Final body weight (g)	1077.33^b	1147.17^a	1119.50^a	985.17^c	0.45
Total bodyweight gain (g)	447.50	517.17	455.67	334.17	0.35
Av. daily weight gain (g)	7.10	8.21	7.23	5.30	1.15
Total feed intake (g)	2397.00	3098.33	2971.00	2924.00	2.05
Daily feed intake (g)	38.05	49.18	47.16	46.41	3.01
Feed conversion ratio	5.36	5.99	6.52	8.75	0.88
Protein intake (g)	4.69	8.19	7.88	7.74	1.49
Protein efficiency ratio	1.14	1.01	1.00	0.69	0.14

^{abc}Means within a row with different superscripts are significantly ($p<0.05$) different fibre level to 13-14% caused a decrease of 125 in feed conversion efficiency and no improvement in health[1,16]. This was a departure from the result of this experiment as higher fibre (11.85-16.55) % levels produced a better feed conversion ratio against those of a low fibre (9.25%) in diet 1 and a higher fibre level (18.75%) in diet 4. The low FCR observed among the rabbits in T_1 and T_4 could be attributable to the level of fibre in their diets and efficiency of feed utilization among others. Fibre is known to reduce levels of other nutrients in the diet and also decrease the digestibility of other nutrients especially energy and crude protein[1-6]. Feed intake and digestion were affected which may have resulted from the increased fibre level in diet 4. Digestive transit time was found to be shorter with barley straw than with purified wood cellulose diets. It was suggested that the level of cell wall constituents and not crude fibre might have been responsible for differences in transit time. The physical form of the fibre also influences digestive transit. As dietary fibre level rises in the range of 5-20% crude fibre, rabbits increase their food intake to maintain a constant energy level but the feed conversion ratio declines and carcass fat content decreases[1,16]. Rations in which nearly all the fibre is highly digestible (usually from root crops such as sugar beet or beetroot pulp) had caused severe diarrhoea probably because of excessive caecal fermentation. When such diets were fed successfully, increases were noted in gastrointestinal motility and the volume of the stomach and caecum. As in other species, success in feeding rations high in easily fermentable carbohydrates may depend on their gradual introduction into the diet. It appears that the fibre content of a rabbit diet should consist mainly of indigestible material if the possibility of digestive disorders is to be minimized, if fibre levels below those usually recommended are to be fed to young rabbits to encourage high growth rates the proportion of indigestible material in the fibre may become increasingly important. The higher level of acid detergent fibre - ADF (indigestible fraction) of 22.95% in diet 3 as against 19.93% in diet 4 may have contributed to the higher feed conversion ratio among the rabbits in the T_3 (7.5%) inclusion level which explains the role of indigestible fibre in the diet of rabbits. A lower level (5-6%) levels provoke hypo motility among the rabbit. A high level of digestible and highly fermentable carbohydrate (cell content fraction) may also result in hypomotility (enteritis), especially the young and growing rabbits.

Feed intake: Infeed intake, grower rabbits fed diets l, 2, 3 and 4 had the average daily feed intake of 38.05, 49.18, 47.16 and 46.41 g, respectively. There was no significant ($p>0.05$) difference in all the growth performance indices considered except in final weight. The higher fibre content in diet 4 may have stimulated increased feed intake, because rabbits, like every other animal, will attempt to maintain their energy balance[1,16]. Thus as the level of the wheat offal and brewers spent grain as well as the two forage meals in the diets, increased the feed intake of the animals increased this is also in agreement with the reports of previous study[4-6] which indicated an increase in fibre level of diets. This increase in the fibre levels of the feed may have brought about an increase in the total voluntary feed intake to maintain a constant energy balance.

Feed conversion ratio: There were no significant ($p>0.05$) differences in feed conversion ratio (FCR) as well as in other growth performance indices measured. Increased fibre levels in diets increased FCR[14,17]. The animals may have met their amino acid requirements on 5, 10 and 15% diets with higher fibre content. High fibre diets improve gut motility and may have increased digestion and availability of amino acids[1,15]. Although there was no significant ($p>0.0$) difference in feed conversion ratio among the treatments, it numerically favoured diet 1 (0%) similar to previous reports of [1,16] since the lower the value of feed conversion ratio, the better the quality of the diet. This was a product of a moderate feed intake and good weight gain. The non-significant difference in all the FCR parameters considered implies that any of the diets could be chosen.

Caecal contents characteristics (Experiment III): Results showed some chemical characteristics as well as the pH and volatile fatty acid concentrations of rabbit caecal content (RCC). The rabbit volatile fatty acids (RVFA) contents obtained in caeca even expressed mg L^{-1} of total VFA in each case, the average The results showed volatile fatty acids of the rabbits caecal content (RCC) as Treatment 1(0%) for acetate ranging from 8.90 mg L^{-1}, Propionate 7.12 mg L^{-1}, Butyrate (11.26), 2 (5%) recorded 3.38, 10.33, 13.96 mg L^{-1} for acetate, propionate and butyrate, respectively. Treatment 3(10%) and 4 (15%) recorded 8.51, 4.19, 8.37 mg L^{-1} and 8.37, 9.08, 11.48 and 14.83 mg L^{-1} in that order. However, the three dominant VFA in the RCC were isolated for consideration in the present study. The total RCC recorded from the innocular (RCC) was 29.8, 29.34, 22.41 and 37.05 for T_1, T_2, T_3 and T_4, respectively. There were no significant differences ($p>0.05$) in treatments 1 and 2. There was a significant ($p<0.05$) difference among the recorded values in treatment 3 and 4 in that order. In ruminant animals, volatile fatty acids (VFA) are

Table 7: Caeca volatile fatty acid contents analysis (mg L^{-1}) (Experiment III)

	pH	Acetate	Propionate	Butyrate	Isobutyrate	Valerianic	Iso valerianic	tVFA
T_1 (0%)	6.51 (0.20)	8.90 (4.46)	7.12 (1.38)	11.26 (0.97)	0.7 (0.24)	0.62 (0.41)	0.68 (0.26)	29.28 (6.09)
T_2 (5%)	6.52 (0.17)	3.38b (6.28)	10.33 (6.10)	13.96 (5.77)	0.42 (0.30)	0.73 (0.45)	0.52 (0.49)	29.34 (18.80)
T_3 (10%)	6.73 (0.09)	8.51 (3.77)^a	4.19 (1.49)^c	8.37 (1.70)^a	0.31 (0.28)^b	0.70 (0.34)^c	0.33 (0.17)^b	22.41 (7.52)^b
T_4 (15%)	6.34 (0.05	9.08 (2.68)^a	11.48 (4.48)^a	14.83 (3.67)^a	0.54 (0.23)^c	0.50 (0.19)^c	0.62 (0.49)^c	37.05 (7.15)^a

Value in parenthesis is the standard error of Mean± SEM

Table 8: Cost: Benefit analysis of grower rabbits fed experimental diets (Experiment III)

	Parameters	T_1 (0%)	T_2 (5%)	T_3 (10%)	T_4 (15%)
A	Cost/rabbit(₦)	*1200	1200	1200	1200
B	Cost of feed/kg (₦)	74.25	62.50	53.42	45.38
C	Cost of medication	*35.00	35.00	35.00	35.00
D	Total feed cost (₦ kg^{-1})	*177.98	193.65	158.71	132.69
E	Feed cost/kg.wt., gain/rabbit	*79.65	100.15	72.32	44.34
F	Total feed consumed/rabbit (g)	2397.00	3098.33	2971.00	2924.00
G	Total weight gain (g)	447.50	517.17	455.67	334.17
H	Total variable cost (₦)	1412.98	1428.65	1393.71	1367.69
I	Total revenue (₦)	1800.00	1800.00	1800.00	1,800.00
J	Gross margin/rabbit (₦)	387.02	371.35	406.29	432.31
K	Cost: Benefit ratio	1.04	1.00	1.09	1.16

*D = (b×f/1000), H = (a+c+d) J = (I-h) E = (g/1000×d)

produced as end products of microbial fermentation in the rumen. In diets high in fibre, acetic acid is the highest VFA produced while on high concentrate diets, propionic acid production is increased. Butyric acid generally is produced in smaller quantities. VFAs are also produced as end products of bacterial fermentation in the rabbit caecum. The production and blood distribution of VFA is different in rabbits when compared with the ruminants. In the present study, the VFA production could probably be a reflection of the unusual microbial population of the rabbit caecum dominated by Bacteroides species which often were butyrate-producing organisms. As fibre levels increased, the proportion of butyric acid was higher. The higher Caecal butyrate levels could explain the protective effect of fibre against enteritis[14,16]. From the foregoing, grower rabbits can tolerate fibre up to 15% in their diets without any deleterious effect and can as well encourage greater production of the bacteroides species which were mostly butyric acids producers as shown in Table 7.

Microbiology of the digestive tract of rabbit (experiment III): Diet can affect the bacterial population of the hindgut of rabbits. With a high fibre diet, low in soluble carbohydrates (Experiments II and III), Caecal microbes were primarily Bacteroides (*piriformis*). With a diet high in soluble carbohydrates and low in fibre (Experiment I), *Clostridia* and *E. coli* were predominant. This has implications for rabbit diarrhoea which was experienced at the onset of the experiment (Experiment I).

Weights of stomach and caeca tissues (experiment III): Weights of the stomach tissue and stomach content were higher in the higher fibre diets (15%) whereas caecal weight was greater in the high starch diets (0%)[14]. A low fibre diet results in greater Caecal size than a high fibre diet. Rabbits fed low fibre, high grain diets had enlarged appendices, containing large amounts of ingesta -similar to previous[14], report. Low molecular mass carboxylic acid (C_2-C_1) monocarboxylic aliphatic acid) are important intermediates and metabolites in biological processes. These carboxylic acids are known as volatile fatty acids (VFA) or short-chain fatty acids (SCFA). The presence of VFA in a sample matrix is often an indication of bacterial activity. VFA analysis is significant in studies of health and diseases in the intestinal tract. In some food, VFA content is an index to quality assurance. VFA Originate from anaerobic biodegradation of organic matter. Result of Analysis, culture and sensitivity of the rabbit caeca contents (T_1, T_2, T_3 and T_4): Culture yielded significant growth of *Escherichia coli* after 48 hrs incubation in the four treatments indicating a normal gut micro-organism for monogastric species.

Economic analysis (Experiment III): The results of the economic analysis of rabbits fed experimental diets are shown in Table 8.

Cost-benefit analysis: The cost per kg of diet was calculated by multiplying the percentage composition of the feedstuffs by the price per kg summing all. Total feed intake×cost per kg feed gave the total feed cost. Feed cost per kg weight gain was calculated as FCR×cost per kg diet. Total feed intake (TFI) is the total feed consumed in each treatment for the duration of the experiment. Total weight gain (TWG) is the total weight gained in each treatment for the duration of the experiment. Total variable cost (TVC) is the cost of inputs during the experiment. Total revenue (TR) is the sales of the live rabbit. Gross margin (GM) is the difference between total revenue (TR) and the total variable cost (TVC). The performance results were summarized in Table 4. Analysis showed that the cost of feed used to produce kg body weight gain of 447.50, 517.17, 455.67 and 334.17 g for T_1, T_2, T_3 and T_4, respectively were N177.98, N193.65, N158.71 and N132.69. The cost of producing 447.50 g -(T_1) and 517.17 g live weight gain in T_2 were significantly ($p>0.05$) higher than those of T_3 and T_4. The initial weight of the rabbits was not significantly ($p>0.05$) different. The result (Table 4) however indicated that it is more profitable to raise rabbits using the T_4 diet because of its cost-benefit ratio of 1.16 which is not significantly ($p>0.05$) different from T_3 (1.09) The analysis showed that discounting the fixed costs and other variable costs such as labour and milling of grass, the cost (N kg^{-1}) of concentrates (leaf meal diets) fed were N74.25, N62.50, N53.42 and N45.38 for T_1, T_2, T_3 and T_4, respectively. This is in agreement with the previous report[1-6] which recorded lower cost value and higher growth performance using leaf meals as a protein supplement in the diet of growing rabbits. However, the feed cost kg^{-1} gains were N79.65, N100.15, N72.32 and N44.34 for T_1, T_2, T_3 and T_4, respectively. There was no significant ($p>0.05$) difference among treatments, since the main objective of feed formulation is for-profit maximization-that is the difference between the returns (growth response) and cost (feed intake) can therefore be judged to be equal economically. However, rabbits in T_3 and T_4 recorded higher values in total revenue and net returns (N406.29 and N432.31) than T_1 (control) and T_2 (N387.02 and N371.35), respectively. In conclusion, the economic implication of this was that inclusion of gamba grass (*A. gayanus*) and *Centrosema* (*C. pubescens*) leaf meals in grower rabbit diets up to 15% (150 g kg^{-1}) inclusion level was more profitable.

CONCLUSION

The feed intake levels were found to be adequate in the three stages administered as it affects all the growth performance indices studied (initial weights, daily feed intake, daily weight gain, feed conversion ratio, daily protein intake and protein efficiency ratio). In a micro biology study, results showed that diet can affect the bacterial population of the hindgut of the rabbit. As shown in diets III and IV when compared to the control diets. The results of this investigation indicated that a 15% dietary inclusion level of *A. gayanus* and *C. pubescens* leaf meals are considered adequate for growing rabbits without any adverse effect on their normal growth and development.

SIGNIFICANCE STATEMENT

The study has shown that the use of *A. gayanus* and *C. pubescens* leaf meals as protein and energy feed ingredients generally improved growth performance, volatile fatty acid content and microbial population of the caeca. The use of *A. gayanus* and *C. pubescens* leaf meals as protein and energy feed ingredients can replace groundnut cake and soybean meal in the diets of monogastric animals at 15% each in the diets of grower rabbits. This practice will reduce the cost of production and hence increase the rate of animal protein intake among consumers. This study has opened new possibilities for the application of A*. gayanus* and *C. pubescens* leaf meals as protein and energy feed ingredients by feed millers instead of groundnut cake and soybean in the diets of rabbits. Feed produced with these ingredients will not only increase savings in foreign exchange for countries that rely heavily on the importation of maize and other feed ingredients. This, by so doing will improve the utilization of locally available feed ingredients and enhance animal protein intake by consumers.

ACKNOWLEDGMENT

We wish to thank Prof. O.C. Onwudike (The Vice Chancellor of Rhema University Aba, Nigeria, for his numerous advice and support during the course of this study.

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