Nigerian J. Anim, Sci. 2024, 26 (1): 232-247

Effects of Gamba grass (*Andropogon gayanus*) and Centro (*Centrosema pubescens*) Leaf meal diets on the growth and Haematological profile of weaner rabbits

[1]Ekwe, O.O.[2]Okafor, C.O.,[3]Effiong, O.O. and[3]Ozung, P.O.

[1]Department of Animal Science, Faculty of Agriculture and Natural Resources Management, Ebonyi State University, Abakaliki. Nigeria,
[2]Department of Geography, Faculty of Environmental Sciences, Alex Ekwueme Federal University, Ndufu Alike Ikwo. Ebonyi State.
[3]Department of Animal Science, University of Calabar, Cross River State, Nigeria.

Corresponding Author's e-mail: *ekweo3@gmail.com ,Tel: +2347035054649*

Target audience: *Animal nutritionist, feed millers and rabbit farmers*

Abstract

A study was conducted to investigate the potentials of two tropical African forages – Andropogon gayanus (Ag) and Centrosema pubescens (Cp) as alternative feed ingredients as replacement for maize and to evaluate the effects of diets containing these two forages on growth and Haematological profile of rabbits. Twenty four rabbits were randomly assigned into four dietary treatment groups of 6 rabbits per treatment in a completely randomized design (CRD). Each treatment was replicated three times with 2 rabbits per replicate. Parameters evaluated were growth performance, haematological and serum biochemical indices. Results showed that there were no significant (P>0.05) differences in all the growth performance indicators and Haematological indices. There were significant (P<0.05) differences in all the serum biochemical indices studied except Alanine amino transaminase (ALT) and very low density lipo-protein (VLDL).Albumin ranged from (30.00 - 31.30) and Creatinine ranged from (45.04 - 46.06) which were within the acceptable values respectively. Result showed that the inclution of Ag and Cp leaf meals in the diets of weaner rabbits at 5 - 15% levels. Conclusively, Ag and Cp leaf meals can be incorporated to an optimal level of 15% in rabbits feed without compromising their health. Therefore leaf meals can be used to replace maize in the diets of weaner rabbits without any deleterious effect on the haematological (blood) and serum biochemical indices.

Keywords: Andropgon gayanus, Centrosema pubescens, growth performance,Haematology, Serum biochemistry, Rabbits

Description of Problem

The high costs of conventional vegetable protein such as groundnut cake and soybean meal, and energy ingredients like 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 (1). To improve its production and consumption in Nigeria where animal protein intake is much below recommended levels, there is need to source for readily available, high quality alternative vegetable protein and energy sources that are cheaper and capable of reducing production cost of feeds. The solution may be found in using the known but neglected tropical grass and legume species such as Andropogon gayanus (Ag) and Centrosema pubescens (Cp).

These are under-utilized but may possess as much agronomic and nutritional potentials 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 and also they are all season tropical forage plants. They are hardy, drought and disease resistant and blossom more in the rainy season. The grasses called 'Egbe Oku" and the legume "Onori"in one of the Igbo dialects. Currently, Nigeria is plagued with food crisis which is partly due to the unprecedented rise in population and the drastic drop in per caput food production particularly in the last decades. This food crisis condition may have emanated from the fact that many farms especially the Livestock/Poultry industries folded as a result of high cost of conventional feed ingredients. Many of the feed industries also closed down as they could no longer sustain themselves on their businesses. The result of this farms closure forced the prices of the available animal products such as meat, milk and eggs to go up thereby making the products unaffordable for low income groups. Staple foods such as garri, beans and rice are not easily affordable by many consumers, but much more, the animal protein intake of many in Nigeria is very low because of very high cost of animal feed leading to very high cost of the 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 an average of 10g of animal protein is consumed per day compared to a recommended daily intake of 35g (2); (3). 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 great difficulty. This situation has led to the closing down of many livestock farms as well as the production of poor quality feed by the feed mill operators in their attempt to make profit.

Based on this precarious health condition occasioned by low animal protein intake, there is an urgent need for ways of ameliorating the food insecurity through the use of non-conventional feed sources such as Ag and Cp in place of maize and the rearing of rabbits which can use these products. Energy feedstuffs constitute between 45 and 60 percent of finished feeds for monogastric animals and at present maize constitutes the bulk of energy component of various rations. 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 by virtue of their high caloric values with several agronomic advantages (4). The nutritional value of some of these plants had already been reported (5);

(1); (6 - 9). Rabbits are known to perform optimally when fed with a mixture of forages and concentrate diets (10-12), (5) ;(13) and (14). Given the abundance of Ag and Cp in our environment, their effective use in rabbit production will contribute greatly to improving the animal protein intake of Nigerian.

Materials and Methods. 21

The experiments were conducted at the Rabbitry Unit of the Department of Animal Science Teaching and Research Farm, Faculty of Agriculture and Natural Resources Management, Ebonyi State University, Abakaliki. Ebonyi State is partly located in the rainforest and derived savanna regions of Nigeria. It has a mean annual rainfall between 1500 mm and 1800 mm and mean temperature of 30oC during very hot weather (February to April) and 21oC during the coldest period of the year -December to January (15).

Source of Rabbits and Forage Plants

The rabbits that were used for this study were purchased from Umosen rabbit Farm, School Road, Uyo at Akwa Ibom State, Nigeria, while the two forages - Gamba grass and Centrosema were harvested from the Teaching and Research Farm of the Faculty of Agriculture and Natural Resource Management, Ebonyi State University.

Experiment I

Chemical composition of Gamba grass (Ag)) and Centrosema (Cp) leaf meals

Forage Processing

(a) Washing and drying: Ag and Cp were harvested and washed separately in a trough using clean water to remove dirt. The leaves were washed once again with 1% salt water and rinsed with clean water. The forages were kept on top of a platform to drain off water lengths (2 to 5 cm) and spread on cellophane for drying. They were air-dried for a period of 4 days and there after milled and bagged.

(b) Milling and bagging: The forages were milled separately using a hammer mill with screen size of 2.0 mm. This screen size was used to allow for proper milling because Ag and Cp leaf meals strongly attract moisture and can reabsorb moisture during and after milling, the leaf meals were stored in separate air-tight plastic containers. 22

© 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, saponnin, 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 according to standard methods (16). The metabolizable energy values of the diets were calculated using the Atawodi 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 was calculated using(18) formula: ME = 35 x CP% +81.8x E.E% + 35.5 x NFE.

(ii) Determination of Anti-nutritional factors/phytochemical composition in Ag and Cp

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 were carried out in triplicates using (17) methods and spectrophotometric method as described by (19), respectively.

(iii) Fibre analysis

(a) Neutral detergent fibre (NDF) Plant cell wall of cellulose, lignin silica and hemicelluloses were separated by the use of neutral detergent.The method of (20) 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:

19.61 of disodium EDTA and 6.86 g disodium tetraborate were dissolved by heating (Na2B2O7.10 H2O) in 150cm3distilled water. 30.8 g sodium lauryl sulphate and 10cm3 alpha ethoxy ethanol in 700cm3 hot water was added to the 1st solution. 4.56 g sodium hydrogen orthophosphate anhydrous (NaHPO4) was dissolved in 150cm3 hot water and was added to the 1st solution. The pH was also adjusted to 6.9 – 7.1 with orthophosphate acid (H3PO4)

Procedure

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

Calculation:

$$NDF = \frac{w2 - w1}{w0} X \frac{100}{1} \quad \text{Equation 1 (Eqn. 1)}$$

W0 = weight of the sample used.
W1 = Weight of filter paper
W2 = Weight of filter paper + Residue after drying

(b) Acid detergent fibre (ADF)

The method of (20) was used. The acid detergent fraction was taken as the difference in weight.

$$ADF = \frac{w2 - w1}{w0} X \frac{100}{1} \quad \text{Equation 2 (Eqn. 2)}$$

(c) Acid detergent lignin

The method of (17) was used. The acid detergent lignin was taken as the difference in weight.

Procedure

1 g of dried sample of Ag and Cp leaf meal each was weighed into a soxhlet flask 100cm3 of a cell solution of cotyletrinethyl ammonium branide (CTAB) (1% m/v) in 0.5m H2SO4. This was fitted into a reflux condenser and boiled gently for 60 minutes (1 hour). The content of the flask was filtered with gentle suction through a sinister 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 was dried at 95oC and reweighed.

Calculation

$$Lignin = w2 - w1 \; X \frac{100}{1} \quad \text{Equation 3 (Eqn. 3)}$$

(d) Cellulose

The method of (17) was used. The cellulose was estimated from the difference between ADF and lignin.

$$Cellulose = \text{Eqn. 2 - Eqn. 3} \; X \frac{100}{1}$$

(e) Hemicellulose

The difference between the NDF and ADF was estimated as hemicelluloses (17).

$$NDF = \frac{Eqn.\ 1 - Eqn.\ 2}{} \ X \ \frac{100}{1}$$

NDF=Neutral detergent fibre 25
ADF =Acid detergent fibre.

(f) Tannin

This was determined using (16). The tannin content was calculated as follows:
%Tannin –An /As x Cx100/w x Five.
Where: An=absorbance of test sample
As =absorbance of standard solution
C= concentration
W=weight of sample used
Vf =total volume of extract
Va=volume of extract analysed.

(g) Oxalate

This was determined using the method (21). The calcium oxalate content was calculated as:

$$\frac{Tx(Vme)[DF]x10^5}{(ME)xMf} \left(\frac{mg}{100g}\right)$$

Where T=titre of KMn04 (ml),
Vme = volume-mass equivalent (1cm3 of 0.05M KMn04 solution is equivalent to 0.00225g anhydrous oxalic acid).
Df = dilution of factor Vt/A (2.4) where Vt is the total volume of titrate (300ml) and A is the aliquot used (125ml),
ME = Molar equivalent of KMnO4 in oxalate (KMn04 redox reaction)
Ms=Mass of sample used.

(h) Trypsin inhibitor activity 26

The trypsin inhibitor activity was measured using the spectrophotometric method described by (19). The trypsin inhibitor activity was expressed as the number of trypsin units inhibited (TU) per unit weight (g) of the sample analysed:

$$TUI/mg = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 0.0IF$$

$$TUI/mg = \frac{b - a}{0.01} \times 0.0IF$$

NDF=Neutral Where b = absorbance of test sample solution
A = absorbance of the blank (control)
F = experimental factor, given by

$$F = \frac{1}{w} \times F$$

Where w =weight of 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(23).

Experiment II

Effect of Ag and Cp leaf meal diets on the growth performance, haematological and serum indices of weaner rabbits.

Experimental Diets

Table 1 shows the composition of experimental diets. The four experimental diets were made up as follows: There was a control diet (T1 - 0% leaf meal) and three other diets in which the forage meals were included at levels of 5% (T2)10% (T3) and 15% (T4) leaf meals, respectively. As the leaf meals increased in the diets, the maize component 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 weaner rabbit.

Experimental Design

Twenty four (24) crossbred weaner rabbits of mixed sexes aged between 4 and 6 weeks were used for experiment study. The animals were weighed and divided into four treatment groups with 6 rabbits in each treatment. Each treatment was replicated three times with 2 rabbits per replicate. They were randomly assigned into four treatments in a completely randomized design (CRD). Rabbits were weighed at the commencement of the experiment to determine the initial weight and subsequently weighed weekly to determine the daily and weekly weight gain using a sensitive Camry Table scale (model; EK5350, MAX; 5kg/11 Ib, d= 1g / 0.1oz). At the end of the experiment, the body weight changes were calculated by subtracting the initial body weight from the final body weight.

Feeding Method

A weighed quantity of feed and fresh cool water was served ad libitum throughout the period of the experiment. The left over feed was collected per replicate every morning weighed and recorded. From this, average daily feed intake for each treatment was determined. The feeding trial lasted for 56 days.

Parameters Measured

The parameters studied were feed intake (FI), weight gain (WG), Feed conversion ratio (FCR), protein intake (PI), protein efficiency ratio (PER), haematological indices and serum biochemistry.

Data Collection

Data were collected on the parameters mentioned above and analyzed General Linear Model (GLM) Procedure of SAS a completely randomized design (P=0.05) (23) and differences between treatment means were separated using (25)

Intake (FI)

$$\text{FI /rabbit/day} = \frac{\text{Quantity of feed given (g) - left over (g)}}{\text{Number of rabbit x 56 days}}$$

Daily weight gain (DWG)

$$\text{DWG/rabbit/day} = \frac{\text{Final live weight - initial live weight (g)}}{\text{Number of rabbit x 56 days}}$$

1. With the data collected on average feed intake (AFI), and average weight gain (AWG) other performance indicators such as feed conversion ratio (FCR) and protein efficiency ratio were calculated.

Feed conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Quantity consumed feed of}}{\text{weight gain}}$$

Protein intake (PI)

PI= protein in feed – protein in faeces

Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Protein consumed (g)}}{\text{weight gain}}$$

Haematological Indices

Blood samples were collected from 12 rabbits at 8 weeks of age, (3 rabbits per treatment) for the determination of the haematological and serum biochemical

indices. Samples were collected from the jugular vein of the rabbits by using disposable needle (5 ml gauge needle) and syringes. The rabbits were fasted overnight (12hrs) and normally bled in the morning (7.00 to 8.00 am) to avoid excessive bleeding. Fasting of the rabbits was done to avoid the temporary elevation of many blood metabolites by feeding. The collection site was cleaned with alcohol and zylene applied to dilate the veins. Sterile cotton was used to cover the punctured vein after collection. The blood was collected in sample bottles containing dipotassium salt of ethylene diamine tetra acetic acid (EDTA) which served as anti-coagulant. The blood was properly mixed with EDTA by gently turning round the bottle to prevent haemolysis of the blood cells. The haematological analyses of blood samples were carried out at the haematological unit of College of Health Science, Ebonyi State University Abakaliki. Blood samples were analyzed for packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts. Haemaglobin concentration (Hb) was determined using Wintrobemicrohaematocrit. Neuberhaematocytometer and cynohaemaglobin procedures respectively as described by (26). Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation according to standard formulae as shown below:

$$MCH = \frac{\text{Haemoglobi n (g per 100ml x 10)}}{\text{RBC counted (10 /L)}}$$

$$MCHC = \frac{\text{Haemoglobin (g/dl x 100}}{\text{PCV (L/L) X 10}}$$

$$MCV = \frac{\text{PW(L/L) X 1000}}{\text{RBC (10 /L}}$$

(a) Serum chemistry

Serum calcium, sodium, phosphorus, magnesium and potassium were determined using atomic absorption spectrophotomer.

(b) Lipid profile

(I) Cholesterol estimate

Principle: In the presence of excess acid such as phosphoric acid and ferric (Fe+++) ions, cholesterol was oxidised to disulphuric acid which was reddish purple in colour (Salkowski reaction). It was read calorimetrically at 560 nm (green yellow filter).

Procedure

Serum and cholesterol were diluted calculated and read as follows:

The serum was diluted at the ratio of 1:20: (0.1ml serum + 1.9ml distilled water).

Cholesterol was diluted at 1:20 with glacial acetic acid. Ten (10) seconds was used for shaking the test tubes in order to mix the contents of each tube. The test tubes were placed immediately in a boiling water bath for exactly 90 seconds and were removed after and cooled in running tap water for 5 minutes. Absorbance was read at 560 nm (green-yellow filter) against the blank, using a dry coveter. The colour was stable for 15 minutes.

$$\text{Calculation: Serum cholesterol (mg/dl)} = \frac{\text{Absorbent test}}{\text{Absorbent standard}} \times 250$$

Serum cholesterol (mmol/L) = mg/dlx 0.0259

(ii) Estimation of serum/plasma high density lipid cholesterol.

Principle: Chylomicrons, very low density lipid (VLDL) and low density lipid

(LDL) were precipitated by phosphoric acid in the presence of magnesium ions leading to high density lipid (HDL) in solution.

Method

1ml sample of serum was pipetted into a centrifuge tube. 0.1ml of PTA reagent was added and mixed properly. 0.05ml of the MgCl was added and properly mixed .The mixture was centrifuged at 2500 rpm for 30 minutes. The supernatant on top was carefully removed with a Pasteur pipette. There was an estimation of cholesterol level in the supernatant using 100mg/dl cholesterol standard.

$$\text{Calculation was made as follows: serum HDL cholesterol (mg/dl)} = \frac{\text{Ab test}}{\text{Ab standard}} \times 115$$

Serum HDL cholesterol (mmol/L = mg/dl x 0.0259

(iii) Estimation of serum/plasma Low Density Lipid cholesterol

LDL cholesterol (mmol/L) = Total cholesterol - (HDL cholesterol + 0.46 x triglycerides) All in mmol/L.

Estimation of serum triglycerides

Principle

Triglycerides from serum or plasma were extracted with isopropanol

Extracted triglycerides were saponified with an alkali, sodium methylate forming glycerol and fatty acids. Triglyceride + Sodium methylate = glycerol + fatty acid. Glycerol was oxidized with sodium periodate formaldehyde. Glycerol + sodium periodate = formaldehyde. Formaldehyde was reacted with acetyl acetone in the presence of ammonium ion to produce a yellow compound diacetyldihydrolutidine which was measured calorimetrically. 32

Data Analysis

Data obtained were analyzed using one way analysis of variance (ANOVA) in a Completely Randomized Design (23). Significant differences between treatment means were separated using (25)

Proximate Composition

The proximate composition of A. gayanus 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 49.48 % nitrogen free extract, respectively (Table 1). The results from this analysis were in agreement with the values reported by (2) and (3). The results of this study confirm the earlier reports which indicated that most of the tropical legumes have appreciable amount of crude protein and that legumes have superior protein content when compared to grasses. High crude fibre contents of different forages assist rabbits in normal digestion transit (11). Forages generally contain appreciable amount of protein, fibre, fat and minerals and that can support growth and production. The mineral composition of the two forages and the experimental diets are presented in Table 2.The mineral contents of the two forages are 0.36% Ca, 2.55% Mg; 2.05% Na; 0.22% 33 Fe; 0.0005% Zn; and 0.00115% Mn; values for Ca.this is in agreement with the report of (2) and (3).

Table 1: Proximate composition (%) of A. gayanus And C. pubescens.

S/No	Parameters	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
2.	C. pubescens	99.9	25.03	13.10	1.02	14.75	49.48	3090.33

DM =(dry matter); CP = (crude protein); CF =(crude fibre); EE =(ether extract); NFE = (nitrogen free extract); GE = gross energy, kcal/kg = kilo calorie/kilogram weight.

Table 2: Minerals composition of the forages and the weaners diets.

S/no	Parameters	Ca	Mg	Na	Fe	Mn
1	C. pubescens	0.36	2.55	2.05	0.22	0.0115
2	A. gayanus	0.24	1.61	1.34	0.22	0.01
3	D1(0%)	1.03	1.26	1.36	0.17	0.01
4	D2(5%)	1.15	1.61	1.41	0.50	0.01
5	D3(10%)	1.21	1.65	1.59	0.52	0.01
6	D4(15%)	1.27	1.68	1.91	0.56	0.005

D1 = (0% forage meals), D2= (5% forage meals), D3 = (10% forage meals) and D4 = (15% forage meals) Ca = (Calcium); Mg = (Magnesium); Na = (Sodium); Fe = (Iron); Mn = (Manganese).

Table 3: Anti-nutritional factors analysis of forages andweaners diets.

Parameters	Solvent	Tannin	Saponin
C. pubescens	N-Hexane	+ + +	-
	H_20	++	+
A. gayanus	N-Hexane	+ + +	+
	H_20	++	-
D_1 (0%)	N-Hexane	+	++
	H_20	-	++
D_2 (5%)	N-Hexane	++	+
	H_20	+	++
D_3 (10%)	N-Hexane	++	++
	H_20	++	+
D_4 (15%)	N-Hexane	++	++
	H_20	++	+

D1= (0% forage), D2= (5% forage); D3=(10% forage); D4 = (15%forage); - = (nil); + = (low); ++ = (high); +++ = (very high) = concentrations of each of the solvents with the two forages, respectively.

Growth Performance Characteristics of Weaner Rabbits

The result of the growth performance of weaner rabbits fed diets containing A. gayanus and C. pubescens leaf meals are presented in Table 5. There were no significant difference ($p>0.05$) in all the growth parameters measured in this study. The initial body weights (450.00g, 433.33g, 430.g and 450.00g), the mean daily body weight gains (13.24g, 12.65g, 13.92g, and 12.20g) for 0%, 5%,10% and 15%),

respectively, showed no significant ($p>0.05$) differences. Diet 3 (10%) and diet 1 (0%-control) had the highest daily weight gain of 13.92g and 13.24g while diet 2(5%) and diet 4(15%) produced lower values of 12.65 and 12.20g/day, respectively.

Feed Intake

The feed intake result showed that rabbits fed diet 1 (0%) recorded the highest feed intake (53.52g/d) while rabbits fed diet T3 (10%) inclusion had the lowest value (48.29g/day). The feed intake values showed a slight tendency towards decreasing intake by the rabbits as the level of A. gayanus and C. pubescens leaf meals in the diets increased. At treatments 2 and 3, the intake level decreased and increased in treatment 4. The decrease intake observed could be related to palatability as the level of the leaf meals increased. The increased consumption level at treatment 4 may have emanated from the low grain (maize) in the diet indicating low energy due to an increase in fibre level of the diet. The energy levels of feed tend to decrease with increase in level of dietary fibre above 15% level in weaner rabbit diet.

Table 4: Percentage composition of experimental diets.

	Graded levels of A. gayanus and C. pub leaf meal			
Ingredients	T1 (0%)	T2 (2.5%)	T3 (5.0%)	T4 (7.5%)
Maize	40.00	35.00	30.00	25.00
A. gayanus (Leaf meal)	-	2.50	5.00	7.50
C. pubescens(Leaf meal)	-	2.50	5.00	7.50
Soybean meal	10.00	10.00	10.00	10.00
Wheat offal	21.50	21.50	21.50	21.50
Brewers spent grain	25.00	25.00	25.00	25.00
Bone meal	3.00	3.00	3.00	3.00
Salt (Nacl)	0.25	0.25	0.25	0.25
*Vit/min Premix	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**
Nutrient composition				
Energy (ME Kcal/kg) -calculated	2285.03	2217.60	2150.18	2082.75
Crude protein (%)	19.08	19.73	20.70	22.13
Crude fibre (%)	8.70	9.75	9.90	10.25
Ether extract (%)	3.15	3.40	3.85	4.20
Nitrogen free extract (%)	45.16	52.83	51.25	49.01
Phosphorus (%)-calculated	1.71	1.73	1.75	1.78
Calcium (%))-calculated	3.19	3.21	3.24	3.26

***Premix** to provide the following per kg diet. Vitamin A. 1500IU; 11.0mg; Riboflavin, 9.0mg; Biotin, 0.25; Pantothenic acid, 11.0mg, Vitamin K3, 3.0mg B2, 2.5mg; B6, 0.3mg; B12, 8.0mg; nicotinic acid, 8.0mg; Fe, 5.0mg; Mn, 10.0mg; Zn, 4.5mg, Co, 0.2mg; Se, 0.01mg

Table 5: Growth performance of weaner rabbits fed diets containing leaf meals.

		Graded levels of A. gayanus and C. pub leaf meal				
		T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	SEM
1	Initial body weight (g)	450.00	433.33	430.00	450.00	0.46
2	Final body weight (g)	1191.67^{b}	1141.67^{b}	1209.33^{a}	1133.33^{b}	45.10
3	Total body weight gain (g)	741.67	708.33	742.50	650.00	1.22
4	Daily body weight gain (g)	13.24	12.65	13.26	11.61	1.20
5	Total feed intake (g)	2997.17	2854.67	2699.33	2976.67	-
6	Daily feed intake (g)	53.52	50.98	48.29	53.15	1.94
7	Feed conversion ratio	4.07	4.12	3.76	4.61	0.40
8	Daily protein consumption (g)	8.56	8.16	7.72	8.77	0.39
9	Protein efficiency ratio	1.55	1.55	1.72	1.33	0.16

abc = Mean in the same row with different superscripts are significantly (P<0.05) different.. SEM = Standard error of mean. T_1 = (0% forage meal); T_2 = (5% forage meal); T_3 = (10% forage meal); T_4 = (15% forage meal).

Feed Conversion Ratio (FCR)

Rabbits fed diets 1, 2, 3, and 4 recorded FCR of 4.04, 4.03, 3.47, and 4.36, respectively. There were no significant (P>0.005) differences in the feed conversion ratio among the treatments

Results and Discussion

Haematology

Results of the effect of A. gayanus and C. pubescens leaf meal diets on the haematological indices of weaner rabbits are shown in Table 6. There were significant (P<0.005) differences in all the haematological indices studied except in red blood cell count RBC (7.30-7.44/L) and mean corpuscular haemoglobin from 17.21-17.79g/dL However, packed cell volume (PCV) recorded values that ranged from 34.20 - 36.10% while haemoglobin (Hb) values ranged from 11.21 - 12.33g/100 ml. White blood cell (WBC) values ranged from 8.28 - 8.91 x 109/L, mean corpuscular volume (MCV) values from 52.96 -72.64 fL mean cell haemoglobin concentration (MCHC) values ranged from 32.36 - 33.09g/dL, neutrophil from 50.47-52.20%, lymphocytes values from 48.02-49.50% and platelets values from 705.08-966.54 x 109 /L.These values fell within the normal range of haematological indices for weaner rabbits (26). These results are in agreement with (27) and (28) who reported that haematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health of an animal. Blood parameters are good indicators of physiological, pathological and nutritional status of animals as changes in haematological parameters have the potentials of being used to elucidate the impact of nutritional factors and additives supplied in diets of any living creature. Blood parameters are good indicators of physiological, pathological and nutritional status of animals and changes in haematological parameters have the potentials of being used to elucidate the impact of nutritional factors and additives supplied in diets of any living creature. For

example, leucocytes are known to increase sharply when infection occurs, as they are one of the first lines of defence of the body (8);(26) and (27)

The results from this study showed that rabbits fed diets containing A. gayanus and C. pubescens leaf meals as alternative energy and protein feed ingredients maintained normal good health when compared with the control group. From the foregoing, it could be concluded that the blood quality of rabbits fed diets 2, 3, and 4 (5, 10, and 15% were similar and comparable to values for rabbits fed the control diets (0% inclusion). Therefore leaf meals can be used to replace maize in the diets of weaner rabbits up to 15% without any deleterious effect on the haematological (blood) indices.

Table 6: Haematological indices of weaner rabbits fed experimental diets.

S/No	Parameters	T1 (0%)	T2 (5%	T3 (10%)	T4 (15%)	SEM
1	PCV (%)	34.20^{a}	34.46^{a}	36.10^{b}	35.60^{b}	0.1
2	Hb (g/100ml)	11.24^{a}	11.26^{a}	12.21^{b}	12.33^{b}	0.1
3	RBC (x 1012/L)	7.42	7.41	7.30	7.44	0.04
4	WBC (x 109/L)	8.28^{b}	8.87^{a}	8.78^{a}	8.91^{a}	0.05
5	MCV (fl)	52.96^{b}	72.64^{a}	54.59^{b}	54.51^{b}	0.57
6	MCH (g/dl)	17.39	17.21	17.79	17.32	0.01
7	MCHC (g/dl)	33.09^{a}	33.13^{a}	32.36^{b}	32.46^{b}	0.20
8	Neutrophils (%)	51.56^{a}	52.20^{a}	50.47^{b}	51.03^{b}	0.28
9	Lymphocytes (%)	48.02^{b}	48.27^{b}	48.60^{a}	49.50^{a}	0.24
10	Platelets (x 109/L)	707.02^{b}	706.15^{b}	969.04^{a}	968.03^{a}	0.35

abc= Means within a row with different superscripts are significantly (P<0.05) different. SEM = Standarderror of mean. T1 = (0% forage meal); T2 = (5% forage meal); T3 = (10% forage meal); T4 = (15% forage meal).

Serum Biochemistry

Results on the serum biochemical indices of weaner rabbits are presented in Table 7. There were no significant (P.>0.05) differences in the average values of serum albumin, urea, uric acid, alanine amino transaminase (ALT) and the concentrations of very low density lipoprotein (VLDL) and low density lipoproteins (LDL) among the rabbits in the four treatments (T1-T4). There were significant (P<0.05) differences in average values of total protein, globulin, glucose and creatinine among the rabbits in the various treatments. Serum biochemistry is a labile biochemical system which can reflect the condition of an organism and the changes happening to it under influence of internal and external factors. In this study, the results are not in agreement with the report of (22). The values obtained for total protein (60.45 -64g/L) and creatinine (45.04 46.0 mmole/L) were higher than the one reported by (22) indicating a higher protein content in the diets. However, these values fell within the normal range of values as reported by (26) and (27) for healthy rabbits. Although there were significant (P<0.05) differences observed in most of the parameters considered in this study, inclusion of A. gayanus and C. pubescens leaf meals up to the 15% level in the diets of weaned rabbits did not adversely affect the serum

biochemical indices and did not also indicate any health problems among the animals. Therefore the leaf meals (A. gayanus and C. pubescens) can replace maize up to 15% level in weaner diets without any deleterious effects on health.

Table 7: Serum biochemical indices of weaner rabbits fed experimental diets

S/No	Parameters	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	SEM	
1	Total protein (g/L)	60.45[b]	61.30[b]	61.00[b]	63.00[a]	0.08	
	Albumin (g/l)	30.00	31.30	30.37	31.04	0.65	
	Globulin (mg/dl)	30.32[b]	30.51[b]	30.93[b]	31.93[a]	0.08	
	Glucose(mg/dl)	62.67[b]	62.72[b]	58.37[a]	56.02[a]	1.20	
	Creatinine (mmol/L)	46.06	46.00	45.14	45.0[4]	0.04	
	Urea (mmol/L)	3.07	3.10	3.14	3.27	0.05	
	Uric acid (mmol/L)	0.08	0.08	0.08	0.08	0.01	
2.	Sodium (mmol/L)	135.68[a]	135.55[a]	137.04[b]	137.00[b]	0.20	
	Bicarbonate (mmol/L)	22.89[a]	23.20[a]	20.83[b]	21.00[b]	0.15	
	Chloride (mmol/L)	97.44[a]	96.5[b]	98.17[a]	95.87[b]	0.24	
	AST (v/l)	8.00[a]	8.14[a]	7.35b	7.03[b]	0.08	
	ALP (iu/l)	6.00	6.03	6.00	6.01	0.17	
3.	Total cholesterol (mmol/L)	26.67[a]	27.06[a]	26.03[b]	25.06[b]	0.02	
	Very low density lipoprotein.	5.54	5.23	6.42	6.66	0.04	
	Low density lipoprotein	0.50	0.50	0.50	0.50	0.02	
4.	High density lipid (mmol/L)	1.80[b]	1.86[b]	2.03[a]	2.00[a]	0.09	
	Triglycerides (mmol/L)	0.54[a]	0.51[a]	0.64[b]	0.66[b]	0.08	

abc= Means within a row with different superscripts are significantly (P<0.05) different. SEM = Standarderror of mean, AST = Aspertate transaminase, ALP = Alanine phosphatase. T1 = (0% forage meal); T2 = (5% forage meal); T3 = (10% forage meal); T4 = (15% forage meal).

Conclusion and Applications

Results of these investigations show the following conclusions:

1. The two forages contain appreciable amount of protein, fibre, fat and minerals.
2. Legume and grass (Centro and Gamba) have higher energy content more that those found in most grains.
3. Minerals contents (Ca, Ma, Na, Fe and Mn) of the two forage were high enough as to support growth and development of rabbits.
4. Anti-nutritional factors (tannin, and sorghum) contents identified were found to be tolerable to the animals throughout the experimental periods.
5. Gamba grass (A. gayanus) is higher in both tannin and saponnin content than Centro (C. pubescens). The contribution of tannin and saponnin from the two forages increased the tannin and saponnin content of the diets which contained forage samples.

Recommendation

The results from this studies if adopted would reduce cost of rabbit

production. This will in turn increase animal protein intake and thereby improve standard of living, improve healthy work force and in general, increase wealth creation, employment generation, increase in gross national domestic product (GDP) through export of the product to other countries where there are needed, increase in the profit margin (income) of rabbit and the rural dwellers and finally reduce rural to urban migration among others. The results also indicated that an optimal level of 15 % each can be incorporated into diets of growing rabbits without any deleterious effect on the health of growing rabbits

References

1. Abasiekong, S.F., Ugwuene, M.C., and Ugwu, I.M. (2001). Performance of growing rabbits as influenced by mixed feeding of concentrate and forage Book of Proceeding 6th Annual Conference Animal science association of Nigeria, Sept., 17 – 19, Univ. Maid pp 171 – 172.
2. Peiretti, P.G., 2005. Prediction of the gross energy of Mediterranean forages. Journal of Food, Agriculture and Environment, 3:12-104.
3. Waziri, A.F., Anka, S.A., Bala, A.Y. and Shehu, H. 2013. A comparative Analysis of Nutrients and Mineral Elements Contant of Andropogongayanus Kunth and Pennisetum pedicellatium Trin–Nigerian Journal of Basic and applied. Sciences. 21(1) 60 – 64. Source: DOI: 10.4314/njbas.v21i1.9
4. FAO (2006). Statistical database of the Food and Agriculture Organization of the United Nations, FAO, Rome, Italy. http://faostat.fao.org/fapstat/
5. Obioha (1992). A guide to Rabbit Production in the Tropics Avena publication. Enugu. Pp.19-28.
6. Onwudike, O.C. (1995) Use of Legume tree crops of Gliricidia sepium and Leucaena leucocephala as green feeds for growing rabbits. Animal Feed Sci. and Tech., pp. 153 – 163.
7. Osakwe, I.I. and Ekwe, O.O., (2007). Variation in Relative Palatability of different forages Fed to rabbits Anim. Research International, University of Nigeria. Nsukka, 4(1):608-610.
8. Akinmutimi, A.H. and Anachebe, O. C. (2008). Performance of weaner Rabbits fed graded levels of yam and sweet potato peel meal in place of maize based diet. Pakistan Journal of Nutrition, p.700-704.
9. Odimba, N.E. (2006). Influence of forage legumes (Centrosema pubescens Calopogonium phaseloides) on the semen characteristics and testicular dimensions of rabbits. B. Agric.Thesis, College of Animal Science and Animal Health Michael Okpara University of Agriculture, Umudike pp. 33.
10. Ogunbode, S.M., Onidiwura, R.B. and Iyayi, E.A. (2011). Crude protein Digestibility in Centrosema pubescens seed meal at terminal ileum broilers. Book of Proceedings 36th Annual Conference Nigeria society of Animal production 13–16thMarch, 2011, University of Abuja, Nigeria. pp.435–437.
11. Cheeke, P.R. (1986). Potential of Rabbit production in Tropical and sub-tropical Agricultural Systems.

Journal of Animal Science 63:158:1856.

12. Fielding, F. (1991) Leucaena leucocephalaas a rabbit feed, 25-31. Edinburgh, UK. MacMillan Education Ltd.
13. Agbakoba, A.M., Ikeorgu, J.E. and Udealor, E.O.(1994). Evaluation of various alternative local feed sources for poultry growers mash in rabbit feeding. FSRE Workshop.S.E. Zone, Natinal Root Crop Research Institute, Umudike, 7th – 12th De. 1995.
14. Ojewola, G.S., Ukachukwu, S.N. and Abasiekong, S.F. (1999). Performance of growing rabbit fed sole concentrate- forage diets and its mix concentrate – forage diets. Journal of sustainable Agriculture and environment (1June, 1999, pp. 51 – 55).
15. Taiwo, A.A., Adejuyigbe, A.D., Adebowale, E.A. Oshotan, J.S. and David, O. O. (2005). Performance and nutrient digestibility of weaned rabbits fed forages supplemented with concentrate. Nigeria Journal Animal Production. 32: 1 and 2, pp. 74-78.
16. Ofomata, G.E.K. (2000). Nigeria in Maps: Eastern States. Ethiope Publishing House Benin City, Nigeria p. 52. 17. AOAC (2006). Official Methods of Analysis (18th Edition) Association of Official Analytical Chemists, Gaithersburg, MD. 18. Pauzenga (1985). Feeding parent stock. Zootecnia International Pp: 22-25. 19. Artifield (2022). Spectrophotometer Chemistry.Libretext.https://chim. Libretexts.o kinetics
20. Hopkin, D.L., Beattie, A.S., Pirlot, K.L.,(1995). Meat quality, Carcass Fatness and growth of short scrotum lamb grazing either forage rape or irrigated perennial pasture. Australian, Journal of Agricultural research, 35(4): https://doi.org/10.1071/EA9950453 .
21. Saura Calixto, J., Canellas Mut, Soler, L.(1985). Characteristics and fatty acid composition of Almond tegument oil.
22. Ahamefule, F. O., Eduok, G.O., Usman, A. Amaefule, K.U., Boa, B.E., Oguike, S.A. (2006). Blood Biochemistry and Haematology of Weaner rabbits fed Sun-dried, Ensiled and Fermented cassava peels Based diets. Pakistan Journal of Nutrition. 5(3) 248 – 253.
23. Sahito, A., Kazi, F.G., Jakhrani; M.A., Sharp, G.A and Manson, M.A. 2002. Elemental investigation of momordia charantia Linn and Syziginm Jambolana linn using Atomic Absorption Spectrophotometer the nucleus, 39:49 – 54.
24. Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics: A biometrics approach (2nd edition.) McGraw-Hill Co. Inc. New York.
25. Duncan, D.B. (2000). Multiple ranges and multiple F-tests. Biometrics, 11:1-42.
26. Mitruka, B. M. and H.M. Rawnsley, (1977). Clinical Biochemical and Heamatological reference values in normal experimental animal. Masson Publication Co. New York, pp: 102-117.
27. Kronfield, O.W. and Mediway, N.C. (1975). Blood Chemistry In: Textbook of Veterinary Clinical Pathology Publication. Williams and

Williams Co., Baltimore, pp. 81- 96.

28. Onyeyili, P.A., Egwu, G.O., Jibike, G. I., Pepple, D.J. and Gbaegbulan, J. O. (1991). Seasonal variations in haematological indices in the grey-breasted guinea fowls (Numidamel egrisgallata, Pallas). Nigerian Journal of Animal. Production. 18(2):108 – 111.

29. Stewart, M. (1991). Animal physiology Publication.The Open University, U.S.A., pp.132-133.