

**PERFORMANCE AND HAEMATOLOGICAL EVALUATION
OF WEANER RABBITS FED *LOOFAH* GOURD SEED MEAL
(*LUFFA CYLINDRICA* { M.J.ROEM})**

FAS Dairo



Ayodeji Dairo

*Corresponding author: email: fasdairo@yahoo.com

Festus A. Sunday Dairo Senior Lecturer, in Agricultural Biochemistry & Nutrition,
Department of Animal Production & Health Sciences, University of Ado-Ekiti,
P.M.B. 5363, Ado-Ekiti, Nigeria. Tel: 234-8033643670

ABSTRACT

Twenty-four weaner rabbits of mean initial body weight of 554.8 ± 55 g, of New Zealand X Chinchilla cross breed were divided into four groups of three replicates with two rabbits each, in a completely randomized design experiment. They were fed experimental diets in which *loofah* gourd seeds ground into meal (LGSM) replaced soybean meal (SBM) on weight basis at 0%, 5%, 10%, and 15% dietary levels in a 56 day feeding trial. Performance, nutrient digestibility and haematological parameters were monitored. The feed intake though not significantly affected ($P>0.05$) ranged from 57.1 g at 15% inclusion of LGSM to 60.13 g for 10%. The values for the final body weight (1564.04 g), daily body weight gain (18.46 g), feed conversion ratio (3.26), and protein efficiency ratio (0.52) were similar for 5% and 10% inclusion of LGSM and significantly highest ($P<0.05$) when compared to 0% and 15% replacement of soybean meal by the test diet. The percent dry matter digestibility at 15% inclusion (57.47%) and nitrogen digestibility values at the same dietary level (55.98%) were significantly lower ($P<0.05$) when compared to other inclusion levels of LGSM. However, the values were similar for other dietary levels of inclusion. Rabbits fed the control diets had the highest values ($P<0.05$) for ether extract digestibility (67.28%). Also 5% LGSM inclusion had the highest ($P<0.05$) crude fibre digestibility (65.51%). The mean corpuscular volume, mean corpuscular concentration and the mean corpuscular haemoglobin concentration were not affected ($P>0.05$) by the inclusion of LGSM in rabbit diet, however the values of the packed cell volume (43.00%) and haemoglobin (12.57 g/dl), were higher for the 5% and 10% replacement of SBM by LGSM. The values recorded for the red blood cell count, white blood cell count, total protein, albumin and globulin were all similar for rabbits on the control diet, 5% and 10% LGSM inclusion levels but significantly higher ($P<0.05$) than those fed 15% LGSM in the diet. Rabbits fed 15% LGSM gave higher values for the blood urea (7.97 g/100ml) indicating wastage of the dietary protein. The investigation showed that rabbits fed 5% LGSM replacement for SBM exhibited a better performance when compared to other dietary levels of inclusion of the test diet.

KEY WORDS: Rabbit, performance, *Loofah* gourd, haematology

INTRODUCTION

Scarcity of feed resource has been the main limitation in the production of livestock products to meet the animal protein requirements of human and other industrial needs. The conventional cereal and vegetable protein sources being used in animal feeds are under pressure of competition through their use in human diets. The feed cost is about 75% in non-ruminant production out of which protein accounts for about 45% [1, 2]. The conventional vegetable protein sources such as soybean and groundnut cake are very expensive in developing countries like Nigeria due to high exchange rate as many of them still import these commodities. The ban on the use of animal by-products in poultry diets by the European Union has further put pressure on these vegetable protein sources [3]. Hence the need for research into plants of lesser importance to man that may serve as a veritable source of vegetable protein because of the ever increasing cost of the high quality conventional sources [4, 5]. Animal protein intake in most developing countries has been found to be below the recommended level. Countries of lower income (among which Nigeria was grouped) had been reported to consume 21 kilogramme of meat and 40 kilogramme of milk per capita per year on the average while those in the developed nations of higher income consumed 76 kilogramme of meat and 192 kilogramme of milk in the first half of the 1990s, a trend that has not changed much [6]. This constitutes about 26% contribution to the daily protein allowance of about 65-75 g per adult in developing countries [6]. One of the easy ways of bridging the gap of animal protein consumption is through rabbit production because of its short generation interval and the suitability for landless producers and the relatively lower capital cost than required for poultry or any other livestock [7]. However, rabbit production is faced with the problem of feed availability especially those of vegetable protein origin, hence the focus on the *loofah* gourd plant. *Luffa cylindrica* M.J.Roem syn. *Luffa aegyptiaca* Muel is a crawling plant that grows in the wild and on abandoned building structures and fence walls in towns and villages in Nigeria. It belongs to the family of *cucurbitaceae* and originated in tropical Asia [8]. It is a climbing annual wild vine with lobed cucumber-like leaves that are dark - green in colouration with rough surface. The plants with yellow flowers bear fruits that are cucumber shaped but larger in size and contain fibrous sponge in which the hard black seeds are enmeshed. Like most oil bearing seeds and legumes, *loofah* gourd seed have been analyzed to contain some anti-nutritional factors such as tannins, oxalates and phytate [9, 10]. This study was carried out to investigate the effect of the inclusion of the *loofah* gourd seed meal (LGSM) on the performance of weaner rabbits.

MATERIALS AND METHODS

Collection of Experimental Test Material and Preparation of Experimental Diets

Loofah gourd fruits were collected from the plants during the dry season of the year between November and March. The plants which have climbing vines bear fruits that normally dry up at this period. The fruits that have shape like cucumber were harvested manually from the wild; the gourd was crushed, split and peeled off. The fibre sponge was also split opened and was shaken vigorously to release the seeds

enmeshed within it. The seeds were later sun dried, freed of dirt and milled as harvested (hulled) in a plate mill. Other feed ingredients were purchased from the open livestock feed market in Ado-Ekiti, Nigeria. The test ingredient, *loofah* gourd seed meal (LGSM) replaced soybean meal weight for weight at graded dietary levels of 0%, 5%, 10%, and 15% with a control to make four experimental diets. The ingredients were adjusted in the diets to achieve the crude protein levels that varied from 15.08 – 15.89 %, and dietary energy of 10.40 – 10.88 MJ/kg to meet the rabbit requirement (Table 1).

Analysis of Test ingredients and experimental diets

The test ingredients and experimental diets were analyzed for proximate composition (dry matter, crude protein, crude fat, crude fibre, carbohydrate and ash) and for anti-nutrients, tannin, phytin and oxalate before the start of the experiment using AOAC standard methods [11].

Tannin and phytin analyses

Milled samples (200 g) were extracted in 10 ml 70% aqueous acetone for 2 hours at 30 ° C in a water bath. A Gallenkamp orbital shaker at 120 rpm was used. Diethyl ether containing 1% acetic acid was used to extract the fats and the pigments in the sample. Tannin was then determined as total phenols in 0.05 ml aliquot in test-tubes by the addition of distilled water to 1ml mark and the addition of 0.5 ml *Folin Ciocalteu* reagent (Sigma) and 2.5 ml sodium carbonate. The absorbance of the solution was measured at 725 nm after 40 minutes using the method of Makkar and Goodchild [12]. The tannin equivalent in the form of phenol was calculated from a standard curve (Table 3).

The phytin content was quantified by adding 8 g of the milled sample soaked into 200 ml of 2% HCl and allowed to stand for 3 hours. The extract was filtered through a double layer filter paper and 50 ml of the duplicate samples of the filtrate was pipetted into 400 ml beaker. Ammonium *thiocyanate* (0.3%w/v) (10 ml) was used as an indicator and distilled water (107 ml) added to obtain a pH of 4.5. Ferrous chloride solution containing 0.00195 gm Fe/ml was then titrated against the solution of the test samples until a brownish yellow colouration persisted for 5 minutes. Phytin-phosphorous was determined using the relationship that each milligram of iron is equivalent to 1.19 mg of phytin phosphorous (Table 3). The phytin content was then calculated by multiplying by a factor of 3.55 according to Young and Greaves [13].

Experimental Site, Design and Procedure

The experiment was conducted at the Rabbitry Unit of the University of Ado-Ekiti Teaching and Research Farm. Twenty-four crossbred (New Zealand X Chinchilla) weaner rabbits of average initial body weight of 444.8 ± 12.5 g were divided into four groups based on weight basis. Each group was later divided into three replicates comprising of two rabbits each. The rabbits were housed individually in single tier hutches in a completely randomized design experiment for 56 days. They were adapted to diets and the cage environment for a period of 14 days exclusive of the

experimental period. The rabbits were de-wormed using piperazine in water and given *Duococin coccidiostat* as prophylaxis before the experiment commenced. An average body weight of 555 ± 55 g was recorded at the commencement of the trial. During the experiment, feed and water were offered *ad-libitum* to the individual rabbit in the hutches at 0700 hr and 14:00 hr. Air dried *Aspilia Africana* at 500 g per rabbit was provided at 17:00 hr daily as supplement to make for the fibre need. The performance indices namely, feed intake, water intake, body weight gain and feed conversion ratio were measured on weekly basis for the duration of the trial.

During the last ten days of the experiment, a digestibility trial was carried out. One rabbit from each of the replicate group was used for the trial to make three rabbits per treatment. The rabbits were transferred to a metabolism cage adapted from a layer cage and one rabbit constituted a replicate for the digestibility trial. They were allowed to get used to the cages for a period of 3 days during which they were fed the experimental diets. They were fasted for a period of 24 hours to ensure total elimination of the GIT content and then fed for a period of four days. Faecal samples were collected for 5 days, including the fasted day to ensure total collection of the faeces. Polythene sheets were spread under the cages to collect rabbit faeces. The daily collections were dried in an oven at 60°C (Gallenkamp) and analyzed for proximate composition as described by AOAC [11]. On the last day of the trial, blood samples were collected into well-labelled blood sample bottles from the marginal ear vein of the rabbit in each replicate group. They were divided into two blood sample bottles for each rabbit in the replicate group. One out of these was centrifuged at 2500 rpm using a table centrifuge (Gallenkamp) and the serum decanted for the analysis of total serum protein, albumin and globulin. The whole blood was put inside the second sample bottles that already contained ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The samples were immediately taken to the laboratory for haematological analysis. From the whole blood, the packed cell volume (PCV), red blood cell count (RBC), white blood cell (WBC) and haemoglobin (Hb) were determined using the Wintrobe microhaematocrit, Neuber haematocytometer and cyanohaemoglobin procedures, respectively as described by Cole [14]. The total protein was determined using the biuret method as described by Reinhold [15]. Doumas and Briggs method was used for the determination of albumin, while that of globulin was obtained as described by Rodkey [16,17]. The whole blood was analyzed for urea by the diacetylmonoxine method of Varley [18]. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Sastry [19]. The LGSM, the experimental diets and the faeces were analyzed for proximate composition as described by AOAC [11]. The energy for the experimental ration was calculated while that of the LGSM was obtained using the prediction equation of Pauzenga [20] to be able to calculate the metabolisable energy in the diets. Data collected were subjected to one way analysis of variance and means were separated using Duncan Multiple Range Test using SAS computer package [21].

RESULTS

The proximate composition and the anti-nutritional factors in the experimental diets and the LGSM sample are presented in Tables 1 and 2, respectively. The results of the performance indices are also presented in Table 3. The feed intake was not significantly influenced ($P>0.05$) by the inclusion of LGSM. The water intake was similar for the control and 5% inclusion of LGSM but subsequently decreased significantly ($P<0.05$) as the test ingredient increased in the experimental diets. The final body weight was significantly higher and similar ($P<0.05$) at inclusion levels of 5% and 10% than the control and the 15% LGSM. Rabbits on 5% inclusion of LGSM had the highest daily body weight gain ($P<0.05$) followed by those fed the 10% in the experimental diets. The control and the 15% inclusion of LGSM had similar daily body weight gain. The feed conversion ratio (FCR) and protein efficiency ratio (PER) values were significantly ($P<0.05$) lower and similar for rabbits on 5% and 10% inclusion of LGSM than those of the control and 15% LGSM. This implies that LGSM was better utilized by rabbits fed at 5% and 10% dietary inclusion.

The nutrient digestibility data is presented in Table 4. The percent dry matter digestibility and nitrogen digestibility values followed similar trends and were significantly lower ($P<0.05$) in 15% LGSM than the remaining test diets where the values were similar. Rabbits on the control diet had higher ether extract digestibility ($P<0.05$) than the test diets. However, the crude fibre digestibility values were significantly ($P<0.05$) higher and similar in the control, 5% and 10% inclusion than 15% LGSM. All the haematological values were significantly affected ($P<0.05$) by the inclusion of LGSM (Table 5) except the mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and the mean corpuscular concentration haemoglobin (MCHC). The PCV, Hb, RBC, and WBC, values were significantly ($P<0.05$) higher in rabbits fed 5% and 10% LGSM than those on the control diet and 15% LGSM inclusion. The total protein, albumin, and globulin recorded higher values in the control, 5% and 10% LGSM than the 15% inclusion. The urea content of the serum was significantly ($P<0.05$) higher for rabbits on 15% LGSM inclusion than those on the other diets.

DISCUSSION

The proximate composition of the LGSM was similar to the values reported for melon seed *Colocynthis citrullis* meal but lower than the values for soybean and groundnut cake which are vegetable sources of high quality protein [22]. The content of the anti-nutritional factors evaluated in LGSM and the experimental diets namely, tannin, phytic acid and oxalate were at tolerable levels for farm animals which agreed with earlier reports of studies on underutilized plants and herbaceous legumes in animal feeds [23]. Water intake increased with the quantity of feed ingested because it is required for digestion and other metabolic processes in the gastro intestinal tract. However, rabbits fed 15% LGSM had the lowest water intake, which may be due to the astringent property of tannin present in the experimental ration at 15% LGSM that conferred a sharp taste as the inclusion rate of the test ingredient increased [24]. This

effect was masked by the presence of other feed ingredients at the lower dietary levels of LGSM. The astringent property may be responsible for the lower feed intake observed in rabbits fed 15% LGSM inclusion in the diet, as a result of the poor palatability that is generally associated with diets containing appreciable level of tannin which also was responsible for the decline in water intake as the test ingredient increased in the diet. Apart from the anti-nutritional factor constituents of LGSM, the reduced water intake obviously did not enhance the effective utilization of the feed because it is very important for proper mastication of food materials into smaller particles that gives enough surface area for effective enzyme action during digestion and absorption despite the fact that rabbits are herbivores and hindgut fermenter [24]. The feed and water intake at the 10% LGSM dietary inclusion may have been responsible for better utilization of the LGSM through proper digestion. The grit-like nature of the test ingredients must have been well-softened in texture that allowed good enzyme actions and absorption in the intestinal lumen of the rabbits. The trend observed in the final body weight was repeated by the FCR and PER values. The digestibility values for dry matter, nitrogen and crude fibre for rabbits fed 5% and 10% LGSM compared with those on 15% LGSM in the diets indicate that the anti-nutritional factors (ANF's) levels in the former two diets containing the test ingredient did not significantly impair the utilization of these nutrients which agreed with reports obtained from literature [10, 25]. The ether extract was better digested in the control diets than those with LGSM because soybean meal was included in the diet which is of higher quality protein than LGSM. The lower values of the nutrient digestibility at 15% LGSM clearly indicate that the ANF's dietary levels especially tannin may have gotten to a threshold where impairment of growth becomes apparent as a result of poor utilization. The PCV and haemoglobin levels were similar for the 5% and 10% inclusion of LGSM; however this was not the case for values obtained for the RBC. The total serum protein, albumin and globulin all followed the same trend. The values for these haematological parameters are all within the range recommended in literature which indicates to an extent some adequacy of the protein content of LGSM [19, 26]. This shows that the rabbits on 5% and 10% LGSM diets were not malnourished. In fact they had better growth than the control (Table 3). The urea values recorded for rabbits fed 15% LGSM indicate that the LGSM protein was wasted or not adequate to sustain normal growth. However, there was no anaemic condition shown by rabbits on 15% LGSM in the diet.

CONCLUSION

The rabbits fed 5% LGSM exhibited the optimum performance indices, and showed good nutrient and haematological values when compared to those on other experimental diets. It indicates therefore that rabbits can be fed 5% inclusion of LGSM for optimum performance. However, inclusion of LGSM at higher levels may require that it undergoes processing that may enhance its digestibility and utilization. Its use in livestock feeding will therefore be an additional source of vegetable protein not competed for as food when compared with soybeans, groundnut, cowpea, coconut or any other conventional plant protein eaten by human.

Table 1: Composition of the experimental diets (%)

Ingredients	% inclusion of LGSM			
	0	5	10	15
Maize	40.00	40.00	38.50	38.00
†PKC	15.00	15.00	15.00	15.00
Rice bran	8.50	8.50	8.50	6.00
Wheat offal	13.50	13.50	15.00	13.00
Soybean meal	15.00	10.00	5.00	5.00
Salt	0.50	0.50	0.50	0.50
Oyster shell	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00
*Premix	2.50	2.50	2.50	2.50
†LGSM	5.00	10.00	15.00	
Total	100.00	100.00	100.00	100.00
Determined Analysis				
Crude Protein (%)	15.89	15.51	15.08	15.60
Crude fibre (%)	5.80	5.80	5.80	5.60
M.E. (calc.) MJ/kg	10.40	10.64	10.82	10.89
Tannin (%)	0.001	0.014	0.030	0.044
Oxalate (mg/g)	0.005	0.135	0.192	0.321
Phytate (mg/g)	0.003	0.014	0.025	0.047

* The premix supplied the following kg⁻¹ of diet: Vitamins A 800 I.U.; D3 (1,4731.C.U); Riboflavin 4.20mg; Pantothenic acid 5.0mg; Nicotinic acid 20.0mg; Folic acid 0.5mg; Choline 300mg; Vitamin K, 2.0mg; Vitamin B12, 0.01mg; Vitamin E, 2.5I.U; Manganese, 56.0mg; Iodine, 1.0mg; Iron 20.0mg; Copper 10.0mg; Zinc 50.0mg and Cobalt 1.25mg.

†PKC: Palm kernel meal

†LGSM: *Loofah* Gourd Seed Meal

Table 2: Proximate composition and some anti-nutritional factors of hulled Loffah Gourd Seed

Parameters	Hull	Coefficient of variation (CV) %
Dry matter (%)	95.65±0.02	3.51
Crude protein (%)	25.59±0.02	5.90
Crude fat (%)	25.64±0.02	7.80
Crude fibre (%)	6.50±0.02	23.51
Carbohydrate (%)	30.62±0.12	37.57
Ash (%)	7.32±0.11	1.50
Tannin (%)	0.36±0.01	6.93
Phytin Phosphorous (mg/g)	0.09±0.02	3.90
Phytic acid (mg/g)	0.33±0.08	5.80
Oxalate (mg/g)	2.93±0.01	3.51

Data expressed as mean ±SD.

Table 3: Performance of rabbits fed graded levels of LGSM

Parameters	% inclusion of LGSM				SEM
	0	5	10	15	
Feed intake (g)	59.08	60.05	60.13	57.14	3.62
Water Intake (ml)	168.91 ^b	182.50 ^a	161.24 ^{bc}	149.23 ^c	13.38
Initial Body Weight (g)	578.29	542.72	530.28	570.14	55.18
Final Body Weight (g)	1419.22 ^b	1619.04 ^a	1564.04 ^a	1146.14 ^c	55.12
**DBWG (g)	15.01 ^c	19.22 ^a	18.46 ^b	10.25 ^c	0.01
FCR	3.93 ^b	3.12 ^c	3.26 ^c	5.56 ^a	0.30
PER	0.63 ^b	0.53 ^b	0.52 ^b	0.89 ^a	0.05

Means with different superscripts differ significantly (P<0.05) from each other.

**DBWG: Daily body weight gain

Table 4: Nutrient utilization of rabbits fed graded levels of LGSM (%)

Parameters	% inclusion of LGSM				SEM
	0	5	10	15	
Dry Matter Digestibility	70.73 ^a	73.20 ^a	70.97 ^a	57.47 ^b	2.09
Nitrogen Digestibility	66.59 ^a	65.22 ^a	66.77 ^a	55.98 ^b	1.34
Ether Extract Digestibility	67.28 ^a	53.83 ^b	52.48 ^b	51.48 ^c	1.31
Crude Fibre Digestibility	64.77 ^a	65.51 ^a	64.03 ^{a,b}	62.40 ^b	0.83

Means with different superscripts differ significantly (P<0.05) from each other.

Table 5: Haematological characteristics of rabbits fed LGSM

Parameters	% inclusion of LGSM				SEM
	0	5	10	15	
PCV (%)	37.67 ^b	43.00 ^a	38.33 ^{a,b}	32.33 ^c	4.71
Hb (g/dl)	11.30 ^b	12.57 ^a	12.27 ^a	10.41 ^c	0.55
RBC (10 ⁶ /mm ³)	5.37 ^b	5.81 ^a	5.09 ^{bc}	4.80 ^c	0.30
WBC (10 ⁶ /mm ³)	7.37 ^b	8.44 ^a	7.99 ^a	7.03 ^b	0.47
Total Protein (g/100ml)	6.50 ^a	6.57 ^a	6.13 ^a	5.47 ^b	0.43
Albumin (g/100ml)	2.77 ^a	2.77 ^a	2.65 ^a	2.53 ^b	0.14
Globulin (g/100ml)	3.73 ^a	3.76 ^a	3.48 ^a	2.93 ^b	0.41
Urea (mg/dl)	6.05 ^b	5.91 ^c	6.09 ^b	7.97 ^a	0.15
MCV (µm ³)	70.58	74.42	73.43	67.35	11.46
MCH (pg)	21.20	21.66	24.05	21.05	1.61
MCHC (%)	29.96	29.64	33.14	32.20	3.60

Means with different superscripts differ significantly (P<0.05) from each other.

REFERENCES

1. **Agbede JO and VA Aletor** Comparative evaluation of weaning foods from *Glyricidia* and *Leucaena* leaf protein concentrates and some commercial brands in Nigeria. *J.Sci. Food Agric.*2003: 84:21 - 30
2. **Sibbald IR** Measurement of bioavailable energy in poultry feeding stuffs. A review. *Can. J. Anim Sci.* 1982: 62 (4):983-1048
3. **Farrell DJ** Matching poultry production with available feed resources: issues and constraints. *World Poult. Sci.* 2005: J. 61 (2): 298 - 307
4. **Amaefule KU and FC Obioha** The substitution of pigeon pea seeds (*Cajanus cajan*) for groundnut cake and maize in broiler finisher. *Nig. J. Anim.Prod.* 1998:25:9-12
5. **Agbede JO** Compositional studies of differently processed ornamental plant seed flour:*Caesalpinia pulcherima*. *Pak. J. Nutr.* 2004:.3 (4):222-22
6. **Delgado C, Rosegrant M, Steinfeld H, Ehui S and C Courbois** *The Livestock revolution*. In Livestock to 2020 The next Food Revolution. Food, Agriculture, and the Environment Discussion Paper 28. 1999: International Food Policy Research Institute Pp1-4
7. **Spore** *Livestock for the landless*. Bi-monthly bulletin of the Technical Centre for Agricultural and Rural Cooperation. No 46 August 1993: Pp 1-4
8. **Tindall HD** *Vegetables in the Tropics*. Macmillan International College Edition.Macmillan Press, 1983:
9. **Liener IE** Implication of anti-nutritional components in soyabean foods. *Crit. Rev. Food Sci. Nutri.* 1994: 34: 31-67
10. **Aletor VA and OA Omodara** Studies on some leguminous browse plants, with particular reference to their proximate, mineral and some endogenous anti-nutritional constituents. *Anim. Feed Sci & Tech.*, 1994 46: 343 - 348
11. **AOAC**. Official Methods of Analysis. 16th Ed. Association of Officiating Analytical Chemist. Washington DC. 1995: 16th edition
12. **Makkar AOS and AV Goodchild** *Qualification of tannins. A laboratory manual*. International Centre for Agriculture Research in the Dry Areas (ICARDA) Aleppo, Syria IV+ 1996:25pp
13. **Young SM and JS Greaves** Influence of variety and treatment on phytin content of wheat. *Food. Res.:* 1940 5:103-105
14. **Cole EH** *Vet. clinical pathology*. 4th Ed. Philadelphia W.B. Saunders. 1986

15. **Reinhold JG** Manual determination of total serum proteins, albumin and globulin fractions by biuret method In: Standard methods of clinical chemistry. 1953: Academic Press New York
16. **Doumas BT and NG Briggs** Serum albumin by bromocresol green binding. *Stand. Methods. Clin. Chem.* 1972 7:175 - 179
17. **Rodkey FL** Globulin by direct determination. *Clin. Chem.* 1965: **11**: 486
18. **Varley H, Gowelock AH and M Bell** *Determination of serum urea using the diacetyl monoxime method.* Practical Clinical Biochemistry, 5th ed. William Heineman Medical Books Ltd. London. 1980.
19. **Sastry GA** Veterinary Clinical Pathology. 3rd Ed. 2004. CBS Publishers & Distributors New Delhi, India. 2004: Pp 10-17
20. **Pauzenga U** Zootechnica International, December, 1985:22-24
21. **SAS** SAS/STAS. Guide for personal computers. *Version 6. Ed.* 1987: Pp 697- 978
22. **Oji UI, Oruwari BM and RO Iwuagu** Performance of growing broiler chickens fed toasted and untoasted melon (*Colocynthis citrullis*) seed meal. *Trop. J. Anim. Sci.* 1999 1 (1): 43 - 49
23. **Agbede JO** Biochemical composition and nutritive quality of the seeds and leaf protein concentrates from under-utilized tree and herbaceous legumes. PhD Thesis, 2000 Federal University of Technology, Akure, Nigeria
24. **Aletor VA** Anti-nutritional factors as nature's paradox in food and nutrition securities. Inaugural Lecture Series 15. Federal University of Technology, Akure. Aug. 12th, 1999; 10 – 21
25. **Amaefule KU, Nwokoro CC and FC Iheukwumere** The effect of feeding graded levels of raw pigeon pea (*Cajanus cajan*) meal on the performance of, nutrient retention and carcass characteristics of weaned rabbits. *Nig. J. Anim.Prod.* 2004: 31:194-199
26. **Jain NC** Essentials of Veterinary Hematology. Lea & Febiger. Philadelphia: P.O.Box, 1993: Pp 55