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DIETARY EFFECTS OF GLOBE AMARANTH (*GOMPHRENA CELOSIOIDES*) LEAF MEAL ON GROWTH PERFORMANCE, HAEMATOPOIETIC CHARACTERISTICS, HORMONAL PROFILE AND REPRODUCTIVE PERFORMANCE OF RABBITS REARED BY RURAL FARMERS IN NIGERIA

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ABSTRACT

This study evaluated the effects of globe amaranth leaf meal (GALM) - based diets on the growth performance, blood characteristics, hormonal profile and reproductive performance of rabbits. Forty growing rabbits (mean body weight: 1500±0.02 g/rabbit) were randomly allotted to five dietary treatments after equalizing for body weight and sex using a completely randomized design (CRD), with eight rabbits per treatment. Experimental diets were formulated such that dietary treatments T1, T2, T3, T4 and T5 had 0, 5, 10, 15 and 20% GALM inclusion levels, respectively. The rabbits were subjected to 60 days initial feeding trial after initial 14 days adjustment period for the determination of growth performance traits, during which blood was collected at the commencement and at the end of the feeding trial for haematopoietic assays. Thereafter, rabbits were allowed to attain sexual maturity and kept further for another 60 days for evaluation of reproductive performance. All results were subjected to one- way analysis of variance (*ANOVA)* and significant mean values were separated. Results showed that growth performance traits showed no significant ($p>0.05$) effect of dietary treatments, GALM did not depress feed intake (66.23 – 69.23 g/rabbit/day) and weight gain (8.59 – 10.49 g/rabbit/day) and no mortality was recorded. The haematological parameters showed significant ($p<0.05$) effect of diets, except for white blood cell counts (7.74-8.64 $\times 10^9$/L), mean corpuscular haemoglobin (21.20-26.00 pg) and mean corpuscular haemoglobin concentration (37 – 38 g/dl) at the end of the feeding trial. All haematological parameters were within normal clinical ranges for healthy rabbits. Serum biochemical indices showed significant ($p<0.05$) effect of diets on most parameters, except aspartate aminotransferase (AST) (21.00 – 28.33 IU), alanine aminotransferase (ALT) (17.00- 19.67 IU) and triglycerides (1.60 – 1.90 mmol/L) at the end of the feeding trial compared to the baseline values. The hormonal profile showed significant ($p<0.05$) differences between dietary treatments, except estradiol (46.50 – 49.50 pg/L). Reproductive performance traits of rabbits were significantly ($p<0.05$) influenced by diets in terms of litter size at birth (1.00 - 3.50) and average birth weight of kittens (151.60 - 295.00 g/kitten). Reproductive performance was not depressed. The study concluded that globe amaranth leaf meal (GALM) up to 20 % inclusion levels in diets could enhance feed intake and weight gain, blood characteristics, hormonal profile and reproductive performance of rabbits. Agricultural extension agents should educate rabbit farmers on the feeding potentials of globe amaranth leaf meal and the recommended level of inclusion.

Key words: Globe amaranth, blood, environment, extension, growth, hormone, rabbit, reproduction

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INTRODUCTION

The nutritional potentials of forages and herbs are enormous and have not been fully utilized in animal feeding trials, probably due to ineffective agricultural extension service delivery in Nigeria. In developing countries, forages can be used to reduce over dependence on grains. The inclusion of forages in animal diets will ultimately reduce the amounts of concentrate feeds for rabbits. Feeding of forages promotes better growth rate compared to rabbits which are not fed forages [1]. There are various forages used by farmers for rabbit production, including *Gomphrena celosioides*, *Aspilia africana, and Pennisetum purpureum*. *Gomphrena celosioides* commonly known as globe amaranth is an herbaceous annual plant of the family amaranthaceae. It is a weed that is commonly used in the treatment of skin infections and as an abortifacient. In Nigeria, it is used in ethno - medical practices for the treatment of various skin diseases and worm infection. It grows along road sides, river banks, old construction sites and fallow lands. As a medicinal plant, it is a cheap renewable source of pharmacologically-active substances and is known to produce certain chemicals that are naturally toxic to bacteria. The plant leaves, seeds, roots and stems have been shown to have a range of medicinal properties such as antibacterial, antiviral, antifungal, antiprotozoal and hepato-protective without adverse effects. Globe amaranth can be used as a leaf meal for feeding rabbits which could be an ideal alternative for synthetic growth promoters in animal production. The leaf meal contains 15.86 % crude protein, 17.60 % crude fibre, 15.90 % ash and 2.05 % ether extract [2]. The nutrient profile makes the leaf meal appropriate for feeding rabbits to maintain growth at 15 % crude protein level [3]. However, it has some anti - nutrients like tannin (0.54 %), phytates (0.85 %), oxalate (1.07%), saponin (0.95 %) and hydrogen cyanide (0.66 %) which are minimal and effects may wear off while processing the leaf meal.

Furthermore, the Globe amaranth is a flowering plant with significant environmental and geographic implications. These implications are linked to its adaptability, ecological benefits and cultural relevance. Globe amaranth flowers are rich in nectar, making them attractive to bees, butterflies and other pollinators. This supports biodiversity and enhances pollination in agricultural and natural ecosystems. The plant's deep root system helps in preventing soil erosion, especially in regions prone to degradation. Its ability to thrive in arid and semi-arid conditions makes it suitable for greening degraded landscapes and conserving water resources. The plant contains antioxidants and bioactive compounds. These properties contribute to traditional medicine and functional foods, particularly in tropical and subtropical areas of the world. Like other plants, globe amaranth contributes to carbon fixation, although modestly, playing a role in mitigating climate change. Its vibrant flowers and low water requirements make it suitable for urban green spaces, contributing to heat island reduction and aesthetic enhancement. It has positive environmental roles such as biodiversity support and soil stabilization, but its spread should be managed to prevent ecological imbalance. Its geographic versatility reflects its importance in addressing environmental challenges like erosion and climate adaptation.

In realization of the importance of animal protein, governments of various developing and developed countries have been pursuing programmes at various levels to boost intensive livestock production, so as to ensure the attainment of the recommendation of Food and Agriculture Organization (FAO) of 35 g of animal protein intake per day [4]. Rabbit (*Oryctolagus cuniculus*) is a micro - livestock species. the production of rabbits is one of the cheapest and easiest ways of producing high quality animal protein, for the increasing human population. Rabbits are prolific and being herbivores can survive and convert cheap fodder into animal protein for human consumption. They do not compete with humans for their food and can convert 20 % of the protein they consume into edible meat completely, compared to 22 - 23 % for broiler chickens, 16 – 18 % for pigs and 8 – 12 % for cattle [5]. Rabbits can convert protein in cellulose-rich plants which is not economical as feed ingredient for poultry that depends on soybean meal and grains which are also consumed by man, as such making, these feedstuffs highly competitive.

Studies on the extensive utilization of forages in feeding trials of rabbits as supplements to concentrate meals, either as pellets or in complete diets have been reported by previous authors [6]. Forages supply protein and energy as well as fibre which aid digestion, which is most suitable for rabbits as they have the ability to properly digest leaf protein, thus the name pseudo-ruminants. There is still paucity of satisfactory research findings on the utilization of globe amaranth leaf meal in rabbit feeding trials, hence its utilization as a test ingredient in this study. This study was designed to determine the dietary effect of globe amaranth leaf meal on growth performance, haematopoietic characteristics, hormonal profile and reproductive performance of rabbits reared by rural farmers in Cross River State, Nigeria.

MATERIALS AND METHODS

Location of study

The study was carried out at the Rabbitry Unit of the Teaching and Research Farm, University of Calabar, Calabar. The study site is located at latitude 40° 57´ N of the equator and longitude 80° 19´ E of the Greenwich meridian, with a land mass of 233.2sq. miles (604km^2). The average annual rainfall is 1830 mm and daily temperature is 24.30°C (770F), respectively and a relative humidity between 70 and 80 % [7].

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Harvesting and processing of globe amaranth leaf meal (GALM)

Fresh leaves of globe amaranth were harvested within the University staff quarters and environs and air dried to constant weight in order to maintain the green colour before milling. Chemical analysis of the leaf meal was done according to methods of AOAC [8]. Milled samples were stored in air-tight containers until the time of feed formulation.

Experimental diets and determination of chemical composition

Five experimental diets were formulated and Globe amaranth leaf meal (GALM) was included at the following levels (0, 5, 10, 15 and 20 %) for treatments T1, T2, T3, T4 and T5, respectively. The gross composition of experimental diets is presented in Table 1. The proximate composition of experimental diets was carried out according to the methods of AOAC [8].

Experimental animals and management

Forty growing cross bred rabbits (20 bucks and 20 does) aged between 6 and 8 weeks old, with initial mean weight of 1500±0.02 g/rabbit were purchased from a rural farm in Akpabuyo Local Government Area, Cross River State, Nigeria for this study. The rabbits were weighed and randomly assigned to five groups on weight and sex equalization basis. Each group/dietary treatment comprised eight (8) rabbits (4 bucks and 4 does). The rabbits were intensively managed in wooden cages in a standard rabbitry. In preparation for the arrival of the animals, the hutches were properly constructed, washed and disinfected with Izal to destroy all residual pathogens. On arrival, animals were allowed to acclimatize for two weeks and during this period, they were dewormed before commencement of the feeding trial. Standard concrete feeders and drinkers were provided and clean drinking water was provided *ad-libitum* throughout the experimental period. The feeding trial lasted 60 days for growth performance determination and another 60 days for reproductive performance trial, making a total of 120 days.

Experimental design

The rabbits were randomly assigned to different dietary treatments after weight and sex equalization in a Completely Randomized Design (CRD). The experimental model used was as follows: $Yij= \mu + Ti + Eij$.

Where: Yij= Observed value μ=Overall mean value Ti=Random treatment effect Eij= Random residual error.

Determination of growth performance parameters

Initial body weight of rabbits per replicate was taken at the beginning of the study and subsequently on a weekly basis. A top loader scale was used to determine the weight of the rabbits.

Weight gain (WG) was determined by the difference method. The initial weight was subtracted from the current weight and the value for weight gain estimated per individual rabbits/day. The formula was as follows:

$$WG = \frac{NW - IW}{7}$$

Where: NW= New weight; IW= Initial weight

The daily amount of feed served and left over were measured with a sensitive electronic scale (Kerro model) and the feed intake estimated as follows: Feed intake = Quantity of feed served - Quantity of left-over feed.

Feed conversion ratio (FCR) was determined as the ratio of the average daily feed intake to average daily weight gain.

$$FCR = \frac{\text{Av. daily feed intake (g/day)}}{\text{Av. daily weight gain (g/day)}}$$

Incidence of mortality per treatment was recorded and calculated as follows:

$$\%\ Mortality = \frac{\text{No. of dead rabbits/ treatment}}{\text{Total no. of rabbits/ treatment}} \times \frac{100}{1}$$

Determination of reproductive performance of rabbits

At maturity, pairing and mating of buck and doe in a particular treatment/diet was done. A pairing ratio of 1:1 (Buck: Doe) was maintained, the doe was introduced to the buck. Animals cohabited for three days to ensure successful mating. Gestation length was taken from the first day of pairing. Pregnancy was diagnosed by a gentle palpation of the abdomen and body weight changes to ascertain pregnant does at 14 - 16 days post mating. Pregnant does were separated and the non - pregnant does were re-mated. Kindling boxes were provided at 3rd week of gestation. The following reproductive parameters were determined. In Does: pregnancy rate, litter size, average birth weight, average weaning weight at 6 weeks and mortality. Milk yield (g/day) was estimated using the formula given by Nguyen *et al*. [9] as 1.18 × (live weight of litter at 21 days - live weight at birth). In Bucks, the weight of testes and the length of the right and left testes were determined accordingly.

Blood collection and assays

Blood samples were collected at the beginning of the study (for baseline data) and also at the end of the feeding trial for haematological and serum assays. Two blood samples (5ml) per rabbit were collected from 4 rabbits (2 bucks:2 does) per treatment (20 rabbits sampled) through the prominent ear vein by means of hypodermic needles and syringes into test tubes. One set of the test tubes contained an anti-coagulant, Ethylenediamine tetra acetic acid (EDTA) for haematological assay, while the other set of test tubes were plain without EDTA for serum biochemical assay.

Determination of haematological parameters
All haematological parameters were determined by standard laboratory methods. The parameters determined were packed cell volume (PCV), erythrocyte counts, haemoglobin concentration, and total white blood cell (leucocyte) counts. Others were granulocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets.

Serum biochemical parameters
Total serum protein determination was by the Biuret method of serum total protein determination. Albumin was determined using Bromocresol Green (BCG) method. The globulin concentration was obtained by subtracting albumin values from the total protein. Alanine and aspartate aminotransferase (ALT and AST) activities were assayed using Randox commercial enzyme kit. Glucose and cholesterol were determined using spectrophotometric method.

Hormonal assay
Blood samples (four per treatment from both sexes) were collected from the marginal ear vein of the experimental rabbits before commencement of the feeding trial as the base line records, and at the end of the feeding trial. The blood samples were collected into a set of sterile plastic bottles and allowed to coagulate to produce sera for hormonal analyses. The test for hormonal parameters in the blood serum (testosterone, follicle stimulating hormone, progesterone and estradiol were carried out with the aid of the tube-based enzyme immunoassay (EIA) method. The protocol used for the hormonal assay was according to the method described in the Kit [BioCheck ELISA Assay, USA]. The hormonal assay was carried out before and after the feeding trial.

Statistical analysis
All data obtained were subjected to one - way analysis of variance using GenStat Release 10.3 statistical package. Significant means were separated using the least significant difference (LSD) method of the same statistical software as described by Steel and Torrie [10].

RESULTS AND DISCUSSION

The growth performance characteristics of rabbits fed globe amaranth leaf meal-based diets are presented in Table 2. There were no significant ($p>0.05$) differences between the treatments in all the parameters. However, the final body weight (2087.00 g/rabbit) for treatment 5 with 20 % GALM inclusion level had the highest numerical value, while treatment 1 with 0% GALM inclusion level recorded the least value (2018.75g/rabbit). Total weight gain increases gradually and treatment 4 with 15 % inclusion level of GALM recorded highest numerical value (593.79g) compared to treatment 1 (481.25g/rabbits). The average daily weight gain recorded increasing numerical values as dietary inclusion level increases. The total feed intake recorded the highest numerical value in treatment 4 (519.79 g) compared to treatment 1 with the least (553.86 g). The growth performance characteristics of rabbits fed GALM based diets showed no significant (p>0.05) difference in all parameters. Akinmutimi and Obioha [2] who fed globe amaranth leaf meal to rabbits also reported non-significant effect in all parameters. There was an increasing trend for total feed intake (TFI), total weight gain (TWG) and average daily weight gain (ADG), but feed conversion ratio (FCR) decreased marginally as the dietary inclusion levels of globe amaranth leaf meal increased. The daily weight gain (DWG) obtained increased marginally across treatments, this implied increased weight as a result of increased dietary inclusion of globe amaranth leaf meal. This was in agreement with the report of Iyeghe – Erakpotobor [11], who stated that rabbits increased body weight when fed diets containing forages. The DWG was lower than the range (15.63-16.07g) reported in Akinmutimi and Obioha [2], who fed globe amaranth leaf meal to rabbits. Mohammed *et al*. [12] also reported a higher range (10.15-14.80g) when different leaf meals were fed to rabbits. Daily weight gain was also higher than the range (5.17 - 6.20g) reported by Okpanachi *et al*. [13], who fed roughages and different leaf meals to rabbits. Variations could be as a result of age, types of feed materials used, and prevailing environmental factors associated with the separate studies. The daily feed intake increased marginally across dietary treatments. This increased range indicated that animals increased feed intake to meet their energy requirements, which was in agreement with reports by Iyeghe- Erakpotobor and Nwagu [14]. The feed conversion ratio recorded a downward decreasing trend, which was comparable with the range (4-8) reported previously by Ngodigha *et al*. [15], who fed agricultural by-products to rabbits. This implied that treatments with 15 and 20 % GALM with the lowest FCR value were the best diets in this study. Adeyemo *et al*. [16] reported that rabbits perform better on feed mixture of concentrate and forages, the reason for increased values of growth parameters as the globe amaranth inclusion levels increased in this study. However, Iyeghe-Erakpotobor [11] reported that nature of feed and age affect the feed conversion ratio. Hence, variations in growth performance characteristics of rabbits fed globe amaranth leaf meal could be due to the age of the rabbits and differences in feed ingredients.

The haematological characteristics of rabbits fed globe amaranth leaf meal-based diets are presented in Table 3. Lymphocytes, granulocytes, red blood cell counts (RBC), haemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular

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haemoglobin concentration (MCHC) and the platelets were significantly different ($p<0.05$) at the base line level and at the end of the feeding trial, respectively. White blood cell counts and mean corpuscular haemoglobin (MCH) were both unaffected at the base line and at the end of the feeding trial. Granulocytes, haemoglobin, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) recorded decreased trends when compared at both the base line and at the end of the feeding trial. White blood cell counts recorded no significant effect at both the base line and at the end of the feeding trial. However, treatment 5 with 20 % inclusion level of GALM had the highest numerical value (8.33 ×109/L) and the least (7.32 ×109/L) was treatment 4 with 15 % inclusion level of GALM for the base line records. The haematological parameters in this study fell within normal clinical ranges for healthy rabbits as reported by Medirabbit [17]. This implies that the GALM and its associated anti - nutrients may not have any serious adverse effects on the haematological characteristics of rabbits. White blood cell counts were not significantly ($p>0.05$) affected, this implied that GALM did not adversely affect the WBC count of rabbits. The range of WBC at the end of the feeding trial was slightly higher than the range (2.85 – 6.00 $\times10^{9}$/L) earlier reported by Ngoshe *et al*. [18] and Shaahu *et al.* [19] who fed carrot leaf / moringa leaf and cassava leaf meal, respectively to rabbits, but were within the range (5.40 - 10.00 $\times10^{9}$/L) as reported by Ahemen *et al*. [20] and Evans *et al*. [21] who fed different leaf meals and some agricultural by products to rabbits. Ubua *et al*. [22] fed neem leaf meal to rabbits and reported a range of 4.00 - 11.30 $\times10^{9}$/L. Differences in WBC counts could be attributed to different leaf meals used in the separate studies. The RBC and Haemoglobin concentration recorded a decreased pattern across dietary treatments, but were within the recommended normal ranges for haemoglobin and RBC. The RBC counts were within the range 6.50 - 6.70 $\times10^{12}$/L as reported by Etim *et al.* [23] who fed water spinach leaf meal to rabbits, but higher than 3.94 - 4.50 $\times10^{12}$/L of Henry *et al*. [24] who fed pawpaw leaf meal to rabbits. Variations could be attributed to differences in dietary leaf proteins in the different studies. Haemoglobin range obtained at the end of feeding trial was higher than range (8.92-13.67 g/dl) reported by Henry *et al.* [24], who fed pawpaw leaf meal to rabbits. Haemoglobin molecule is an oxygen carrier from the lungs to other tissues; excess haemoglobin gives dark blood colouration and heart attack. Animals in this study did not manifest obvious adverse effect. The packed cell volume (PCV) recorded a decreased pattern as the dietary inclusion level of GALM increased across treatments. The PCV range was higher than the range (31-38 %) reported by Ahemen *et al*. [20], who fed moringa leaf meal to rabbits. Okosun *et al*. [25] reported that PCV value of less than 30 % indicates anaemia, especially when the red blood cell and hemoglobin levels are low as well. Rabbits in this study did not display any form of infection as the PCV was well above 30%. The haemoglobin and PCV pattern in this study showed a

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relationship which agrees with Etim *et al*. [23], that PCV and haemoglobin exhibit a correlation which is common in healthy and normal rabbits. The lymphocytes recorded marginal increase and were significant ($p<0.05$) across dietary treatments at the end of the feeding trial. The values recorded were within the standard recommended range for rabbits, and slightly higher than 55 - 73 % reported by Shaahu *et al.* [19] and Okosun *et al.* [25] who fed false yam (*Icacina tricantha*) and cassava leaf meal, respectively to rabbits. Increased significant range indicated that globe amaranth leaf meal affected the lymphocyte counts. The granulocytes recorded a decreased trend which was significantly affected by the leaf meal. Granulocytes (neutrophils, basophils, eosinophils) phagocytize foreign bodies and prevent infections [26]. However, ranges were within recommended values for rabbits. The mean corpuscular volume (MCV) recorded a decreased marginal pattern as dietary inclusion of globe amaranth leaf meal increased. The range obtained was higher than (29 – 28 fl) reported by Okpanachi *et al*. [13] and Shaahu *et al*. [19] who fed different leaf meals to rabbits but was within the range (69 -71 fl), as reported by Henry *et al*. [24] who fed pawpaw leaf meal. The mean corpuscular volume (MCV) recorded no significant ($p>0.05$) effects meaning that the leaf meal diets did not negatively affect the blood mean corpuscular volume as well as other red blood cell indices.

The blood serum biochemical indices of rabbits fed varying levels of globe amaranth leaf meal-based diets are presented in Table 4. Base line results were significantly ($p<0.05$) different for all parameters except for Potassium (K^+), high density lipoprotein (HDL), low density lipoprotein (LDL), and at the end of the feeding trial all parameters recorded significant ($p<0.05$) differences except for ALT, AST, very low-density lipoprotein (VLDL) and triglycerides (TGC). All parameters recorded increasing trends across dietary treatments except for AST, Total cholesterol, VLDL and TGC which recorded reducing values. Serum biochemical characteristics of rabbits fed GALM based diets revealed significant ($p<0.05$) effect for most parameters except alanine amino transferase (ALT), aspartate amino transferase (AST), very low-density lipoproteins (VLDL) and triglycerides (TGC) at the end of the feeding trial. All parameters were within the recommended ranges which indicated that globe amaranth leaf meal did not have any adverse effect on serum biochemical parameters of rabbits. The serum glucose recorded a decreased pattern as dietary inclusion levels of GALM increased. The range obtained was within range (2.67 – 3.40 mmol/L) as reported by Jiwuba *et al*. [26], who fed Moringa leaf meal and Audu *et al*. [27], who fed diets containing *Ficus sycomorus* and *Parkia biglobosa* leaf meals to rabbits. Decreased blood glucose level could be attributed to the anti – nutrients in the leaf meal, in which Audu *et al*. [27] reported that anti-nutrients like saponins present in forages reduce blood serum glucose. The Total cholesterol recorded increased significant ($p<0.05$) effect as dietary inclusion of GALM increases, at the end of the feeding trial. The range obtained was higher than range (1.17-2.60 mmol/l), reported by Audu *et al*. [27]. Serum triglyceride range obtained at the end of the feeding trial was higher than 1.3-1.50 mmol/L, as reported by Selim *et al*. [28]. Ranges for total cholesterol and triglycerides were within the standard recommended ranges for healthy rabbits. Observed decreased pattern reported for triglycerides probably suggests a general decrease in lipid mobilization, indicating that GALM has indirect inhibitory effects exerted on some key enzymes involved in cholesterol biosynthesis. The high-density lipoprotein (HDL) and low-density lipoprotein (LDL) recorded an increased significant ($p<0.05$) effect at the end of the feeding trial. Hence, ranges obtained were higher than HDL (0.70- 9.05 mmol/L) and LDL (0.55-0.78 mmol/L) reported by Enyeniyi *et al*. [29]. The HDL and LDL exhibited a relationship which was not in agreement with the report by Akinmolodu and Akinloye [30], who stated that the HDL and LDL concentrations are usually inversely related for normal and healthy rabbits. The total protein and albumin recorded decreased patterns at the end of the feeding trial, ranges were within the recommended standards for rabbits. The total protein was within the range (7.03-8.15 g/dl), as reported by Aljoni and Abduljawad [31], who fed moringa leaf meal to rabbits. The albumin range was slightly higher than the range (3.41-3.91 g/dl), reported by Adeyemi [32]. Globulin recorded a decreased pattern as dietary inclusion levels increased. The range (3.33 - 3.60 g/dl) was lower than the range (5.26- 6.94 g/dl), reported by Otchoumou *et al.* [34]. Total Protein is useful in differentiating acute conditions from persistent diseases that have suddenly deteriorated [12]. Rabbits in this study did not show any form of adverse disease condition. The liver enzymes, ALT and AST were not significantly ($p>0.05$) affected indicating that the GALM did not affect these enzymes adversely. The enzymes, ALT and AST ranges obtained were within the ranges (11.67-28.67 UI) and (11.33- 28.67 UI), as reported by Ozung [33] who fed diets containing cocoa pod husk meal to rabbits. Onyekwere *et al*. [35] who fed moringa leaf meal, reported lower range (16.87 – 19.84 IU) for ALT and (18.72- 25.62 IU) for AST, respectively. Variations could be as a result of differences in dietary leaf meals used in the separate studies. Lower ALT and AST indicate better immunity and optimum liver functions [30]. Sodium and potassium levels decreased significantly, while calcium increased significantly as the dietary inclusion levels of GALM increased at the end of the feeding trial. Parameters were within the recommended standards for rabbits, which indicated that the diets did not affect the blood mineral levels. The sodium and potassium ranges were lower than ranges (7.90-10.50 mmol/L) and (0.48-6.8 mmol/L), reported by Ubua *et al*. [22], who fed neem leaf meal to rabbits. Decreased values of sodium and potassium implied that the anti-nutrients may have interfered with the mineral balance of the blood of rabbits. Urea recorded a fluctuating significantly increased trend at the end of the feeding trial. Urea range was higher than the range (3.4-4.9 mmol//L), reported by Jiwuba *et al*. [26]. The increased trend of urea across treatments indicated protein adequacy of the diets used in this study.

The hormonal profile of rabbits fed varying levels of globe amaranth leaf meal-based diets is presented in Table 5. Progesterone and follicle stimulating hormone were significantly (p<0.05) different at the baseline data. At the end of the feeding trial, progesterone, follicle stimulating hormone and testosterone recorded significant (p<0.05) differences between dietary treatments. Parameters recorded a marked increase in values across treatments, except for follicle stimulating hormone for the doe on treatment 4 (15% GALM), which remain approximately the same (11.00 IU/L) for both the base line records and at the end of the feeding trial. The hormonal characteristics of rabbits fed GALM based- diets recorded significant (p<0.05) effect for most parameters except for estradiol which remained unaffected and testosterone that became significantly affected at the end of feeding trial. Progesterone range obtained at the end of the feeding trials was higher than (14.50-18.20 ng/ml), as reported by Henry *et al*. [24] who fed pawpaw leaf meal and Onyekwere *et al*. [35] who fed garlic meal to rabbits. Increased progesterone indicated that the GALM contains some minerals which increased the hormonal level as reported by Cheah and Yang [36] that some plant leaves and extracts contain minerals and vitamins which increase the hormonal level in rabbits. Follicle stimulating hormone range obtained at the end of the feeding trial recorded fluctuating decreased significant (p<0.05) effect as dietary inclusion of GALM increased, which indicated the effect of diets. Values obtained at the end of the feeding trial were within the range (9.00-20.5 IU/l) as reported by Ogbuewu *et al.* [37], who fed ginger meal to rabbits. Decreased trend indicated that the anti - nutrients in GALM may have affected the important vitamins and minerals necessary for enhancing the level of follicle stimulating hormone production in the blood and Ogbuewu *et al*. [37] confirmed that some plants contained some bioactive ingredients that have negative effect on biosynthesis and release of hormones. The follicle stimulating hormones for the buck range obtained at the end of the feeding trial was higher than (5.0-11.5UI/L) reported by Ogbuewu *et al*. [37]. Decreased range of follicle stimulating hormones of the buck indicated that the anti -nutrients in the GALM might have affected vitamins and minerals that can enhance follicle hormone level. The decreased range of follicle stimulating hormones for the buck and female rabbits (does) indicate reduced spermatogenesis and oogenesis which effect manifested in the low litter size recorded in this study. Estradiol recorded non - significant (p>0.05) difference, this implied that estradiol was not affected by the dietary treatments. The range obtained at the end of the feeding trial was higher than the range (28.61-36.72 pg/ml) reported by Henry *et al*. [24], but lower than (166.35-

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175.05 pg/ml) reported by Ogbuewu *et al.* [37]. Testosterone range obtained at the end of the feeding trial recorded increased significant ($p<0.05$) effect indicating the effect of GALM. The range obtained at end of the feeding trial was higher than 9.14-4.54 ng/ml reported by El- Mary *et al*. [38] who fed diets containing ginger powder to rabbits; Henry *et al.* [24] and Onyekewere *et al*. [35] who fed pawpaw leaf meal and garlic bulb powder to rabbits, respectively. Variations could be as a result of differences in the feed materials used, genetic makeup and prevailing environmental conditions during the separate studies. Increased range of testosterone with GALM which El- Mary *et al*. [38] confirmed that micronutrient elements present in some forages are the bases for increased serum hormones. These nutrients influence sertoli cells and leydig cells involved in the secretion of some reproductive hormones (androgens). Increased testosterone level as dietary inclusion level of GALM increased indicated that the leaf meal contains some androgenic analogue which acts on the hypothalamus to stimulate testosterone release, through the action of gonadotropin releasing hormones (GnRH).

The reproductive performances of rabbits fed globe amaranth leaf meal-based diets are represented in Table 6. Parameters recorded no significant effect except for average litter size at birth and average birth of kitten were significantly ($p<0.05$) affected by treatments. Conception rate was the same for all treatments except treatment 4 (50 %) with 15 % inclusion level. The average litter size was highest in Treatment 1 (3.5) and treatments 2 and 3 recorded the lowest (1.00) value. Average birth weight recorded high significant ($p<0.05$) effect in treatment 3 with 10 % inclusion level of GALM (295.00 g) and treatments 1 (151.71 g/kitten) and 5 with 20 % inclusion level of GALM (151.60 g/kitten) were the lowest. Average weight of kitten at day 21 was high in treatment 2 (361.00 g) and treatment 1 (223.4 g) low. Milk yield recorded the high value in treatment 2 (757.56 g) and the lowest was in treatment 5 (162.00 g). The average weaning weight was high in treatments 2 (550 g) and 4 (389.40 g) was the lowest. Number of rabbits alive at weaning was high in treatments 1 (7.00) and treatment 3 (0.00) was the lowest. Mortality rate was highest in treatments 3 (100 percent) and 4 recorded none. Cases of abortion was seen in treatments 3, 4 and 5, respectively. The reproductive performance of rabbits fed globe amaranth leaf meal-based diets recorded no significant ($p>0.05$) effect for most parameters, except average litter size and average birth weight. The average birth weight recorded a fluctuating decreasing trend. Values obtained were within the range (179.75-231.67 g) reported by Adeyemi [32] and Onyekwere *et al.* [35] and slightly higher than range (120-192 g). Decreased range in birth weight indicated the leaf meal affected litter weight; hence increase inclusion level up to 20 % reduced the litter weight. The average litter size recorded fluctuating decreased pattern. The range was lower than 3.75-6.80, as reported by Adeyemi [32], Henry *et al*. [24] and

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Onyekwere *et al.* [35], but was within the range (6.0-1.00), as reported by Ozung [33], who fed cocoa pod husk meal to rabbits. Variations could be as a result of age, different feed materials used or environmental factors. The litter size in this study fell below the average value (4.27-6.75) reported for Dutch and New Zealand rabbits and (6.50-7.25) in cross bred rabbits [39]. Low litter size indicated that GALM did not favour the number of litters in breeding females, this was evidenced in reduced follicle stimulating hormone reported in the female rabbits. The gestation length (30-32days) was within the range (31.00-32.75 days), as reported by Adeyemi [32]. The relationship between gestation length, litter size and birth weight corroborated with the report of McClure [40], that asserted that increased gestation length in rabbits resulted in higher birth weight and reduced litter size. Pregnancy rate recorded uniformity for all treatments. The result was different from that reported by Adeyemi [32] who made use of moringa leaf meal in diets of rabbits. The weight of rabbits at day 21 and milk yield decreased marginally, showing dietary effect, and the weight of rabbit at 21 days was within the range (250-200 g), reported Ozung [33]. The milk yield range was comparable with the range (517-836g) reported by Otchoumou *et al.* [34]. Decreased pattern of milk yield indicated that globe amaranth leaf meal may have affected the milk production, this contradicted the reports of Otchoumou *et al.* [34] who reported increased milk yield on moringa leaf meal diets. The average weaning weight also recorded the same pattern. The range was within the range (368- 862 g) reported by Onyekwere *et al*. [35] and lower than (617- 580 g) reported by Otchoumou *et al.* [34].

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The study concluded that globe amaranth leaf meal (GALM) can be included as an alternative plant protein source in diets meant for rabbits. For optimum growth performance, blood and hormonal profile as well as reproductive performance of rabbits, GALM should be included at levels up to 20 % in animal diets.

The study recommended that globe amaranth leaf meal (GALM) at not more than 20 % should be included in diets meant for rabbits. Agricultural extension workers are encouraged to educate rabbit farmers on the usefulness of this lesser known and under-utilized plant leaves as a means of boosting animal protein intake, especially in the rural communities of Nigeria. The massive cultivation of the plant is also advocated in view of its numerous environmental benefits in the ecosystem.

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Authors' contributions

Authors made the following contributions to this study: Ozung, P.O. and Ekpo, P.E. conceived and designed the study and developed the manuscript. Anoh, K. U., Ekpo, P.E. and Eyong, I.I. executed the experiment and analyzed blood samples. Ebegbulem, V. N., Idiku, F. O. and Akintoye, O. A. analyzed the data and arranged results into Tables. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Conflict of interest

None

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Table 1: Gross composition of experimental diets

Ingredient	T1 (0 % GALM)	T2 (5 % GALM)	T3 (10 %GALM)	T4 (15 % GALM)	T5 (20 % GALM)
Yellow maize	44.00	44.00	44.00	44.00	44.00
GALM	0.00	5.00	10.00	15.00	20.00
SBM	15.80	15.80	15.80	15.80	15.80
Rice husk	12.50	12.50	12.50	12.50	10.50
Wheat offal	20.00	15.00	10.00	5.00	3.50
PKC	3.00	3.00	3.00	3.00	1.50
Bone meal	2.00	2.00	2.00	2.00	2.00
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Crayfish dust	2.00	2.00	2.00	2.00	2.00
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Determined analysis:					
%CP	16.00	16.14	16.43	16.57	16.71
%CF	8.06	8.06	8.17	8.23	8.27
ME (Kcal/kg)	2448.85	2487.85	2525.85	2585.85	2591.21
Proximate composition of GALM					
DM: 95.40%, CP:16.05%, CF:31.15%, ash: 5.06%, ether extract: 8.75% & NFE: 38.99%					

GALM: Globe amaranth leaf meal; SBM: soybean meal

Composition of premix: Vitamin A = 3000000, D2 = 6000000, E=4000mg, K3=5000mg, B1=320mg, B2=100mg, Ferrous sulfate = 800mg, Zinc oxide =1000mg.

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Table 2: Growth performance characteristics of rabbits fed globe amaranth leaf meal-based diets

Performance trait	T1	T2	T3	T4	T5	SEM
No. of rabbits per diet	8	8	8	8	8	-
Initial wt. (g/rabbit)	1537.50	1537.50	1525.00	1475.00	1500.00	37.86
Final wt. (g/rabbit)	2018.75	2050.00	2068.75	2068.79	2087.00	27.87
Total wt. gain (g/rabbit)	481.25	512.50	543.75	593.79	587.00	27.65
Av. Daily wt. gain (g/rabbit/day)	8.59	9.15	9.70	10.49	10.45	0.49
Total feed intake (g/rabbit)	519.79	528.84	530.95	553.86	532.25	23.50
Av. Daily feed intake (g/rabbit/day)	64.97	66.23	66.38	69.23	66.53	1.78
FCR	7.56	7.24	6.84	6.59	6.37	0.94
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00

Means along the same row are not significantly ($p>0.05$) different

Av: Average and FCR: Feed conversion ratio

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Table 3: Haematological characteristics of rabbits fed globe amaranth leaf meal - based diets

	Baseline data at the onset of the study						Data at the end of feeding trial						
Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**Ref. values***
WBC ($\times10^9$/L)	7.66	7.62	7.90	7.32	8.33	0.29	8.33	8.04	8.64	7.74	8.72	0.20	5.2-12.5
Lymphocytes (%)	56.60^b	56.03^b	56.27^b	75.87^a	73.00^a	1.79	70.90^b	71.95^b	73.77^b	79.04^a	78.66^a	0.89	43-80
Granulocytes (%)	21.25^b	18.43^{ab}	27.00^a	14.20^{bc}	9.10^c	1.77	23.50^a	20.25^b	20.10^b	16.60^c	13.30^c	0.45	-
RBC ($\times10^{12}$/L)	6.41^a	6.25^a	6.37^a	5.04^b	5.51^b	0.02	7.25^a	7.30^a	7.30^a	6.60^b	5.80^c	0.10	5.2-12.5
Haemoglobin(g/dl)	17.05^a	25.75^a	16.95^a	14.70^b	14.70^b	0.35	18.96^a	18.80^{ab}	18.85^{ab}	16.95^b	14.95^c	0.40	10-17.4
PCV (%)	43.26	41.50	41.12	36.83	34.00	2.21	50.90^{ab}	54.09^a	50.00^{ab}	45.77^b	39.33^c	1.19	30-50
MCV (fl)	68.00^{ab}	66.00^b	64.33^b	73.67^a	70.50^{ab}	1.67	70.50	70.50	70.50	69.97	67.80	1.86	57.5-75
MCH (pg)	27.45	27.45	26.80	29.20	26.70	0.83	25.95	21.20	26.00	25.85	25.80	1.46	17.1- 23.9
MCHC (g/dl)	39.40^b	41.30^a	41.75^a	39.95^a	38.05^c	0.48	37.00	37.50	37.00	37.00	38.00	0.98	28.2-40
Platelets ($\times10^9$/L)	253.00^b	247.50^b	262.80^b	306.50^a	316.50^a	6.46	354.50^{ab}	324.50^b	329.00^{ab}	386.37^{ab}	394.00^a	14.25	200-650

^{a,b}Means on the same row with different superscripts are significantly ($p<0.05$) different
WBC: White blood cell counts
RBC: Red blood cell counts
PCV: Packed Cell Volume
MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Haemoglobin
MCHC: Mean Corpuscular Haemoglobin Concentration
*Ref. values obtained from Medirabbit [17]

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Table 4: Serum biochemical indices of rabbits fed varying levels of globe amaranth leaf meal – based diets

	Baseline data at the onset of the study						Data at the end of feeding trial						
Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**Ref. values***
Total protein (g/dl)	7.10^{a}	5.47^{b}	6.47^{ab}	3.73^{c}	5.27^{b}	0.32	8.30^{a}	6.90^{b}	5.87^{bc}	5.37^{c}	5.60^{c}	0.37	5-7.5*
ALT (IU)	19.67^{a}	13.67^{c}	18.30^{ab}	16.66^{b}	15.33^{b}	0.32	18.33	18.33	17.00	19.67	19.65	1.69	12-67*
AST (IU)	34.33^{b}	25.67^{c}	37.67^{a}	36.00^{ab}	23.00^{c}	2.90	28.33	25.67	21.00	27.00	23.00	2.80	10-98
Glucose (mmol/L)	3.07^{a}	2.53^{c}	2.97^{b}	2.70^{c}	2.70^{c}	0.13	4.67^{a}	4.07^{a}	3.17^{b}	2.87^{c}	2.93^{c}	0.22	4.2-8.9
Sodium (mmol/L)	157.33^{a}	134.00^{c}	137.67^{c}	147.33^{b}	141.00^{c}	5.40	164.67^{a}	153.33^{a}	140.33^{b}	136.00^{c}	132.67^{c}	5.84	130-155
Potassium (mmol/L)	4.83	4.83	5.27	4.93	3.33	0.25	5.57^{a}	5.47^{a}	5.70^{a}	4.67^{b}	3.87^{c}	0.33	4.0-8.0
Urea (mmol/L)	6.33^{b}	3.03^{c}	6.10^{b}	7.23^{a}	7.13	0.24	6.23^{b}	4.43^{c}	5.90^{ab}	6.73^{b}	7.53^{a}	0.37	9-25.5*
Albumin (g/dl)	4.57^{a}	3.50^{b}	4.20^{a}	2.53^{c}	3.30^{b}	0.18	4.70^{a}	3.77^{b}	3.33^{bc}	2.55^{c}	3.30^{bc}	0.19	2.7-5.0**
Globulin (g/dl)	2.53^{a}	1.97^{b}	2.27^{ab}	1.07^{c}	1.90^{b}	0.24	3.60a	3.01a	2.57b	2.40c	2.33c	0.29	1.5-3.6
Calcium (mg/l)	10.13^{a}	8.17^{b}	9.47^{a}	7.07^{c}	7.77^{c}	0.22	7.30^{c}	7.37^{c}	8.10^{b}	8.30^{ab}	8.90^{a}	0.41	5.5-12.6
T. Cholesterol (mmol/L)	7.50^{c}	8.93^{b}	9.00^{ab}	9.27^{ab}	10.33^{a}	0.32	5.97^{c}	6.80^{bc}	7.56^{ab}	8.30^{b}	9.57^{a}	0.39	0.1-2.00
HDL (mmol/L)	1.03	1.27	1.30	1.37	1.47	0.08	1.40^{c}	1.50^{c}	1.73^{b}	2.23^{a}	2.20^{ab}	0.22	-
LDL (mmol/L)	2.33	2.63	2.33	2.27	2.47	0.47	3.00^{c}	4.47^{ab}	4.96^{ab}	5.53^{b}	6.45^{a}	0.42	-
VLDL (mmol/L)	0.63^{c}	0.75^{ab}	0.75^{ab}	0.77^{ab}	0.82^{a}	0.03	0.32	0.36	0.38	0.34	0.40	0.04	-
TGC (mmol/L)	3.17^{a}	3.63^{ab}	3.77^{ab}	3.83^{ab}	4.10^{a}	0.15	1.60	1.80	1.90	1.70	1.70	0.18	1.4-1.8

$^{a, b}$Means on the same row with different superscripts are significantly ($p<0.05$) different
ALT: Alanine Amino Transferase
AST: Aspartate Amino Transferase
T. Cholesterol: Total cholesterol
HDL: High Density Lipoproteins
LDL: Low Density Lipoproteins
VLDL: Verl Low Density Lipoproteins
TGC: Triglycerides
Ref.values: *Medirabbit [17]

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Table 5: Hormonal profile of rabbits fed varying levels of globe amaranth leaf meal – based diets

	Baseline data at the onset of study						Data at the end of feeding trial					
Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Progesterone (ng/ml)	29.00^{ab}	28.00^{b}	28.00^{b}	31.00^{a}	30.50^{ab}	0.59	42.00^{b}	46.00^{a}	47.00^{a}	46.00^{a}	48.50^{a}	0.79
FSH (Doe) (IU/L)	8.50^{b}	9.00^{ab}	10.00^{ab}	11.00^{a}	10.50^{ab}	0.48	13.00^{bc}	17.00^{a}	15.50^{ab}	10.50^{c}	12.50^{bc}	0.72
FSH (Buck) (IU/L)	6.00^{b}	6.00^{b}	7.00^{ab}	11.00^{a}	8.00^{ab}	0.52	12.00^{ab}	13.00^{ab}	10.00^{b}	15.50^{a}	13.50^{a}	0.75
Estradiol (E2) (pg/L)	34.50	36.00	26.50	37.00	36.50	0.56	48.00	46.50	49.50	48.50	48.00	0.34
Testosterone (ng/ml)	7.00	6.00	6.00	6.00	5.50	0.47	8.00bc	7.50c	8.00bc	9.50ab	10.50a	0.34

^{a,b}Means on the same row with different superscripts are significantly ($p<0.05$) different

FSH: Follicle Stimulating Hormone

Table 6: Reproductive performances of rabbits fed globe amaranth leaf meal-based diets

Parameter	T1	T2	T3	T4	T5	SEM
No. of Does bred	2.00	2.00	2.00	2.00	2.00	-
Pregnant Does after 14 days (Nos.)	2.00	2.00	2.00	2.00	2.00	-
Pregnancy rate (%)	100.00	100.00	100.00	50.00	100.00	0.01
Av. Gestation length (days)	30.00	32.00	32.00	30.00	30.00	0.01
Av. Litter size at birth (Nos.)	3.5^{a}	1.00^{b}	1.00^{b}	2.50^{ab}	2.50^{ab}	0.37
Av. Birth weight (g/kitten)	151.71^{b}	240.00^{a}	295.00^{a}	190.00^{ab}	151.60^{b}	5.09
Av. Weight of kitten (g) at 21 days	223.43	361.00	0.00	229.20	194.00	8.14
No. of kittens alive at weaning (6^{th} Wk.)	7.00	2.00	0.00	5.00	5.00	1.11
Av. Weaning weight (g/kitten)	339.39	550.00	0.00	389.40	393.40	52.14
Milk yield (g)	588.82	757.56	0.00	231.28	162.00	35.79
Mortality (%)	14.00	50.00	100.00	0.00	60.00	0.14
Mortality at birth (No.)	1.00	2.00	4.00	0.00	3.00	0.61

$^{a, b}$Means on the same row with different superscripts are significantly ($p<0.05$) different

Milk yield = 1.18 × [Live weight of litter at 21 days – Live weight at birth] [9]

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