HAEMATOLOGICAL CHANGES IN BROILER FINISHER CHICKENS FED DIETS CONTAINING GRADED LEVELS OF BITTER LEAF (*Vernonia amygdalina*) MEAL

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ABSTRACT
A four -week study was conducted to evaluate the haematological responses of broiler finisher chickens fed graded levels of bitter leaf (*Vernonia amygdalina*) meal. A total of 150-day-old Abor-acre strain of broiler chicks were used in this study. The birds were randomly allocated to five dietary experimental treatments consisting of thirty birds each in a completely randomized design (CRD). Each treatment was further divided into three replicates of 10 birds each. The bitter leaf meal was included at 0, 10, 20, 30 and 40 g/kg diets to represent treatments T_1, T_2, T_3, T_4 and T_5 respectively. At the end of the feeding trial, 5ml of blood samples from two birds per replicate were collected for haematological analysis. The results revealed significant ($p<0.05$) differences in all the parameters measured. The red blood cells count (RBC) and lymphocytes were significantly higher at T_4 (3.15 $\times 10^6$/l and 83.44 %) and T_5 (3.28 $\times 10^6$/l and 85%) levels respectively. The haemoglobin level also followed the same trend with observed increase in birds fed diets T_4 and T_5. The mean corpuscular hemoglobin (MCH), MCHC, platelets and white blood cells count all tended to increase significantly ($p<0.05$) with increasing levels of bitter leaf meal in the diets, and the highest values obtained from birds fed diets T_4 (30 g/kg) and T_5 (30 g/kg) of feed. This study concluded that bitter leaf meal in diets of finisher broiler chickens favoured the production of most of their haematological parameters at 30 and 40 g/kg diet.
Keywords: Blood, broiler chickens, bitter leaf, hematopoietic process

INTRODUCTION
Intensive poultry production has been identified as one of the means of attaining sufficiency in the supply of animal protein to the diets of average Nigerians (Ukoha *et al*., 2019). However, due to the need for massive production, many supplements, additives and leaves have found their way into poultry feeds. These, in many cases have certain impacts either positively or negatively on the health of these birds. Bitter leaf is one of such novel leaves and little work has been done on the effect of bitter leaf in the blood chemistry of broiler birds. Feed additives are ingredients added to poultry diets to enhance productive efficiency, health status and reduction in morbidity (Tokofai *et al*., 2020). The major economic benefits of feed additives are mainly to lower production cost because of improvement in feed utilization and efficiency (Mohammed and Zakariya, 2012). One of such of phytogenics in poultry diets is bitter leaf. It is a shrub or small tree that grows throughout tropical Africa. It is commonly called bitter leaf because of its abundant bitter components (Ekpo *et al*., 2007). Banjoko *et al*. (2019), reported that the leaf contains a considerable amount of anti-nutritional factors like tannins, (40.60mg 100^{-1} g DM), saponin (25.50mg 100^{-1} g DM) and cyanide (60.10mg 100^{-1} g DM). Owen (2011) reported the proximate content of bitter leaf as dry matter (86.40%), crude protein (21.50%), crude fibre (13.10%), ether extract (6.80%) and ash (11.05%). According to Dakpogan, (2006), bitter leaf is said to contain high bioactive compounds such as flavonoids, alkaloids and phenolics, anti-bacterial antiviral, anti-inflamatory and anti-lipidaemic properties. These properties could really enhance feed efficiency and promotes growth as well as improve animal immune system, through their action in the blood indices of birds. This study therefore investigated the haematological changes in broiler birds fed graded levels of bitter leaf meal.

MATERIALS AND METHODS
Experimental site
The experiment was carried out at the Poultry Unit of the Teaching and Research farm, University of Calabar, Calabar, Nigeria. Calabar is located at latitude 4° 57^{I}N of the equator and longitude 8° 19^{I}E of

the Greenwich Meridien. The average annual rainfall is 1830 mm and daily temperature is 24.30°C and relative humidity is between 70 and 80% (NMA 2021).

Source/preparation of bitter leaf meal

Fresh bitter leaves were purchased from WATT market in Calabar. Cross River State. The leaves were plucked from the stem and air-dried to constant weight but retaining the green colour and thereafter milled and a portion stored for chemical analysis, while the remaining lots included in broiler finisher diets at 0, 10.00, 20, 30 and 40 g/kg of feed, constituting T_1, T_2, T_3, T_4 and T_5 respectively.

Experimental diets

A total of 150, day-old Abor Acre strain of broiler birds were purchased from ZARTECH farms, Ibadan. A commercial starter (VITAL feed) was fed to the birds for 4 weeks before feeding with the experimental diets (commercial finisher Vital feed of 20.56% CP and 3100 Kcal/kg ME, supplemented with bitter leaf meal). The birds at finisher phase were randomly distributed into five experimental treatments of thirty birds each and three replicates of 10 birds each in a Completely Randomized Design (CRD) experiment in a deep litter system of management.

Proximate analysis

The dry matter was determined after oven drying to a constant weight. Nitrogen content determined by the Kjeldahl method of AOAC (2006) method and crude protein calculated as N x 6.25, the ash content was determined as the residue remaining after incinerating the sample at 600°C in a muffle furnace. The AOAC (2006) methods were employed for ether extract and crude fibre determination.

Haematological Indices

At the end of the feeding trial (4 weeks), blood samples (5mls) each were collected from prominent veins of the wing web of 3 birds per replicate. The samples were put dispensed into labeled ethylene diamine tetra acetic acid (EDTA) anti-coagulant bottles for haematological assay.

Data analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) for Completely Randomized Design experiment according to the method of Steel and Torrie (1980), Duncan Multiple Range Test (Duncan, 1955) was used in separating the means.

RESULTS AND DISCUSSION

The proximate composition of the test ingredient (bitter leaf) in Table 1, indicated the crude protein level of 20.34%, crude fat of 3.99%, crude fibre of 14.38 %, ash content of 8.15%, nitrogen free extract of (53.14%) and dry matter of 89.92%. These values are quite contrary to the reports of Okunlola (2019). These variations in the proximate compositions of bitter leaf meal according to Ebenebe *et al*. (2019), may have been influenced by soil nutrients, soil type and location, where the bitter leaf were source. The effect of graded levels of dietary bitter leaf meal on the haematological indices of broiler finisher birds is represented in Table 2. There were significant ($p<0.05$) differences in all the measured parameters across the dietary treatments. Birds fed 30g/kg and 40g/kg of bitter leaf diets recorded the highest ($p<0.05$) concentrations of white blood cell (WBC), lymphocytes(LYM), red blood cells(RBC), Haemoglobin (HBC), packed cell volume (PVC), mean corpuscular haemoglobin (MCHC), mean corpuscular value (MCV) and platelets (PLT). The lowest concentration of the above parameters was found in birds fed 0g/kg. Blood biochemical examination is an assessment of the physiological and nutritional status in the animals (Mitruka and Rawnsley (1977). The higher Hb values obtained for birds fed diets containing higher inclusion level (30 and 40 g/kg) of bitter leaf meal is indicative of relatively better nutritional profile and protein quality of bitter leaf meal which is in line with the report of Owen and Ahakin (2011), that HBC levels were positively correlated with protein quality and level of the diets. The value of haemoglobin obtained in this study ranged from 9.11 - 16-85g/dl, this range was higher than the normal range for chickens (7.13-11.34gl), as stated by Gelman and Hawskley (1995), and did not equally agree with the range of 8.7-9.3g/dl reported by lheukwumere and Herbert, 2012) for indigenous chickens. The results obtained in this research also is not in consonance with the range of 6.3-7.8g/dl as reported by Mmereola (1966).

The hematocrit, red blood cells, and mean corpuscular hemoglobin values obtained in this study were higher than the normal range for chickens reported by Iheukwumere and Herbert (2012). This is an indication of a better bone marrow. The significant increase in the concentration of PCV and RBC contents of the blood of broilers reared with bitter leaf meal is an indication that the oxygen carrying

capacity of the blood would have been increased (Adulojie, 2000) and this will help prevent anaemia (Moss, 1999). The lymphocytes concentration was highest in birds fed 40g/kg bitter leaf meal diet. A decreased in white blood cells and lymphocyte would predisposed the animal to reduced immunological response to infections. In addition, a reverse is the case here, where birds fed with bitter leaf meal had a better immune system that can resist infections. The blood platelets count increased significantly with the level of bitter leaf meal in the diets. It showed that bitter leaf can boost blood hemostasis when the birds are exposed to injury.

Table1: Proximate composition of bitter leaf meal

Nutrients (%)	Composition
Dry matter	89.92
Crude protein	20.34
Ether extract	3.99
Crude fibre	14.38
Ash	8.15
NFE	53.14

Table 2: Haematological indices of broiler chickens fed graded levels of bitter leaf meal

Parameter	0.00 g/kg	10g/kg	20g/kg	30g/kg	40g/kg	SEM
WBC(× 10^3/L)	80.40	86.48	90.87	94.23	94.66	2.11
Lym (%)	80.55	79.44	79.21	83.44	85.00	3.60
RBC (× 10^6/L)	2.42	3.00	3.13	3.15	3.28	0.09
HBC (g/dl)	9.11	10.18	12.12	14.93	16.85	1.01
PCV(%)	29.67	33.00	33.96	35.90	35.90	2.25
MCV (fl)	121.82	137.08	136.59	140.22	142.05	5.90
MCH (Pg)	67.69	70.03	71.66	81.89	80.92	3.90
MCHC(g/dl)	41.29	43.67	44.00	45.72	45.66	3.0
Plt (× 10^3/L)	14.82	16.00	16.21	17.20	17.50	1.19

WBC=white blood cell, Lym=Lymphocytes, RBC=Red blood cells, HBC=Haemoglobin, PCV=packed cell volume, MCV=Mean corpuscular value, MCH=Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin, Plt=Platelets, SEM =Standard error of mean

CONCLUSION AND RECOMMENDATION

This study concluded that bitter leaf meal in diets of finisher broiler chickens improved their haematological indices and up to 30 and 40 g/kg of the leaf meal should be added in broiler finisher diets.

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