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Comparative Response of Bitter Leaf (*Vernonia amygdalina*) Infusion Administration on Performance, Haematology and Serum Biochemistry of Broiler Chicks

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ABSTRACT

Growing concern about antibiotic growth promoters in animal nutrition and its health implication to consumers of poultry products has initiated efforts towards use of alternative growth promoting agents especially medicinal plants. This study aimed at investigating the response of broiler chicks administered bitter leaf (*Vernonia amygdalina*) infusion. Sixty day-old Marshal broiler chicks were allotted in a Completely Randomized Design (CRD) to four treatment groups identified as T1, T2, T3 and T4 consisting of three replicate with five birds per replicate. 50 g of dried bitter leaf was infused for 12 h in 1 L of hot boiled water and given to the birds at T1 (0, 25 (T2), 50 (T3) and 75 mL L^{-1} (T4) of drinking water *ad-libitum*. Data collected on growth performance traits revealed that feed intake was not influenced due to treatment, but final body weight, weight gain and feed conversion ratio were increased. Carcass, drumstick, thigh and breast weights and dressing percentage improved in treated groups. There was a reduction of 14.58-23.44% in abdominal fat in the treated group compared to T1. Although birds on T4 gave the highest revenue, the best cost-benefit ratio was obtained from birds on T3. Except for packed cell volume and white blood cell, the results showed no variations in other haematological parameters measured. Cholesterol, low density lipoprotein and glucose concentration in the serum was reduced in the treated group compared to the control. There was no influence on total protein and albumin: globulin ratio in all treatment groups. T3 had the highest value for albumin. Administration of bitter leaf infusion improved the growth performance, haematological and serum biochemical indices of broiler chickens and can be applied in broiler nutrition.

Key words: Bitter leaf infusion, body weight, carcass weight, cost-benefit ratio, feed conversion ratio, serum biochemical indices

INTRODUCTION

The feed industry is faced with a number of challenges, not only regarding the availability of feed ingredients but also the ability to produce high quality products in a cost-effective manner (Chauynarong *et al*., 2009). Khan *et al*. (2012) noted that various feed additives are used in poultry to maximize net returns and carcass quality. The effects of any feed ingredient on the haematological factors of the chicken are of immense assistance in deciding whether, or not, such a feed ingredient will be used as poultry feedstuff (Mitruka and Rawnsley, 1997). Certain

hematological factors such as packed cell volume, red blood cell, hemoglobin, etc., can be associated with certain production traits and serve as means of assessing clinical and nutritional health status of animals. For instance it has been established that high Packed Cell Volume (PCV) and high hemoglobin content (Hb) are associated with high feed conversion ratio (Mitruka and Rawnsley, 1997), while high percentage white blood cells especially lymphocytes are associated with the ability of the chicken to perform well under very stressful conditions.

Feed additives are ingredients added to poultry diets to enhance production efficiency, improve health and reduce morbidity (FAO., 1998). A major feed additive which has been used for decades in poultry production is the antibiotics. Bans on the use of antibiotics as feed additives have accelerated and led to investigations of natural alternative feed additives in animal production (Polat *et al*., 2011). Wang *et al.* (1998) reported that compared with synthetic antibiotics or inorganic chemicals, these plant-derived products have proven to be natural, less toxic, residue free and are thought to be ideal feed additives in food animal production. Rich store houses of medicinal plants exist everywhere especially in Africa which offers a vast reservoir of plant that has been categorized (Aluyi *et al*., 2003). Akinyemi *et al.* (2005) noted that the use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. Bitter leaf (*Vernonia amygdalina*) is a shrub or small tree and one of the edible vegetables in Nigeria and other parts of African sub regions (Igbakin, 2009). It performs both medicinal and nutritive functions (Adaramoye *et al*., 2008). WHO (1996) describes a medicinal plant as any plant in which one or more of its organ contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. It is popularly called bitter leaf because of its abundant bitter principles (Ekpo *et al*., 2007). The leaves contain a considerable amount of anti-nutritive factors like high level of tannic acid and saponin. Proximate composition of *Vernonia amygdalina* Leaf Meal (VALM) shows a chemical composition of 527.83 ME kcal kg^{-1}, 86.40% DM, 21.50% CP, 13.10% CF, 6.80% EE, 11.05% Ash and the result on mineral composition indicate that *V. amygdalina* has 3.85% Ca, 0.40% Mg, 0.03% P, 0.006% Fe, 0.33% K and 0.05% Na (Owen *et al*., 2009). It is one of the natural feed additives which can be of great productive and health importance in the broiler industry.

The present study was designed to evaluate the efficacy of different levels of bitter leaf aqueous extract on growth performance, economics of production, carcass quality and blood parameters of broiler chicks.

MATERIALS AND METHODS

Location of study: The study was carried out at the Poultry Experimental Unit of the Department of Animal Science, Ebonyi State University, Abakaliki, Nigeria. Abakaliki is located within the Southern Guinea Sanannah Zone at between latitude 06° 4'N and longitude 08° 65'E with a day length range of 12-14 h all year round. It has an annual mean rainfall range of between 1500-2250 mm with mean daily temperature ranges of 27°C and relative humidity of 85%.

Preparation of infusion: Fresh leaves of bitter leaf were bought from the local market in Abakaliki, Nigeria. These were washed thoroughly without squeezing with clean tap water to remove dirt (sand and dust). Washed leaves were air-dried at room temperature for two weeks until they became brittle. The dried leaves were pulverized using a hammer mill and stored in an air-tight plastic container. Fifty gram of pulverized leaves was infused in 1 L of hot boiled water

Table 1: Proximate composition of feed

Nutrients	Starter	Finisher
Crude protein	22.00	18.00
Fat/oil	6.00	6.00
Crude fibre	5.00	5.00
Calcium	1.00	1.00
Available phosphorus	0.45	0.40
Lysine	1.20	0.85
Methionine	0.55	0.35
Salt	0.30	0.30
ME (kcal kg^{-1})	2900	2900

for 12 h (overnight). The infusion was thereafter, filtered using a filter paper and the filtrate administered to the chicks according to the treatment dose. Infusion was prepared daily.

Experimental animals, design and management: This study which lasted for 54 days was performed with sixty day-old straight-run Marshal strain broiler chicks procured from Obasanjo Farm, Ota, Ogun state, Nigeria. It was initiated by raising communally the day-old chicks for seven days. On the 8th day, the birds were randomly allotted into four treatments designated T1- 0, T2- 25, T3- 50 and T4- 75 mL^{-1} of bitter leaf infusion per litre of drinking water. Each of the treatment was replicated three times, with five birds per replicate in a Completely Randomized Design (CRD). Water was made available to the chicks *ad libitum*. All chicks were fed *ad libitum* with same commercial diet. Proximate composition of the feed as given by the feed manufacturer is presented in Table 1.

Data on growth performance of broiler chicks were obtained by records from the daily feed intake and weekly body weight. These were used to calculate the feed conversion ratio (feed consumed divided by weight gain) at the end of the experiment. At the end of the experiment, one bird was selected from each replicate, i.e., three birds per treatment. The selected birds were fasted for 12 h and weighed prior to slaughtering. The carcass weight, weight of prime cuts (drumstick, thigh, breast, back and giblets) and abdominal fat weight were obtained. The weights of the cuts and abdominal fat were expressed as relative percentage weight of the carcass. A cost-benefit analysis was done to evaluate the economic efficiency of administering bitter leaf infusion to broiler chicks. Data obtained from the cost/kg feed consumed/bird, cost of production and revenue were estimated and applied in the cost-benefit analysis.

Data on blood parameters were also obtained at the end of the feeding trial. Three birds each were randomly selected from each replicate group and 2 mL of blood collected from the wing vein with sterile needle into well labeled sterilized bottles that contained EDTA as anticoagulant. These were used to determine the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and the hemoglobin (Hb) count according to Dein (1984). Another 2 mL of blood was collected from the birds without anticoagulant into a vacutainer to determine the following serum biochemical indices (total protein, albumin, glucose, globulin, cholesterol, LDL and HDL level in the blood).

Data were analyzed statistically using one-way analysis of variance (ANOVA) and significant means were compared using Duncan's New Multiple Range Test at 5% significance level. The analysis was done using SPSS 13 for windows. The experimental model applied was:

$$X_{ij} = \mu + T_i + E_{ij}$$

Where:
X_{ij} = Any observation made in the experiment
μ = Observed mean
T_i = Effect of dosage (i = 25, 50 and 75 mL)
E_{ij} = Residual error

RESULTS AND DISCUSSION

The effect of oral administration of bitter leaf aqueous extract on the performance of broiler chickens is presented in Table 2. The result indicates that no difference ($p>0.05$) was observed in daily feed intake among the four treatment groups. However, there were significant variations ($p<0.05$) in the final body weight, daily weight gain and feed conversion ratio-ranging between 268.72-413.57, 5.07-7.88 and 0.35 0.30 g, respectively-between T1 and the other treatments (T2, T3 and T4). However, birds on T4 had the best performance, numerically, when compared to the other treated groups. The result of the present study is in line with the findings of Tangka (2003) and Durunna *et al.* (2011) who reported improved growth performance of animals fed bitter leaf. The improvement observed in the improved weight gain is correlated with the lower FCR observed in the treated group. The lower the FCR the higher it is for the birds to convert feed consumed to meat. Olobatoke and Oloniruha (2009) reported that inclusion of bitter leaf powder in cockerels feed significantly improved FCR.

Adaramoye *et al.* (2008) opined that the improvement observed could be associated with the beneficial effect of bitter leaf in enhancing the gastro intestinal enzyme thereby improving digestion and assimilation of nutrients. Huffman *et al.* (1996) also reported that bitter leaf enhanced gastro intestinal enzymes (chymotrypsin) production which may improve not only the utilization of feed but could aid in the digestion of sporozites and other intestinal parasites that

Table 2: Effects of oral administration of bitter leaf aqueous extract on performance of broiler chickens

Parameters	T1 (0 mL)	T2 (25 mL)	T3 (50 mL)	T4 (75 mL)	±SEM
Growth performance traits					
Initial body weight (g)	170.67	165.33	166.67	158.67	10.45
Final body weight (g)	1966.43^{b}	2235.15^{a}	2352.30^{a}	2380.00^{a}	120.12
Daily weight gain (g)	33.26^{b}	38.33^{a}	40.48^{a}	41.14^{a}	1.94
Daily feed intake (g)	94.26	97.20	100.54	104.08	11.60
Feed conversion ratio	2.84^{b}	2.54^{a}	2.49^{a}	2.53^{a}	0.10
Economic analysis					
Cost/kg feed consumed/bird	559.91^{c}	577.39^{bc}	597.18^{ab}	618.23^{a}	13.86
Cost/kg live weight gain	311.80^{b}	278.96^{a}	273.23^{a}	279.32^{a}	14.52
Cost of prod.(₦)**	822.91^{c}	840.39^{bc}	860.18^{ab}	881.23^{a}	19.97
Revenue (₦)***	1474.82^{c}	1676.36^{b}	1764.23^{a}	1785.00^{a}	26.28
Cost-benefit ratio	1.26^{c}	1.01^{b}	0.95^{a}	0.98^{ab}	0.02
Carcass quality					
Live weight before slaughter (g)	1633.33	1900.00	2216.67	2166.07	298.37
Carcass weight (g)	1233.33^{b}	1516.67^{ab}	1777.33^{a}	1816.67^{a}	204.18
Dressing percentage	75.92^{c}	79.80^{b}	80.02^{b}	83.87^{a}	1.16
Abdominal fat*	4.70^{a}	3.60^{c}	4.01^{b}	3.98^{b}	0.06
Drumstick*	11.87^{c}	12.26^{b}	12.32^{b}	13.31^{a}	0.14
Thigh*	14.35^{c}	17.41^{a}	17.20^{a}	15.12^{a}	0.28
Wing*	10.68	11.85	10.53	10.81	0.92
Breast*	22.81^{a}	21.93^{a}	17.20^{b}	23.62^{a}	1.29
Giblets*^{y}	11.03	11.41	11.42	11.01	1.02

^{ab}Means on the same row followed by different superscript are significantly different ($p<0.05$); ^{y}Giblets-heart, liver, gizzard and neck, *Weight of cuts/organs expressed as % of carcass weight, **Cost of production = Cost of feed+cost of medication+cost of day old chick and ***Revenue based on ₦750 kg^{-1} live weight

could cause decreased utilization of feed. Mohammed and Zakariyau (2012), contrarily, observed that inclusion of bitter leaf as a feed additive did not significantly improve weight gain and FCR in broilers.

Table 2 also reveals that the cost/kg feed consumed/bird as well as cost of production increased with increase in the quantity of bitter leaf extract in the drinking water. However, the cost of feed/kg live weight gain was least for birds on T3 while those on control had the highest cost (a difference of up to ₦38.57). Although T4 gave the highest revenue, the best revenue, numerically, was obtained from birds on T3 (a margin of ₦0.28 between T3 and T4). This, further, translated to a better cost-benefit ratio. The present result is in consonance with the findings of Kyvsgard (2002) which noted that broilers fed bitter leaf performed better in terms of cost of feed per kg gain than those on control.

Though no variations ($p>0.05$) existed in the live weight of birds in all treatments, there were significant variations ($p<0.05$) between the treatments groups in carcass weight and dressing percentage, particularly, between T1 and other treatments (Table 2). Birds on T4 had a better performance. Their carcass weight was 32.11% heavier than those on T1 and their dressing percentage 9.48, 4.85 and 4.59% better than those on T1, T2 and T3 respectively. There was a reduction ($p<0.05$) of 14.58-23.44% in abdominal fat in the treated group compared to T1. Isika *et al.* (2012) observed that dressed weight, eviscerated weight and abdominal fats were higher ($p<0.05$) in the control diet than in the test diet when 0.25% bitter leaf, ginger and a mixture of the two herbs were included the diets of broiler chicks. Javed *et al.* (2009) observed a significant increase in dressing percentage in broiler chicks in response to an aqueous extract of plant mixture. Oleforuh-Okoleh *et al*. (2014) demonstrated that abdominal fat was significantly reduced in broiler chicks treated with ground ginger and garlic. It is supposed that the improvement in the FCR resulted in improved muscle development hence the observation made in the treated groups. Furthermore, this could also be attributed to the hypolipidaemic effect of bitter leaf which ensured the development of leaner meat.

Table 3 presents the blood parameters of the experimental birds. The hemoglobin concentration (Hb) and Red Blood Cell (RBC) of the experimental birds were not statistical different ($p>0.05$), though they increased numerically as levels of aqueous extracts of bitter leaf increased.

Table 3: Blood parameters of broiler chicks administered aqueous extracts of bitter leaf

Parameters	T1 (0 mL)	T2 (25 mL)	T3 (50 mL)	T4 (75 mL)	±SEM
Haematological					
PCV (%)	24.33^{b}	32.00^{a}	30.66^{a}	31.33^{a}	1.12
Hb (g dL^{-1})	9.74	10.64	10.22	10.44	0.74
RBC ($x10^{6}/mm^{3}$)	3.99	4.23	4.35	4.11	1.72
WBC ($x10^{3}/mm^{3}$)	473.00^{c}	522.00^{a}	493.33^{b}	498.66^{b}	6.14
MCV (fl)	60.98	75.65	70.48	76.22	3.88
MCHC (%)	33.33	33.25	40.03	33.32	8.06
MCH (pg)	24.89	25.13	22.39	26.19	1.36
Serum biochemistry					
Cholesterol (mg dL^{-1})	132.12^{a}	113.99^{b}	86.23^{c}	112.21^{b}	7.34
LDL (mg dL^{-1})	137.91^{a}	112.14^{b}	79.91^{c}	103.89^{b}	2.47
HDL (mg dL^{-1})	33.51^{c}	69.60^{a}	63.16^{a}	47.69^{b}	3.86
Total protein (g L^{-1})	40.33	42.00	43.66	43.06	1.96
A/G ratio	1.02	1.09	1.15	1.09	0.33
Albumin (g L^{-1})	20.33^{b}	21.94^{ab}	23.33^{a}	22.42^{a}	0.94
Glucose (FBS) (mg dL^{-1})	138.60^{a}	131.00^{b}	129.20^{b}	127.20^{b}	2.93

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

Osho *et al.* (2014) demonstrated that oral administration of bitter leaf extract on broiler chickens did not have a significant effect on the Hb and RBC in treated birds. The Packed Cell Volume (PCV) and total White Blood Cell (WBC) count of the birds were significantly increased in the treated group. Owen and Amakiri (2011) made similar observation, where with the exception of White Blood Cell (WBC), all other hematological indices measured were influenced by increasing levels of *Vernonia amygdalina* leaf meal. Aregheore *et al*. (1998) suggested that the presence of some phytochemicals in bitter leaf extract induces the animal to respond as if it has infection. Thus increase in WBC could also be attributed to the presence of anti-nutritional compounds in *Vernonia amygdalina*. The PCV values were within the normal range of 25-45%.

Results in Table 3 also reveals that administration of bitter leaf extract significantly ($p<0.05$) reduced serum cholesterol in the blood plasma. The LDL of the birds on T2 were similar ($p>0.05$) to T4, but significantly higher than ($p<0.05$) those on T3 and lower than those on T1. Nwanjo and Nwokoro (2004) in their study on effects of aqueous extract of *Vernonia amygdalina* on rats concluded that the plant possesses anti-oxidant property. Ojiako and Nwanjo (2006) documented that administration of bitter leaf significantly attenuated the LDL cholesterol level of streptozotocin diabetic rats. Owen *et al*. (2011a) also reported serum cholesterol and LDL lowering potentials of bitter leaf meal. Increased activity of the enzyme catalase involved in esterification of cholesterol in the plasma could have influenced the decrease in serum cholesterol in the bitter leaf extract administered birds. Birds on bitter leaf aqueous extract had better HDL values compared to those on control. This indicated that the tendency of having high serum lipid can be prevented by supplementation of natural additives.

The fasting blood glucose of broilers on bitter leaf aqueous extract was significantly reduced with increasing level of bitter leaf in the drinking water. A reduction of 5.5-8.23% of glucose level between the control and the treated group was observed. The finding affirms the work of Osinubi (2006) and Owen *et al.* (2011b) which suggested that *Vernonia amygdalina* has hypoglycemic effect. Akah and Okafor (1992) reported that bitter leaf possess both hypoglycemic properties. Two mechanisms have been adduced for the hypoglycemic effect observed in the administration of bitter leaf. The works of Atangwho *et al*. (2007) on the effect of *V. amygdalina* leaf on kidney function of diabetic rats depicts that one of the mechanisms is the targeting of insulin synthesis/production from the beta-cells of the islet of Langerhans and the second on peripheral carbohydrate mechanism.

Total protein and albumin tests are usually used to evaluate the health status of animals. These tests are often used in diagnosing diseases and in monitoring changes in health status of farm animals. The total protein is a composite of the albumin and globulin content in the blood and is a reflection of the nutritional status of the birds. Low levels of albumin indicate incidence of disease related to the liver or kidney. It could also be associated with presence of infection. Total protein of the birds on T3 was significantly higher than those of other treatments. Similar trend was observed in the albumin. This finding concurs with earlier report by Owen *et al*. (2011b).

The inclusion of bitter leaf aqueous extract in the diets of broiler chickens had no adverse effect on the performance traits as well as economic production parameters evaluated. Furthermore, administration of the extract reduced the total cholesterol, LDL and glucose in the blood plasma. The best administration level with respect to cumulative effect on all parameters investigated was 50 mL L^{-1} of drinking water. This implies that the administration of this natural herb at this level of inclusion could be important in improving the productivity and health status of broiler chicks.

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