Effect of Varying Dietary Levels of Bitter Leaf (*Vernonia Amygdalina*) Meal on the Serum Biochemistry of Finisher Broilers

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ABSTRACT
A twenty eight (28) day research to investigate the effect of varying inclusion levels of bitter leaf meal on the serum biochemical profile of finisher broiler chickens was undertaken. A total of ninety six (96) four weeks old broilers were randomized into four treatment groups of three replicates of eight birds per replicate in a completely randomized design. Bitter leaf meal was included in the feed at 0 g, 25 g, 50 g and 75 g/25 kg of feed for treatments I, II, III and IV respectively. Results obtained revealed improvement in the serum biochemical profile with increase in the inclusion levels of bitter leaf meal as total protein increased from 3.21 g/dl to 3.63 g/dl, albumin from 1.89 g/dl to 2.25 g/dl, while creatinine, AST, ALT and cholesterol were significantly reduced across treatments It was concluded that the inclusion of bitter leaf meal significantly improved the serum biochemical indices of finisher broilers within the confines of the inclusion levels used. It was recommended that bitter leaf meal be added up to 75 g/25 kg feed of broiler chickens as it proves to have significantly improved ($p<0.05$) serum biochemical parameters.
Key Words: bitter leaf, serum, broilers, protein, cholesterol creatinine

INTRODUCTION

The poultry industry has proved to be one of the most profitable agro-industries with the capacity to effectively tackle the problems of unemployment in the rural areas (Singh, 2010). Poultry industry can be adopted under a wide range of climatic conditions and can generally be combined conveniently with other farm enterprises. A huge gap exists between availability and requirement of poultry products which is due to the slow growth in the poultry sector for the past two decades (Singh, 2010).Unfortunately, this enterprise is facing series of constraints which invariably affects its productivity.

Research reports show that the major factors militating against increased poultry production and/or sustainable animal production especially the monogastrics industry such as poultry, rabbits, pigs and fishes is the low availability of feed at economically feasible price and incidence of pest and disease (Esonu *et al*., 2006; Agbabiaka *et al*., 2012). Most of the concentrates feedstuff are very expensive because of their multi-purpose use: farm products as staple food for the ever-increasing human population, component of livestock diet formulation and major raw-material in the brewing industry (Agbabiaka *et al.,* 2012).

Consequently, the price has continued to rise because the demand far outweighs the supply of these products.

Feed additive is applied in a broad sense, to all products other than those commonly called feedstuffs, which could be added to the ration with the purpose of obtaining some special effects (Onibi *et al*., 2009). The main reason for adding feed additives is to enhance growth rate, better utilization of feed, higher resistance to diseases thereby lowering mortality rate in poultry birds. These feed additives which are growth promoters are mostly called non-nutritive feed additives (Singh and Panda, 1992). Most times, synthetic drugs and feed additives are given as supplement to the broilers thereby enhancing their growth, but the adverse effect associated with their use have shown many disadvantages in their utilization. These include: high cost, health complications and long residual properties in the carcass of the birds etc. feed additives are substances of both chemical and biological origin, which purpose for being added to livestock ratio is to enhance the growth performance and feed utilization, and thereby improving production efficiency and economics of birds. Their system of action varies from one growth promoter to another. Positive influence can be observed in improved appetite, improved feed utilization, intestinal micro-flora regulation, immune system boost, increased vitality, etc (Morshedul *et al.,* 2015).

Vernonia amygdalina (*VA*) is a shrub or small tree that grows throughout tropical Africa. It is popularly called bitter leaf because of its abundant bitter taste (Ekpo *et al.,* 2007). Research has shown that *Vernonia amygdalina* could be used as a growth promoter by enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (Olobatoke and Oloniruha, 2009). Proximate composition of *Vernonia amygdalina* leaf meal (VALM) shows a chemical composition of 527.83 ME kcal/ kg, 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 % Calcium, 0.40 % Magnesium, 0.03 % Phosphorus, 0.006 % Iron, 0.33 % Potassium and 0.05 % Sodium (Owen and Amakiri, 2011).

The result of blood analysis is usually used to assess the health status of an animal. Khan and Zafar (2005) reported that serum parameters are observed as good indicators of the physiological status of animals and their changes are important in assessing the response of animals to various physiological situations. Results of this research will aid in the adoption of the use of bitter leaf (*Vernonia amygdalina*) meal by farmers and nutritionist as a substitute in place of antibiotics to improve the serum metabolites of broiler chicken. The objective of this study is to determine the effect of varying levels of *Vernonia amygdalina* (bitter leaf) meal in the diet on the serum biochemistry of broilers chickens.

MATERIALS AND METHODS

Experimental Location

The study was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Production, Faculty of Agriculture, Kogi State University, Anyigba in Dekina Local Government Area of Kogi State. The experimental location falls within the Guinea savanna zone of Nigeria on longitude 07^0 29'N and latitude 07^0 11^1E of the Greenwich meridian (Ifatimehin *et al.,* 2006). The zone is favourable for agronomic conditions and for the production of forage crops, bitter leaf (*Vernonia amygdalina*) inclusive, which is cultivated both as food and medicinal crop by the local community.

Experimental design and Management of Birds

Ninety-six (96) four weeks-old broiler chickens were used for the experiment after raising them from day old. On the twenty-eight day, the birds were weighed and randomly allocated to four experimental treatments in a Completely Randomized Design (CRD). Dried bitter leaf (*Vernonia amygdalina*) meal was incorporated at 0 g, 25 g, 50 g and 75 g/25 kg of feed in T1, T2, T3, and T4 respectively. Each treatment had 24 birds with three replicates of eight (8) birds each which were raised on deep litter system. The experiment lasted for four weeks. Feed and drinking water were provided *ad libitum* and standard routine management practices were followed.

Table 1: Proximate Composition of Bitter Leaf Meal and the Commercial Diet

Proximate composition	*Vernonia amygdalina*	Commercial Diet
Dry matter	92.60 ± 0.61	89.08
Crude protein	23.01 ± 0.17	17.30
Crude fiber	17.14 ± 0.05	5.50
Ash content	13.70 ± 0.10	6.00
Ether Extract	4.33 ± 0.06	6.54
Nitrogen Free Extract	46.03 ± 0.09	53.64

Collection, Processing and Preparation of the Experimental Diet

Fresh bitter leaves (*Vernonia amygdalina*) were collected from Kogi State University Livestock Unit premises. The samples were air-dried and further ground into powder by a hammer mill at Anyigba market. The obtained powder was then packed in a polyethylene bag and preserved in the feed storage room until used as additive in the feed. *Vernonia amygdalina* were manually incorporated into the commercial diets at 0 g, 25 g, 50 g and 75 g/25kg of feed. The proximate composition of the experimental diet and commercial diet are presented in Table 1.

Collection of Blood Samples for Serum Analysis

At the end of the experiment, blood samples were collected from two birds per replicate via wing vein puncture using 5mls syringe into plain blood sample bottles (EDTA free). The blood samples were then analysed for Total Protein (TP), Albumin, Globulin, Glucose, creatinine, urea, Aspartate amino-transferase (AST) and alanine amino-transferase (ALT) in accordance with procedures outlined by AOAC (2009)

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Science version 16. Significant differences between means were separated using the fisher's least significance difference (LSD).

RESULTS

The result of the serum biochemical evaluation of broiler chickens fed varied inclusion levels of bitter leaf meal is presented in Table 2. Observed result indicates that total protein, globulin, creatinine and aspartate amino transferase were significantly ($p<0.05$) affected by treatments. Total protein ranged between T1 (3.21 g/dl) and T4 (3.63 g/dl). Value range of 2.25 g/dl to 2.89 g/dl was recorded for the globulin content while cholesterol values ranged from 0.30 to 2.62 mmol/L. Albumin and aspartate amino transferase showed value range of 0.73 to 0.80 g/dl and 61.33 to 65.33 mg/dl respectively. No significant difference ($p>0.05$) were observed for albumin and alanine amino transferase which had value ranges of 1.24 - 1.38 g/dl and 12.00 iu/dl - 12.80 iu/dl respectively.

Table 2: Effect of Varied Inclusion Levels of Bitter Leaf Meal on the Serum Parameters of Finisher Broilers

		Levels of	Inclusion			
Parameters	**T1 (0 g)**	**T2 (25 g)**	**T3 (50 g)**	**T4(75 g)**	**SEM**	**LOS**
Total Protein (g/dl)	3.21^{b}	3.23^{b}	3.61^{a}	3.63^{a}	0.06	*
Globulin (g/dl)	1.89^{c}	1.99^{bc}	2.12^{b}	2.25^{a}	0.04	*
Albumin (g/dl)	1.32	1.24	1.49	1.38	0.08	NS
Creatinine (mg/dl)	0.80^{a}	0.74^{ab}	0.71^{b}	0.73^{b}	0.01	*
AST (iu/dl)	65.33^{a}	63.50^{ab}	61.83^{b}	61.33^{b}	0.49	*
ALT (iu/dl)	12.80	12.50	12.50	12.00	0.21	NS
Cholesterol (mg/d)	2.62^{a}	2.53^{ab}	2.47^{b}	2.30^{c}	0.05	*

^{a,b,c,d} = means with different superscripts along the same row show significant difference at $p<0.05$, AST alanine aminotransferase, AST = aspartate amino transferase, T1= 0 g, T2 = 25 g, T3 = 50 g, T4=75 g bitter leaf inclusion.

Total protein significantly differed ($p<0.05$) across treatments. Highest value of (3.63 g/dl) was recorded for 75 g/25 kg feed (T4). Total protein increased with increase in the quantity of bitter leaf included in the diet. A range of 3.21 g/dl to 3.63 g/dl was obtained for total protein. Values obtained in this experiment are in agreement with data recorded by Akinmutimi *et al*. (2004) for normal range of Total Protein for broilers. Variation in Total Protein could be attributed to an improved digestion capacity conferred by the bitter leaf present in the feed thereby making more protein available to the birds. This implies superiority of the 50 g/25 kg and 75 g/25 kg of feed bitter leaf inclusion level in diet in protein balance in comparison with the control and T1. Bonsi *et al*. (2005) showed that the higher serum total protein value signifies better utilization of the protein content of the feed. This thereby implies improvement of the adequacy of the diets for the chicks with increase in the inclusion level of bitter leaf.

Albumin was not significantly affected ($p>0.05$) by treatment. T4 (1.38 g/dl) recorded the highest value. A range of 1.24 g/dl to 1.38 g/dl was recorded which falls within the normal range of 1.27 g/dl t0 3.97 g/dl reported by Bowes *et al.* (2000) and Durotoye *et al.* (2003). Dairo *et al*. (2016) reported that albumin helps to prevent haemorrhage. This therefore implies higher blood clotting ability with improved albumin content.

Significant treatment differences ($p<0.05$) were observed for globulin. Highest value was recorded for T4 (2.25 g/dl) with an average range of 1.89 g/dl to 2.25 g/dl. Globulin and albumin are very important components of total protein. Globulin is majorly implicated for the conferment of immunity in livestock (Dairo *et al*., 2016). Values obtained for total protein and globulin though significantly different ($p<0.05$) are within normal range of 3.16 g/l to 8.88 g/l and 1.05 g/dl to 6.40 g/dl respectively reported by Mitruaka and Rawnsley (1997) for healthy chicks. This implies that the chickens' immunity was not compromised at any of the levels of inclusion. Reports by other researchers indicated that provided the level of bitter leaf inclusion is within the tolerable range for birds, significant increase in the serum total protein and its derivatives is expected with an increase in the inclusion level of the bitter leaf in the diets of birds (Bonsi *et al*., 2005; Owen and Amakiri, 2011; Banjoko *et al*., 2019). Reduced levels of creatinine in response to increasing quantity of the bitter leaf inclusion may indicate improved utilization of the protein in the feed.

ALT is found in highest amount in the liver and is used to identify acute liver failures (Orlewick and Vovchuk, 2012) as the enzyme is released into the serum immediately after a hepatocellular damage. In this study, the ALT levels were not significantly affected ($p>0.05$) though there was reduction in value with increase in inclusion level of bitter leaf.

This implies that the inclusion of bitter leaf may improve liver function which may have resulted in lowering of ALT (Wasserman, 2013).

The various treatment groups had general normal levels of serum AST. There is enough evidence that broilers show some levels of inflammatory changes in the liver through their life (Singh *et al.* 2011). The decreased level of AST might be lowered by the inclusion of bitter leaf meal which helps to increase digestion and utilization capacity of the treatment group thereby making more nutrients available to the birds. Increase in AST and ALT values may cause chronic liver damage in fast growing broilers (Dudley *et al.,* 1982) and this might result to an occurrence of sudden death syndrome (SDS) as reported by Qujeq and Aliakbarpour (2005). As clearly shown in the present study, the effect of bitter leaf inclusion significantly reduced the AST level thereby creating a more healthy state for the birds.
Significant decrease in the cholesterol level with increase in the inclusion level of bitter leaf was observed. Value range of 2.30 to 2.62 mg/dL cholesterol was obtained. Radwan *et al*. (2007) reported that with high cholesterol levels, broilers tend to develop fatty deposits in their blood vessels. This eventually leads to difficulty in blood flow and eventually lameness and sudden death of broiler chickens.

CONCLUSION AND RECOMMENDATION

From the result obtained, inclusion of bitter leaf meal showed significant effect ($p<0.05$) on some of the serum parameters of the broiler chickens. The chickens showed improved total protein, globulin, creatinine, aspartate amino transferase and cholesterol levels across treatments with increasing levels of inclusion of bitter leaf meal. The albumin and alanine amino transferase parameters showed no significant difference ($p>0.05$) across treatments. However, values obtained for the control indicates adequacy of nutrient as shown in the serum metabolites. The values of the serum parameters show that the inclusion of bitter leaf had no deleterious effect on the bird but rather improved the serum parameters.
Based on the findings of this research, it is recommended that bitter leaf meal be added up to 75 g/25 kg feed of finisher broilers as it proves to have significantly improved ($p<0.05$) serum biochemical parameters.

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