


Assessment of calcium, phosphorus and enzymes of broiler finisher chickens' fed with bitter leaf (*Vernonia amygdalina* Delile) meal diets

Determinación de calcio y fósforo y enzimas de pollos asaderos en fase de acabado alimentados con dietas de harina de hojas de *Vernonia amygdalina* Delile

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ABSTRACT

The serum calcium, phosphorus and enzymes of 144 Marshall broiler chickens weighing 500 – 610 g were studied after 28 days exposure to dietary treatment with bitter leaf (*Vernonia amygdalina*) leaf meal (VALM). Four diets were formulated by substituting groundnut cake with VALM at 0, 5, 10 and 15% levels in a completely randomized design experiment. Blood samples were collected and assayed to obtain serum and enzyme indices vis-à-vis Calcium (Ca), Phosphorus (P); Aspartate transaminase or Serum glutamic oxaloacetic transaminase (SGOT), Alanine transaminase or Serum glutamic pyruvic transaminase (SGPT) and Alkaline phosphatase (ALP). Results obtained showed a significant ($P<0.05$) dietary treatment effect on SGOT, SGPT and ALP. The data showed that broiler chickens reared on 15% VALM had higher ($P<0.05$) activities of SGPT (9.0 iu/l), SGOT (58.3 iu/l) and ALP (120.0 iu/l) in their serum whereas mean values for 0 and 5% VALM treated broilers in SGPT, SGOT and ALP were similar ($P>0.05$). The values obtained for Ca were 1.2, 1.3, 1.4 y 1.6 mmol/l and those for P were 1.1, 1.2, 1.3 and 1.3 mmol/l respectively for 0, 5, 10 and 15% levels of VALM inclusion. The concentrations of serum Ca and P studied were similar ($P>0.05$) among the treatments indicating normal Ca and P levels in the birds. The results indicated that although significant ($P<0.05$) differences existed in all the enzyme indices evaluated; some of them still fall within the normal ranges established for chickens. From the above results, it was concurred that VALM is appropriate for use as feed ingredient in broiler chickens' diets.

Key words: Bitterleaf, broilers, calcium, enzymes, groundnut cake, phosphorus

RESUMEN

El calcio y fósforo en suero y las enzimas de 144 pollos asaderos Marshall pesando 500-610 g se estudiaron después de 28 días de exposición al tratamiento dietético con la harina de hojas de *Vernonia amygdalina* (HHVA). Se formularon cuatro dietas mediante la sustitución de la torta de maní con HHVA a niveles de 0, 5, 10 y 15% en un experimento con un diseño completamente aleatorizado. Se tomaron muestras de sangre y se analizaron para obtener los índices de suero y de las enzimas vis-à-vis de calcio (Ca), fósforo (P), aspartato transaminasa o transaminasa oxaloacética glutámica en suero (TOGS), transaminasa alanina o transaminasa pirúvica glutámica en suero (TPGS) y la fosfatasa alcalina (FAL). Los resultados obtenidos mostraron un efecto del tratamiento dietético significativo ($P<0,05$) sobre TOGS, TPGS y FAL. Los datos mostraron que los pollos asaderos criados con 15% HHVA tuvieron mayores actividades ($P<0,05$) de TPGS (9,0 iu/l), TOGS (58,3 iu/l) y FAL (130,0 iu/l) en suero mientras que los valores promedios de los pollos tratados con 0 y 5% HHVA fueron similares ($P>0,05$) para TOGS, TPGS y FAL. Los valores obtenidos para Ca fueron 1,2; 1,3; 1,4 y 1,6 mmol/l y aquellos para P fueron 1,1; 1,2; 1,3 y 1,3 mmol/l para los niveles 0, 5, 10 y 15% de inclusión de HHVA. Las concentraciones estudiadas del Ca y P en suero fueron similares ($P>0,05$) entre los tratamientos lo cual indica niveles normales de Ca y P en los pollos. Los resultados indicaron que, aunque existieron diferencias significativas ($P < 0,05$) en todos los índices enzimáticos evaluados, algunos de ellos todavía caen dentro de los rangos normales establecidos para los pollos. De los resultados anteriores, se concluyó que HHVA es apropiada para su uso como ingrediente en la alimentación de las dietas de pollos asaderos.

Palabras clave: *Vernonia amygdalina*, pollos asaderos, enzimas, metabolitos, torta de maní

INTRODUCTION

There has been a continuous search for alternative plant protein sources in rations over the years as a replacement for a more costly, more competitive and sometimes scarce soya bean meal, groundnut cake and fish meal (Nwambe *et al.*, 2008; Owen *et al.*, 2010). This is because conventional protein sources are becoming expensive due to stiff competition between man and his animals. The inability to match their production with demand in Nigeria has resulted in shortage of these ingredients, which therefore necessitates the need to exploit cheaper sources to replace the conventional sources for livestock production. This has resulted in huge research interest in a bid to find alternative feed ingredients rich in protein and capable of substituting the expensive conventional protein sources in Nigeria. One of such feed ingredients is bitter leaf (*Vernonia amygdalina*) leaf meal (VALM).

Vernonia amygdalina Del. (compositae) popularly known as bitter leaf is a shrub of 2 – 5 m tall with petiolate of about 6.0mm wide (Ojiako and Nwanjo, 2006). The plant is widely distributed in Nigeria especially in the Southern part of the country (Taiwo *et al.*, 2009). It is cultivated in Nigeria mainly for its nutritional value (Igile *et al.*, 1995a). *Vernonia amygdalina* leaf meal (VALM) being an alternative proteinous feed resource 20 – 34% CP (Aregheore *et al.*, 1997; Owen *et al.*, 2009b) can be used as protein supplement, energizer and even can serve medicinal purpose. Phytochemicals contained in *V. amygdalina* include saponins, sesquiterpenes, lactones and flavonoids, steroid glucosides such as Vernoniosides A₁, A₂, A₃, A₄, B₁, B₂, B₃, D and E have been isolated from the plant (Ohigashi, 1994; Aregheore *et al.*, 1997; Igile *et al.*, 1995b). The plant (especially the leaf) has been found useful in the ethnotherapy of diabetes (Akah and Okafor, 1992; Uhegbu and Ogbuehi, 2004; Nwajo, 2005), asthma, schistosomiasis, malaria (Masaba, 2000), measles, diarrhoea, tuberculosis, abdominal pain and fevers, cough, (Akinpelu, 1999).

Before the adoption of a given feed/food resources in livestock feeding, evaluator tests for its composition, nutritional value and health implications of its consumption are essential to permit credible enlightenment programmes with regard to its quality and use (Aletor *et al.*, 2002; Owen, *et al.*, 2008a; Owen *et al.*, 2008b; Owen *et al.*, 2009a). There is information dearth on the effects on serum

metabolites and enzymes of broiler finisher chickens fed bitter leaf (*Vernonia amygdalina*) meal diets hence this study.

MATERIALS AND METHODS

The study was conducted in Poultry Research Unit of the Department of Animal Science; Rivers State University of Science and Technology, Port Harcourt South- South of Nigeria. The fresh leaves of bitter leaf with average age of 6 months were harvested in the Agip Estate, a few kilometers away from the University Farm; washed and air-dried for 60 days and sun-dried for one additional day to ensure perfect drying, milled to obtain bitter leaf (*Vernonia amygdalina*) leaf meal (VALM) and packed in jute bags. The meal was used to replace Groundnut cake (GNC) at 0, 5, 10 and 15% levels. Four experimental diets were formulated as shown in Table 1 below:

A total of 144 Marshal brooded broiler weighing 500 – 610g were used in a study that lasted for 28 days. The birds were housed in a deep litter with wood shavings as litter material. Before the arrival of the birds, the pens were cleaned, washed and disinfected. The birds were divided into 4 groups of 36 birds each. Each treatment group was further sub-divided into 3 replicates of 12 birds each in a completely randomized design.

Feed and water were offered *ad libitum*. Routine management practice and medications were maintained while the study lasted. At the end of the study, 3 birds per treatment were randomly selected and bled by severing the jugular vein. Blood samples were collected without anticoagulant for blood chemistry evaluation at the Department of Chemical Pathology Laboratory of University of Port Harcourt Teaching Hospital. The serum metabolite and enzyme parameters determined includes Calcium (Ca), Phosphate (P), Serum Glutamic Pyruvic Transaminase (SGPT) known as Alanine Transaminase (ALT), Serum Glutamic Oxaloacetic Transaminase (SGOT) Known as Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP). All the data collected were subjected to Analysis of Variance (ANOVA) according to Steel and Torrie (1980) and Means were partitioned where necessary using Duncans New Multiple Range Test (DNMRT) as outlined by Obi (1990).

RESULTS AND DISCUSSION

Mean values of biochemical parameters are presented in Table 2. The results showed that SGPT, SGOT and ALP were all significantly ($P < 0.05$) affected by dietary treatments. The data showed that broiler chickens reared on 15% VALM had higher ($P < 0.05$) supply of SGPT (9.00 iu/l), SGOT (58.30 iu/l) and ALP (120.00 iu/l) relative to birds fed 0% and 5% VALM. The effects of VALM inclusions on serum Ca and P of broiler finisher were not significant ($P > 0.05$).

Transaminases are the most commonly used

indicators of cellular necrosis and high levels in serum may indicate liver malfunctioning (Rosenthal, 1977). They occupy a central position in amino acid metabolism; increase in their activities in the serum could have a consequential effect on the amino acid metabolism in these tissues. Furthermore, it may indicate some sort of injury to the organs. Such damage may cause the enzymes to leak from the injured organs to the blood stream (Oloyede *et al*, 2007). In this study, the birds placed on different levels of VALM based diets showed significant increase in glutamate pyruvic transaminase activities when compared to the GNC based diet. Although values of GPT of birds on test diets were higher when

Table 1: Composition of the Treatment Diets of Broiler Finisher

Ingredients	Dietary levels of <i>Vernonia amygdalina</i> leaf meal (%)			
Maize	57.00	57.00	57.00	57.00
Groundnut cake	24.00	23.00	20.00	17.00
Leaf meal	0.00	5.00	10.00	15.00
Fish meal	3.00	3.00	3.00	3.00
Wheat offal	5.00	3.00	2.00	1.00
Brewers' dried grain	5.00	3.00	2.00	1.00
Oyster Shell	2.50	2.50	2.50	2.50
Bone Meal	2.50	2.50	2.50	2.50
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25
Calculated chemical composition				
Crude Protein	19.82	19.50	18.70	17.90
Ether Extract	5.34	5.34	5.29	5.23
Crude Fibre	3.58	3.69	3.92	4.18
Calcium	2.03	2.03	2.35	2.51
Phosphorus	0.89	0.87	0.82	0.76
Ash	3.13	3.50	3.88	4.25
Metabolisable energy	2948.35	2756.80	2678.30	2585.20

*To provide following per kg of feed: vitamin A – 1000iu, Vitamin D3 – 20000iu, Vitamin B1 – 0.7mg, Nicotinic acid – 25mg, Calcium pantothenate – 12.5mg, Vitamin B12 – 2.5mg; Vitamin K3 – 2.5mg; Vitamin E – 25mg; Cobalt – 0.40mg; Biotine – 0.5mg

Table 2. The effects of varying levels of *Vernonia amygdalina* on serum metabolites and enzymes of broiler finishers.

Parameters	Treatments			
	A (0% VALM)	B (5% VALM)	C (10% VALM)	D (15% VALM)
Ca mmol/l	1.20	1.30	1.40	1.60
P mmol/l	1.10	1.20	1.30	1.30
SGPT iu/l (mean ± SEM)	7.00 ^b ± 0.20	7.00 ^b ± 0.15	9.00 ^a ± 0.10	9.20 ^a ± 0.12
SGOT iu/l (mean ± SEM)	54.50 ^c ± 0.45	55.00 ^c ± 0.30	57.00 ^b ± 0.15	58.30 ^a ± 0.18
ALP iu/l (mean ± SEM)	80.00 ^c ± 0.15	85.50 ^c ± 0.20	102.00 ^b ± 0.18	120.00 ^a ± 0.21

Within rows, means ± SEM with different superscript differs significantly at $p < 0.05$. SEM: Standard error of mean

compared to the control diet which, however, still falls within the normal range in chickens which ranged from 1-37 iu/l (Ker *et al*, 1982). Glutamate oxaloacetate transaminase is associated with the mitochondria and cytoplasm. Alteration in its activity could imply alteration in the cytosolic content. The mitochondria is regarded as the engine house of the cell and exposure of this organelle to assault of any form could imply cell death. The activities of GOT was observed to be higher in birds fed various VALM based diets when compared with the birds placed on the control diet. Similar trend was also observed for ALP activity in the serum. It was observed to be highest in birds fed 15% VALM based diets.

These observations could be as a result of the negative effect of anti-nutrients in VALM (Ohigashi, 1994; Aregheore *et al*, 1997; Igile *et al*, 1995b). There was no significant ($P>0.05$) dietary effect on the serum electrolytes (Ca and P) studied. These ions are needed to maintain proper osmotic and electrolyte balance in the body fluid of the animal. The fact that the dietary treatment did not influence those ions might suggest that the inclusion of VALM up to 15% in broiler finishers' diet would be adequate to support normal osmotic and electrolyte environment in the broiler chicken.

CONCLUSION

The present study investigated the effect of the inclusion of VALM on the serum metabolites and enzymes of broiler chickens. From the result obtained, it was observed that some anti-nutrients are present in VALM which cannot be totally eliminated by the processing techniques applied like drying and milling. It could also be inferred that the normal range of some biochemical indices on the treatment and non-significant effect on the serum Ca and P measured might substantiate the nutritional adequacy and safety of VALM in broiler finishers' diet.

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