Replacement Value of Enzyme Fortified Feather Meal on Haematology and Biochemical indices of Layer Chickens raised in a deep litter

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Abstract

High cost of animal feed has been a major challenge in animal production, hence the need for alternative feed resources that reduces the cost of feed and increases the production that will meet the growing demands for animal protein consumption. The research was carried out to examine the replacement value of enzyme-enhanced feather meal on the haematology and serum biochemical parameters of layer chickens. A total of one hundred and fifty Isa brown layers were randomly distributed into five treatment groups in a completely randomized design, with each group replicated three times. The experimental diets 1-5 were formulated so that diet 1 contained 0% enzyme-enhanced feather meal, while diets 2, 3, 4, 5 contained 1, 2, 3 and 4% levels of enzyme-enhanced feather meal, respectively. Blood samples were taken from the birds, with one sample from each replicate, resulting in a total of nine samples per treatment for the haematological and biochemical evaluations. Results from the study showed significant difference on all haematological indices and for serum biochemical indices except for albumin, globulin, creatinine, K, bicarbonate and chloride ions which recorded non-significant difference. The study suggested that enzyme fortified feather meal could be used in layer's diet up to 4% without any adverse effect.

Keywords: haematology, serum biochemical, lsa brown layers, enzyme fortified feather meal

Valeur de remplacement de la farine de plumes enrichie en enzymes sur l'hématologie et les indices biochimiques des poules pondeuses élevées sur litière profonde

Résumé

Le coût élevé des aliments pour animaux a été un défi majeur dans la production animale, d'où la nécessité de ressources alimentaires alternatives qui réduisent le coût des aliments et augmentent la production pour répondre aux demandes croissantes de consommation de protéines animales. La recherche a été menée pour examiner la valeur de remplacement de la farine de plumes enrichie en enzymes sur l'hématologie et les paramètres biochimiques sériques des poules pondeuses. Un total de cent cinquante pondeuses Isa brown ont été réparties au hasard en cinq groupes de traitement dans un dispositif complètement aléatoire, chaque groupe étant répété trois fois. Les régimes expérimentaux 1 à 5 ont été formulés de sorte que le régime 1 contienne 0 % de farine de plumes enrichie en enzymes, tandis que les régimes 2, 3, 4, 5 contenaient respectivement des niveaux de 1, 2, 3 et 4 % de farine de plumes enrichie en enzymes. Des échantillons de sang ont été prélevés sur les oiseaux, avec un échantillon par répétition, résultant en un total de neuf échantillons par traitement pour les évaluations hématologiques et biochimiques. Les résultats de l'étude ont montré une différence significative sur tous les indices hématologiques et pour les indices biochimiques sériques sauf pour l'albumine, la globuline, la créatinine, le K, les ions bicarbonate et chlorure qui ont enregistré une différence non significative. L'étude a suggéré que la farine de plumes enrichie en enzymes pourrait être utilisée dans l'alimentation des pondeuses jusqu'à 4 % sans aucun effet néfaste.

Mots-clés : hématologie, biochimie sérique, pondeuses Isa brown, farine de plumes enrichie en enzymes

Running title: Haematology and biochemical evaluations of layers

Introduction

Poultry plays a significant role in producing and providing animal protein as efficiently as possible in the least amount of time (Chen *et al.,* 2016). In Nigeria, poultry production is regarded as a source of income and a means of obtaining economic independence (Ogundipe, 2002). Feeding, a crucial part of poultry production, contributes 70-80% of the overall cost of production as a result of competition among humans, industry and farm animals for the same food items, as food, feed or raw materials (Assam *et al.,* 2022). Many farmers are looking for alternate ways to increase feed utilization for overall animal performance and profitability due to high cost of feed in broiler production (Anyanwu *et al.,* 2022).

Haematological indices are metrics associated with blood and the organs that form it (Bamishaiye *et al.,* 2009). Khan and Zafar (2005) reported that haematological parameters are reliable markers of an animal's physiological state. Animals with a complete blood composition are more likely to perform well (Isaac *et al*., 2013). The physiological haemoglobin roles include carrying carbon dioxide out of the animal's body and carrying oxygen to the tissues for the animal's metabolism of food, which releases energy for other bodily processes (Soetan *et al.,* 2013). Evidence for a variety of conditions, including stress, infection, malnutrition, and health status, can be obtained by comparing the animal's haematological and biochemical indices with a reference interval (Clifford and Briggs, 2007).

The enzyme, Biozyme is a combination of three enzymes; lipase, protease and amylase which are normally produced by the pancrease and are important in the digestion of fats, proteins and sugar (Biozyme Manual, 2010).

Materials and methods

Experimental site: The study was carried out at the Teaching and Research Farms of Imo State University Owerri. Owerri is situated in Nigeria's tropical rain forest region with the coordinates of longitude $7^0$03E, latitude $5^0$48'N and elevation of 73 meters above sea level. The annual evapo-transpiration is 1450mm, with a mean annual rainfall of 1750mm.

Experimental birds

A total of 35-week old 150 ISA Brown hens were split up into five treatment groups, each consisting of 30 birds. In a completely randomized design (CRD), each group was assigned at random to one of the experimental diets. Three replicates of 10 birds were created for each treatment group.

Management operations

The birds were housed on a deep litter pen. They had unlimited access to feed and water. Vaccination and medication schedule were strictly adhered to. Biosafety was also ensured. Daily routine management of washing of the feeder and drinker were done. Litters were changed as at when due.

Processing of experimental materials

The poultry feathers were sourced from commercial slaughter houses, washed and boiled with a batch cooker at internal pressure of 50-60Pa (Pascal) for 60-90 minutes (Tesfaye *et al.,* 2017) under intense pressure until the hydrolysis process sufficient to convert the feathers into a more soluble form. After boiling, the feathers were washed, sundried and blended to make feather meal.

The fish meal and other feed ingredients used for the purpose of the research were procured from a reliable feed mill in Owerri. Proximate analyses of the feather meal and fishmeal were conducted at Precision Analytical and Research Laboratory Ibadan, Nigeria. The Mineral analysis was performed using the procedure described by Grueling (2000), while the gross energy was calculated using Gallencamp Oxygen Adiabatic Bomb Calorimeter (AOAC, 1995).

Experimental diets

Five experimental diets for the layer diets were prepared, where experimental diet one which was

used as the control contains 0.0% enzyme fortified feather meal. Diets two, three, four and five contained 1, 2, 3, and 4% enzyme fortified feather meal with 100g of biozyme® per 100kg of feed respectively according to manufacturer's prescription. The composition of the diet is shown in Table 1.

Collection of the data

Collection of blood samples: Three birds were chosen at random from each replicate at the conclusion of the feeding trial (90 days), making it a total of nine birds per treatment for biochemical and haematological studies. Ten millilitres (10mL) of blood sample were collected from the birds through the wing vein with a 10mL sterile syringe. For the haematological evaluation, 5 mL was placed in large bottles with ethylene diamine tetra-acetic acid (EDTA), an anticoagulant. In order to create sera for serum biochemical analysis, the remaining 5 milliliters of blood were placed in a vial bottle (without an anticoagulant) and left to coagulate.

Ratiff and Halls (1973) method was used to calculate packed cell volume (PCV), Schalm *et al.* (1975) method was used to calculate other haematological parameters.

Data analysis: The statistical package for social sciences (SPSS, 2012) was used to perform a one-way analysis of variance. The Duncan Multiple Range Test, as described by the same SPSS software, was used to separate means.

Table 1: Composition of the ingredients for the layer diets (%)

	Diets treatments				
Ingredients	**T1(0%)**	**T2 (1%)**	**T3(2%)**	**T4(3%)**	**T5(4%)**
Maize	46.00	46.00	46.00	46.00	46.00
Soyabean	12.00	12.00	12.00	12.00	12.00
Fish meal	4.00	3.00	2.00	1.00	0.00
EFFM	0.00	1.00	2.00	3.00	4.00
Groundnut cake	6.00	6.00	6.00	6.00	6.00
Palm kernel cake	11.20	11.20	11.20	11.20	11.20
Bone meal	4.00	4.00	4.00	4.00	4.00
Oyster shell	2.00	2.00	2.00	2.00	2.00
Lime stone	3.00	3.00	3.00	3.00	3.00
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.10	0.10	0.10	0.10	0.10
Vita/min premix	0.25	0.25	0.25	0.25	0.25
Common salt	0.30	0.30	0.30	0.30	0.30
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrient Content of the Experimental diets					
Crude protein (%)	18.81	18.85	18.90	18.95	18.99
Calcium (%)	3.41	3.40	3.39	3.38	3.37
Phosphorus (%)	0.99	0.98	0.97	0.96	0.94
Crude fibre (%)	4.35	4.34	4.32	4.31	4.29
ME (Kcal/kg)	2653.24	2662.95	2672.66	2682.37	2692.08

ME=Metabolisable energy, EFFM=Enzyme fortified feather meal

Table 2: Proximate and mineral composition of the test materials

Nutrients	Ingredients	
	Fish meal	**Feather meal**
Dry Matter (%)	94.06	90.75
Crude protein (%)	55.93	60.44
Crude fat (%)	5.91	20.79
Crude fibre (%)	1.84	0.32
Total ash (%)	23.10	7.31
Nitrogen free extract (%)	8.80	4.21
Metabolisable energy (Kcal/kg)	3085.35	4086.36
Mineral compositions		
Calcium (%)	6.09	1.87
Phosphorus (%)	3.05	0.71
Magnesium (%)	2.00	0.08
Iron Fe (%)	1.50	0.05
Copper cu (%)	3.70	0.001
Zinc (%)	0.003	0.012

Results

Table 1 presented the composition of the ingredients formulated for the layer chicken. The five experimental diets contained equal amounts of maize (46%), soyabean (12%), groundnut cake (6%), palm kernel cake (11.2%), bone meal (4%), oyster shell (2%), limestone (3%), lysine (0.15%), methionine (0.10%), and vitamin/premix (0.25%).

For the replacement feed material (fish meal), experimental diet 1 (T1) which served as the control contained 4%, T2 contained 3%, T3 contained 2%, T4 contained 1% while T5 contained 0%.

For the test material (EFFM), experimental diet 1(T1), contained 0%, T2 contained 1%, T3 contained 2%, T4 contained 3% and T5 contained 4%.

Table 2 presented the result of the proximate and mineral composition of the fish meal and feather meal respectively. The fish meal had higher composition of the dry matter (94.06%), crude protein (55.93%), crude fat (5.91%), crude fibre (1.84%), total ash (23.10%) and nitrogen free extract (8.80%) than the feather meal which had lower compositions of the analysis, but higher metabolizable energy (Kcal/kg) (4086.36) more than the fish meal.

Haematological indices

Table 3 shows the haematological indices of the layers fed diets with varied quantities of enzyme-fortified feather meal (EFFM). The study found significant differences ($P<0.05$) between treatments in haemaglobin (Hb), packed cell volume (PCV), red blood cell (RBC), erythrocyte sedimentation rate (ESR), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and white blood cell differentials (WBC). However, there was no trace of basophil seen anywhere within the treatment means.

Table 3: Haematological values of the laying hens fed diets containing different quantities of enzyme fortified feather meal

Parameters	0.0	1.0	2.0	3.0	4.0	SEM
	(%)					
Hb (g/dL)	13.00^{ab}	13.07^{a}	12.73^{bc}	12.43^{c}	12.80^{ab}	0.10
PCV (%)	42.67^{ab}	43.33^{a}	40.33^{bc}	38.33^{c}	41.00^{ab}	0.95
RBC (x10^{12}/L)	12.17^{ab}	12.90^{ab}	12.60^{bc}	12.33^{c}	12.77^{ab}	0.12
MCV (*fl*)	132.87^{ab}	133.50^{a}	131.97^{bc}	131.07^{c}	132.07^{bc}	0.45
MCH (*pg*)	20.06^{b}	20.26^{a}	20.20^{ab}	20.20^{ab}	20.06^{b}	0.03
MCHC (g/dL)	30.43^{b}	30.37^{b}	31.70^{ab}	32.47^{a}	31.33^{ab}	0.46
ESR (mm^3/h)	26.67^{c}	26.67^{c}	26.67^{c}	40.00^{a}	33.33^{b}	2.06
WBC (x10^9/L)	11.40^{a}	11.20^{b}	11.30^{ab}	11.24^{b}	11.23^{b}	0.04
Heterophils (%)	54.00^{a}	53.67^{a}	53.33^{a}	51.67^{b}	51.00^{b}	0.05
Eosinophil (%)	1.33	1.67	1.33	1.67	1.33	0.13
Lymphocytes (%)	43.33	42.67	44.00	45.33	45.67	0.48
Basophils (%)	0	0	0	0	0	0
Monocytes (%)	1.33^{b}	2.00^{a}	1.33^{b}	1.33^{b}	2.00^{a}	0.13

means with different superscripts on the horizontal rows are significantly different (P<0.05)

Note: Hb=haemoglobin, PCV=packed cell volume, RBC=red blood cell, MCV=mean cell volume, MCH= mean cell haemoglobin, MCHC=mean cell haemoglobin concentration, ESR=erythrocyte sedimentation rate, WBC=white blood cell

SEM: Standard Error of mean

Serum biochemical properties

The serum biochemical properties of layers fed different quantities of enzyme fortified feather meal are summarized in Table 4. Results showed that the albumin, globulin, K and HCO_3^-, Cl^- were similar *(P>0.05)* across the treatments while other properties measured differed *(P<0.05)* across the treatment means.

Table 4: Biochemical parameters of laying birds fed varying levels of enzyme fortified feather meal for fish meal

Parameters	0.0	1.0	2.0	3.0	4.0	SEM
Total serum protein	65.00^{a}	66.67^{a}	64.00^{ab}	63.67^{ab}	61.00^{b}	0.61
Albumin (g/L)	21.33	23.33	22.00	21.00	22.00	0.48
Globulin (g/L)	43.67	43.33	42.00	42.67	39.67	0.62
Urea (mmol/L)	7.63^{b}	8.00^{a}	7.37^{b}	7.30^{b}	6.70c	0.12
Creatinine (mmol/L)	51.33^{b}	55.67^{a}	53.00^{b}	52.00^{b}	51.33^{b}	0.52
Cholesterol (mmol/L)	8.27^{b}	8.50^{a}	8.20^{bc}	8.23^{b}	8.00^{c}	0.05
K^+ (mmol/L)	1.23	1.23	1.23	1.20	1.20	0.02
HCO_3- (mmol/L)	10.17	10.23	10.23	10.10	10.17	0.02
Na+ (mmol/L)	42.33^{b}	45.33^{a}	42.33^{b}	42.33^{b}	42.33^{b}	0.43
CL- (mmol/L)	20.67	22.00	21.67	21.33	21.33	0.21
ALP (iu/L)	1.20^{ab}	1.27^{a}	1.13^{b}	1.17^{ab}	1.23^{ab}	0.02

SGOT (iu/L)	11.23^{ab}	11.30^{a}	11.17^{ab}	11.10^{b}	11.17^{ab}	0.02
SGPT (iu/L)	6.93^{ab}	7.17^{a}	6.87^{b}	6.80^{b}	6.50^{c}	0.07

Means on the horizontal rows with different superscripts are significantly different (P<0.05)

K^{+}= Potassium ion, HCO3-=bicarbonate, Na^{+}=sodium ion, CL-=chlorine ion, SGOT=serum glutamic-oxaloacetic transaminase, SGPT=serum glutamic-pyruvic transaminase

SEM: Standard Error of mean

Discussion

Haematological indices from the study shows that values of Hb, PVC, RBC, MCV, MCH, MCHC, ESR differed significantly across the treatments although with no clear trend. These values were much reduced in birds fed 3% diets, while the ESR was significantly higher in this treatment group (T4) more than the others. Significant reductions in the white blood cell and heterophils were also observed with increased inclusion levels of EFFM (T4 and T5). However, all the values in this study were within the normal range for chicken (Mitruka and Rawnsley, 1997; Bonnous and Steadman, 2000; Merek Veterinary Manual, 2006).

On the other hand, serum biochemical indices of total protein (TSP), urea, creatinine, cholesterol, Na+, ALP, SGOT and SGPT showed treatment effects, although did not follow any clear pattern. Serum biochemical constituents (TSP, albumin, and globulin) have been used as predictors of serum/blood dietary proteins and also routinely used for the detection of organ diseases in domestic livestock (Guleken *et al.,* 2020). The albumin and globulin values were similar among the groups indicating that the inclusion of enzyme fortified feather meal in the layer diets had no negative effect on protein metabolism of the experimental hens. The serum TSP and albumin indicates the quality and availability of protein, hence the enzyme fortified feather meal diets had sufficient protein enough to maintain appropriate blood protein levels in the blood (Kwari *et al.,* 2014). Values obtained in the serum protein fell within the reference range reported for chicken (Bamgbose *et al.,* 2007). Laying hens on diet 5 recorded the lowest serum urea value while hens on diets T3 – T5 had lower but similar creatinine values in hens on the control treatment indicating that the birds did not suffer any impairment in protein synthesis or utilization (Denli *et al.,* 2009). Serum urea is a by-product from the metabolism of proteins by the liver and is removed from the blood by the kidney. The serum potassium, bicarbonate and chloride values were similar (*P>0.05*) among all treatment means. Serum electrolytes are used in maintaining cellular iconicity, fluid balance, PH and regulation of neural and muscular functions as reported by Cheesebrough (2000). The electrolyte values were within the reported range for chicken (Obih, 2018). Inclusion of enzyme fortified feather meal in the experimental diets did not show any negative effects on the haematology and biochemical properties of the layer chickens even up to 4% level inclusion. The study therefore suggests that in order to bring down the cost of feeding in layer chicken production which accounts for about 60% of the overall cost of production, use of alternate feed source such as feather meal should be incorporated in layer chicken feed formulation.

Conclusion

Inclusion of enzyme fortified feather meal did not record any deleterious effect on the heamatological results of the layers even up to 4% level inclusion. Hence, the study suggested that inorder to bring down the cost of feeding in layer chicken production, use of agro-byproducts such as feather meal should be incorporated in layer chicken feed formulation.

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