

**PHYSIOLOGICAL INFLUENCE OF MULTI-ENZYME  
SUPPLEMENTATION ON BROILER CHICKENS FED NORMAL  
ENERGY AND PROTEIN LEVEL DIET**

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**ABSTRACT**

*A total of 120 day-old Abor Acre mixed sex broiler chickens were used in a completely randomized design experiment to investigate the Physiological influence of multi-enzyme supplementation on finisher broilers fed normal energy and protein level diet. Each dietary treatment was replicated four (4) times with fifteen (15) birds per replicate. Multi-enzyme was supplemented at 1g/kg of experimental diet. Results showed that multi-enzyme supplementation to normal energy and protein dietary levels decreased live weight, organ weights and carcass percentage weight of broiler chickens. This suggested that the practice of supplementing multi-enzyme to normal energy and protein starter to finisher broiler diets will negatively affect performance and should be discouraged.*

**Keywords:** Broiler chickens, multi-enzyme supplementation, organ, carcass, hematological, serum indices.

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**INTRODUCTION**

Nutritional level is one of the factors which affect the physiology of animals [1]. It also plays a vital role in the assessment of physiological and pathological status of birds [2]. Therefore nutrition could certainly affect the entire body physiology in terms of maintenance, growth, reproduction and health [3]. Nutrition, genetics, environment and age might also influence the blood profile of animals [4]. Diets for broilers are based mainly on gains such as corn, sorghum and soybean, which have become expensive in recent years [5]. However, the dynamics of the scarcity of the feed materials for poultry placed premium on the study of the actual required dietary nutrient in poultry feeding which must be well balanced for optimum performance and production [6].

The identification and alleviation of factors that inhibit nutrient utilization are necessary for poultry production [7]. The non-starch polysaccharides (NSP) in ingredients such as soybean meal and other cereal grains are the main factors which reduce nutrient bioavailability [8].

Meanwhile, the introduction of synthetic enzymes for enhanced utilization in monogastric feeding to meet dietary energy and protein needs has been worked upon [9]. It has been documented that supplementation of exogenous enzyme to cereal-based diet is often followed by improved performance [10]. Adeyemi *et al.* [11] reported that enzyme supplementation reduced gizzard weight in contrast to the findings of Omojola and Adesehinwa [12] who reported that the inclusion of exogenous enzyme to broiler diets did not affect the relative weights of kidney, gizzard, heart and liver. On the other hand, Alam *et al.* [13] reported significant improvement in bodyweight and body weight gain and reduced feed intake with enzyme supplemented diet, with no significant effect on the haematological and most serum chemistry profiles of the birds with the inclusion of enzyme. Hajati *et al.* [14] reported significant increase in carcass yield and decreased the relative size of the digestive organs as a result of enzyme supplementation.

Increasing energy and protein density of diets for broiler chicken has given inconclusive results; hence this study aimed at investigating the influence of multi-enzyme supplementation on finisher broilers fed normal energy and protein level diet in south-east tropical zone of Nigeria.

## **MATERIALS AND METHODS**

A total of 120 day-old Abor Acre mixed sex broilers from Nastech hatchery, Oyo State, Nigeria were used for this study. The birds were allocated to the experimental dietary treatment groups in a completely randomized design. Each treatment was replicated 4 times with 15 chicks per replicate; the dietary treatment was formulated to meet the nutrient recommendation according to feeding standard of broilers [15]. The composition of the diet is shown in Table 1. Maxi-Grain<sup>R</sup> multi-enzyme, (Polchem Hygiene Laboratories Pvt. Ltd India), was supplemented at the level of 1g/kg of the experimental diets.

Brooding was done with kerosene stoves under metal hovers, while light was provided with 100 watt electric bulbs for additional five hours at night each day. Water and experimental diet were provided *ad libitum*. Routine vaccination against common diseases (Newcastle, Gumboro, and Typhoid) and medications were administered to the birds during the experiment.

### **Experimental Design**

The experimental design for this study is a Completely Randomized Design, with the model;

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:  $Y_{ij}$  = Single observation on broilers influence,  $\mu$  = Overall mean,  $e_{ij}$  = Experimental error.

### **Data Collection**

At the end of the 7<sup>th</sup> week (day 49), 4 birds per treatment were randomly selected, starved over-night but allowed access to drinking water, weighed and slaughtered by severing the jugular vein. They were thoroughly bled and the internal organs and intestine were eviscerated through a slit made between the end of the keel bone and the cloacae.

### **Organ and Carcass Measurement**

The weights of the small intestine, heart, liver, kidney, gizzard, spleen and lungs were taken in groups using electronic sensitive scale (Model; CS200 Ohaus, China) and expressed as percentage of live weight. The carcass cut parts; breast, shank; thigh, drumstick, wings, neck, back and head were dissected out, weighed using electronic sensitive scale and expressed as percentage of dressed weight.

### **Blood collection**

A sterile needle was used to collect 5 ml of blood from the jugular vein of each bird before slaughter. Two millilitres of the blood was discharged into a sample bottle containing Ethylene Diamine Tetra-acetic

Acid (EDTA) as anti-coagulant and used for the evaluation of haematological parameters [16]. The remaining 3 ml of blood was gently discharged into a clean test tube and allowed to coagulate to separate blood sera for serum biochemistry analysis. The biochemical parameters evaluated included globulin, albumin, total protein, creatinine, urea, cholesterol and glucose [17].

### Statistical Analysis

Data collected were subjected to one way analysis of variance (ANOVA) for a Completely Randomized Design while differences between treatment means were separated using Duncan's Multiple Range Test of SPSS 15 [18](SPSS, 2011).

**Table 1. Percentage Composition of the experimental diet.**

<b>Feedstuff</b>	<b>Starter</b>	<b>Finisher</b>
Maize	57.32	63.29
Soybean meal	38.98	33.61
Palm oil	0.45	3.10
Lysine	0.20	0.10
Methionine	0.10	0.10
Bone meal	2.55	2.50
Vitamin premix	0.25	0.25
Nacl	0.15	0.15
Total	100	100
CP (%)	23	21
ME (kcal/kg)	3000.98	3204.54

\*Vitamins and mineral premix supplied the following per kg starter diet: Vitamin A:  $1 \times 10^7$ ; Mn 80 g; vitamin K: 2,250 mg, Fe 20 g; Thiamine 1,750 mg; Cu 5 g; Riboflavin 5,000 mg; Iodine 1.2 g; Pyridoxine 2,750 mg; Se 200 mg; Niacin 2,750 mg; Co 200 mg; Pantothenic acid 7,500 mg; Zn 50 g; Folic acid 7,500 mg.

\*Vitamins and premix supplied the following per kg finisher diet: Vitamin A 800 I.U.; D3 (1, 4731.C.U); Riboflavin 4.20 mg; Pantothenic acid 5.0 mg; Nicotinic acid 20.0 mg; Folic acid 0.5 mg; Choline 300 mg; Vitamin K, 2.0 mg; Vitamin B12, 0.01 mg; Vitamin E, 2.5I.U; Manganese, 56.0 mg; Iodine, 1.0 mg; Iron 20.0 mg; Copper 10.0 mg; Zinc 50.0 mg and Cobalt 1.25 mg.

### RESULTS

Live weight and organ characteristics of finisher broilers fed normal energy and protein level diets supplemented with or without multi-enzyme are shown in Table 2. Broilers fed multi-enzyme supplemented diet had significantly ( $P < 0.05$ ) lower live weight, weight of the kidney and small intestine but with significantly ( $P < 0.05$ ) higher weight than those broiler chickens fed diet without multi-enzyme supplementation.

Influence of multi-enzyme supplementation on Carcass characteristics of finisher broiler chickens fed normal energy and protein level diet is shown on Table 3. Dress percentage, breast and back-cut of broilers fed normal energy and protein diet level without multi-enzyme supplementation were significantly ( $P < 0.05$ ) higher than those fed normal energy and protein diet supplemented with multi-enzyme.

Haematological parameters of finisher broiler chickens fed normal energy and protein level diet with and without multi-enzyme supplementation are shown on Table 4. There were no significant ( $P > 0.05$ ) differences in haematological parameters of broiler chickens fed either multi-enzyme or non multi-

enzyme supplemented diet except for WBC and MCH where broilers fed non-multi-enzyme supplemented diet had significantly ( $P<0.05$ ) lower values than those fed multi-enzyme supplemented diet.

**Table 2. Live weight and organ characteristics of finisher broilers fed normal energy and protein level diet supplemented with and without multi-enzyme**

Parameters	Non-Enzyme supplemented	Enzyme-supplemented	SEM
Live weight (g)	1840.00 <sup>a</sup>	1573.33 <sup>b</sup>	108.73
Liver (%)	1.81	2.15	0.30
Kidney (%)	0.50 <sup>a</sup>	0.46 <sup>b</sup>	0.08
Lungs (%)	0.38	0.40	0.01
Spleen (%)	0.13	0.16	0.02
Gizzard (%)	1.95	2.37	0.21
Heart (%)	0.44 <sup>b</sup>	0.52 <sup>a</sup>	0.03
Small Intestine (%)	3.04 <sup>a</sup>	2.80 <sup>b</sup>	0.11

<sup>ab</sup>Means in the same row with different superscripts differ significantly ( $P<0.05$ ); SEM= standard error of mean.

**Table 3. Carcass characteristics of finisher broilers fed normal energy and protein level diet supplemented with and without multi-enzyme.**

Parameters	Non-Enzyme supplemented	Enzyme-supplemented	SEM
Dressed percentage	69.78 <sup>a</sup>	63.68 <sup>b</sup>	0.73
Wings (%)	13.28	14.45	0.48
Drum stick (%)	16.12	15.90	0.51
Thigh (%)	17.79	17.80	0.31
Breast (%)	30.86 <sup>a</sup>	27.40 <sup>b</sup>	0.59
Back cut (%)	24.96 <sup>a</sup>	22.45 <sup>b</sup>	0.80

<sup>ab</sup>Means in the same row with different superscripts differ significantly ( $P<0.05$ ); SEM= standard error of mean.

**Table 4. Haematological indices of finisher broiler chickens fed normal energy and protein level diet supplemented with and without multi-enzyme.**

Parameter	Non-Enzyme supplemented	Enzyme-supplemented	SEM
PCV (%)	29.00	31.25	0.42
RBC ( $\times 10^6/\mu\text{l}$ )	2.97	3.10	2.40
WBC ( $\times 10^3/\mu\text{l}$ )	6.15 <sup>b</sup>	10.00 <sup>a</sup>	1.76
Hb Con.(g/dl)	7.45	8.18	0.12
MCV (fl)	99.48	102.01	6.72
MCH (pg)	25.55 <sup>b</sup>	30.12 <sup>a</sup>	1.85
MCHC (g/dl)	25.69	26.18	2.55

<sup>ab</sup>Means in the same row with different superscripts differ significantly ( $P<0.05$ ); SEM= standard error of mean.

Effect of multi-enzyme supplementation on the serum biochemical indices of finisher broilers fed normal energy and protein level diet supplemented with and without multi-enzyme is presented in Table 5.

Significant ( $P < 0.05$ ) higher urea, cholesterol and glucose levels were observed among broilers fed normal energy and protein level diet supplemented with multi-enzyme than those fed the same diet without multi-enzyme supplementation.

**Table 5. Serum biochemical indices of finisher broiler chickens fed normal energy and protein level diet supplemented with and without multi-enzyme.**

Parameter	Non-Enzyme supplemented	Enzyme-supplemented	SEM
Globulin (g/dl)	1.87	1.64	0.28
Albumin (g/dl)	1.15	1.01	0.23
Total protein. (g/dl)	2.64	2.65	0.22
Creatinin (mg/dl)	0.45	0.26	0.03
Urea (mg/dl)	2.67 <sup>b</sup>	4.12 <sup>a</sup>	0.26
Cholesterol (mg/dl)	132.82 <sup>b</sup>	150.00 <sup>a</sup>	34.37
Glucose (mg/dl)	186.75 <sup>b</sup>	194.54 <sup>a</sup>	7.38

<sup>ab</sup>Means in the same row with different superscripts differ significantly ( $P < 0.05$ ); SEM= standard error of mean.

## DISCUSSION

The beneficial effect of enzyme supplementation of poultry diets based on viscous cereals are well established [19]. Earlier report showed that supplementation of exogenous enzyme to cereal-based diet is often followed by improved performance [10]. Thus, enzyme supplementation has become increasingly popular in poultry feeds [20]. This study has shown that enzyme supplementation to normal energy and protein diet significantly ( $P < 0.05$ ) decreased live weight, kidney, heart and small intestinal percentages of finisher broiler chickens.

Dressed percentage, breast and back-cut of finisher broiler chickens fed multi-enzyme supplemented diet had significantly ( $P < 0.05$ ) reduced percentage weight than those fed non multi-enzyme supplemented diet. The significantly ( $P < 0.05$ ) decreased live weight and carcass cut-parts of finisher broiler chickens fed normal dietary energy and protein level diet could be as a result of increased nutrient availability probably due to multi-enzyme activity. Multi-enzyme activity results in fast nutrient requirement attainment by the birds and thus, reduced feed intake and consequently poor performance and organs weight reduction of the broiler birds. This is in line with the previous report that soybean meal contains non-digestible carbohydrates which could become available to broilers with proper enzyme supplementation [21]. Zanella *et al.* [22] reported that exogenous enzyme supplementation of broilers diets based on corn and soybean improved nutrient digestibility which is in corroboration with the findings of this study but contradicts with performance improvement. The poor performance observed with broilers fed multi-enzyme supplemented diet could be attributed to dietary nutrient level; this is in accordance with the report of Zhu *et al.* [20] who stated that exogenous enzyme supplementation allowed for reduction in the energy formulation of diets.

Examination of blood provides the opportunity to clinically investigate the presence of several metabolites while helping in the assessment of physiological status of animals [23]. Significant ( $P < 0.05$ ) higher WBC, MCH, urea, cholesterol and glucose levels above normal ranges of finisher broiler chickens fed multi-enzyme supplemented diet could be due to the activities of multi-enzyme that may have increased the nutrient density of the experimental diet. However, the enzyme supplementation did not manifest any anemic condition in the birds.

In conclusion, therefore, the results of this study suggests that supplementing normal energy and protein corn-soybean diet with multi-enzyme negatively affected internal organ weights and the over-all performance of finisher broiler chickens. Consequently, the results suggest that supplementation of multi-enzyme to normal energy and protein starter to finisher broiler chicken diets will negatively affect performance and should be discouraged.

## REFERENCES

1. Amaefule, R. A., Etuk, I. F., Iwuji, T. C., Ogbuewu, I. P., Obikaonu, O. H. and Amaefule, K. U. (2020). Haematological indices of grower pigs fed low protein and low energy diets supplemented with multi-enzyme. *Nigerian Journal of Animal Production*, 2020, 47 (1):167 – 173.
2. Amaefule, R. A. (2016). Physiological responses of broiler birds to low energy and low crude protein diets supplemented with multi-enzyme in the humid tropical environment. M.Sc thesis Department of Animal science. Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
3. Oke, Y. K., Herbert, U., Ebuzoeme, C. O. and Nwachukwu, E. N. (2007). Effect of genotype on haematology of Nigerian local chickens in a humid tropical environment. Proceedings of the 32<sup>nd</sup> Annual Conference of the Nigerian Society for Animal Production. Calabar, Nigeria. 18<sup>th</sup> – 21<sup>st</sup> March, PP; 123-125
4. Addas, P. A., David, D. L., Edward, A., Zira, K. E. and Midau, A. (2012). Effect of Age, sex and management system and some haematological parameters of intensively and semi-intensively kept chicken in mubi, Adamawa State, Nigeria. *Iranian Journal of Applied Animal Science*, 2 (3): 277 - 282.2.
5. Branson, A. and Hernández, G. (2012). Mexico-poultry and products semi-annual sector integration and strong demand continue, in global agricultural information network. <http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Poultry%20and%20Products%20Semi-annual%20Mexico%20City%20Mexico%203-20-2012.pdf>. Accessed 16<sup>th</sup> October, 2015.
6. Dairo, F. A. S., Adeseinwa, A. O. K., Oluwasola, T. A. and Oluyemi, J. A. (2010). High and low dietary energy and protein levels for broiler chickens. *African Journal of Agricultural Research*, 5: 2030 - 2038.
7. Zou, J., Ping, Z., Keying, Zhang, X. D. and Shiping, B. (2013). Effect of exogenous enzyme and dietary energy on performance and digestive physiology of broilers. *Journal of Animal Science and Biotechnology*, 4: 14.
8. Malathi, V. and Devegowda, G. (2001). *In vitro* evaluation of nonstarch polysaccharide digestion of feed ingredients. *Poultry Science*, 80 (3): 302 - 305.
9. Aletor V. A., Hamid, I. I., Niess, E. and Pfeffer, E. (2000). Low protein amino acid-supplemented diets in broiler chickens: Effect on performance, carcass characteristics, whole body composition and efficiencies of nutrient utilization. *Journal of the Science of Food and Agriculture*. 80 (5): 547 - 554.
10. Wang, Z. R., Qiao, S. Y., Lu, W. Q. and Li, D. F. (2005). Effect of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diet. *Poultry Science*, 84: 875 - 881.
11. Adeyemi, O. A., Jimoh, B. and Olufade, O. O. (2013). Soybean meal replacement with cassava leaf: blood meal mix with or without enzyme in broiler diets. *Archivos de Zootecnia*, 62: (238): 279.
12. Omojola, A. B. and Adesehinwa, A. O. K. (2007). Performance and carcass characteristics of broiler chickens fed diets supplemented with graded levels of Roxazyme G. *International Journal of Poultry Science*, 6: 335 - 339.
13. Alarm, M J., Howlider, M. A. R., Pramanik, M. A. H. and Haque, M. A. (2003). Effect of exogenous enzyme in diet on broiler performance. *International Journal of Poultry Science*, 2: 168 - 173.

14. Hajati, H., Rezaei, M. and Sayyahzadeh, H. (2009). The Effects of Enzyme Supplementation on Performance, Carcass Characteristics and Some Blood Parameters of Broilers Fed on Corn-Soybean Meal-Wheat Diets. *International Journal of Poultry Science*, 8 (12): 1199 - 1205.
15. National Research Council (1998). Nutrient requirement of poultry. 10<sup>th</sup> revised edition, National Academy Press, Washington, DC.
16. SPSS (2011). *Statistical Package for Social Students*, 2011 version 15 for Windows.
17. Coles, H. E. (1986). *Veterinary Clinical Pathology*. 4<sup>th</sup> edn., W. B. Saunders Co., Philadelphia.
18. Das, A. and Mohanty, P. K. (2017). Hematological and serum biochemical analyses of wild male blackrats, *Rattus rattus* (Linnaeus, 1758). *International Journal of Science and Research*, 6 (2): 1515 - 1519.
19. Annison, G. (1992). Commercial enzyme supplementation of wheat based diets raises ileal glycannase activities and improves apparent metabolizable energy, starch and pentosan digestibilities in broiler chickens. *Animal Feed Science Technology*. 38: 105 - 121.
20. Zhu, H. L., Hu, L. L., Hou, Y.Q., Zhang, J. and Ding, B. Y. I. (2014). The effects of enzyme supplementation on performance and digestive parameters of broilers fed corn-soybean diets. *Poultry Science, Journal*, 93: 1704 – 1712.19.
21. Cowan, W. D. (1993). Understanding the manufacturing, distribution, application and overall quality of enzymes in poultry feeds. *Journal of Applied Poultry Research*, 2: 293 - 299.
22. Zanella, L., Sakomura, N. K., Silversides, F. J., Figueirido, A. and Pack, M. (1999). Effect of enzyme supplementation of broiler diet based on corn and soybean. *Journal of Poultry Science*, 78: 561 - 568.
23. Amakiri, A. O., Owen, O. J. and Jack, D. O. (2009). Effect of refined petroleum product (kerosine) flame and fumes on haematological characteristics of broiler chickens. Proceedings of the 34<sup>th</sup> Annual Conference of the Nigerian Society for Animal Production, Uyo, Nigeria. 441 – 44.