MORPHOMETRY AND HISTOPATHOLOGY OF BROILER CHICKENS FED GINGER BY-PRODUCT MEAL TREATED WITH EXOGENOUS ENZYME †

[MORFOMETRÍA E HISTOPATOLOGÍA DE LOS POLLOS DE ENGORDE ALIMENTADOS CON UN SUBPRODUCTO DE GINGER TRATADO CON ENZIMA EXÓGENA]

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SUMMARY

Background. It has been reported that feeding of dried fermented ginger increased intestinal villus height, villus area, and cell area in broiler chickens. It has been reported that essential oils originating from plants have antimicrobial activity which have toxic effects in poultry when administered at very high doses. **Hypothesis.** Toxins may destroy some organs immediately (intra- cellular) or later (extracellular) affecting either biochemical functions or structure of organs depending on the dosage and therapy. **Methodology.** An experiment was conducted to evaluate morphometry and histopathology of chickens fed Ginger By-product Meal (GBM) diets. In this experiment, GBM was supplemented with a multi enzyme preparation (Maxigrain©), using 270 broiler chicks allocated to six experimental diets (0, 15 and 30% GBM; 0 and 0.01% enzyme) in a completely randomized design for eight weeks. **Results.** The inclusion of GBM had a significant effect ($P < 0.05$) on the weight of intestine, gizzard, intestinal length and abdominal fat. The weight of the gizzard increased while the abdominal fat decreased. There was also an increase in the length and weight of the intestine. Enzyme supplementation brought about a significant ($P < 0.05$) decrease in the weight of the breast but a significant ($P < 0.05$) increase in the weight and length of the intestine. **Conclusion.** The diets had no negative effect on most of the organs except the intestine where there was sloughing of the micro-villi caused by GBM and enzyme.
Key words: Broiler chickens; Ginger By-product Meal; enzyme; carcass; organ and histopathology.

RESUMEN

Antecedentes. Se ha reportado que la alimentación de jengibre fermentado seco aumentó la altura de las vellosidades intestinales, el área de las vellosidades y el área celular en los pollos de engorde. También, que los aceites esenciales procedentes de plantas tienen actividad antimicrobiana que tiene efectos tóxicos en las aves de corral cuando se administran a dosis muy altas. **Hipótesis.** Las toxinas pueden destruir algunos órganos de forma inmediata (intracelular) o posterior (extracelular) afectando las funciones bioquímicas o la estructura de los órganos, dependiendo de la dosis y la terapia. **Metodología.** Se realizó un experimento para evaluar la morfometría y la histopatología de pollos alimentados con dietas con un subproducto de Ginger (GBM). En este experimento, GBM se complementó con una preparación de múltiples enzimas (Maxigrain ©), utilizando 270 pollos de engorde asignados a seis dietas experimentales (0, 15 y 30% de GBM; 0 y 0.01% de enzima) en un diseño completamente al azar durante ocho semanas. **Resultados.** La inclusión de GBM tuvo un efecto significativo ($P < 0.05$) sobre el peso del intestino, molleja, longitud intestinal y grasa abdominal. El peso de la molleja aumentó mientras que la grasa abdominal disminuyó. También hubo un aumento en la longitud y el peso del intestino. La suplementación con enzimas produjo una disminución significativa ($P < 0.05$) en el peso de la pechuga, pero un aumento significativo ($P < 0.05$) en el peso y la longitud del intestino. **Conclusión.** Las dietas no tuvieron ningún efecto negativo en la mayoría de los órganos, excepto en el intestino donde se desprendieron las microvellosidades causadas por GBM y enzimas.
Palabras clave: pollos de engorde; harina de subproductos de jengibre; enzima; canal; órgano e histopatología.

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INTRODUCTION

About 85-90% of poultry feed consists of plant materials that contain large amount of dietary fibres. Ginger by-product is the residue obtained after extraction of juice from ginger rhizome, it has high levels of fibre. Ginger contains pungent substances such as zingerone, shogaols and gingerols which can adversely affect feed intake and suppress gastric contraction in animals (Suekawa *et al*., 1984; Nidaullah *et al*., 2010). Poultry however do not produce enzymes like cellulase, hemicellulases, xylanase and β-glucanase which are required for the digestion of cell wall components of plant materials (Panda *et al*., 2011). Enzymes permit utilization of high levels of agro-industrial by-products by disrupting the plant cell walls which brings about improved performance of monogastrics, enhance the absorption of nutrients by improving nutrient digestibility, destruction of anti-nutritional factors, manipulation of gut flora population, as well as through their effect on the intestinal morphology (Brenes *et al*., 1993; Bedford, 1996; Acamovic, 2001; Attia *et al*., 2003; Ngxumeshe and Gous, 2009). Maxigrain© contains cellulase, phytase, beta-glucanase and xylanase. Bone (1979) reported that abnormalities in the weights of the internal organs such as liver and kidney arise because of increased metabolic rate of the organs in an attempt to reduce toxic elements or anti-nutritional factors to non-toxic metabolites. There is a paucity of data on the effect of feeding GBM and dietary enzyme on morphometry and histopathology of broiler chickens. The objectives of the study are to evaluate morphometry and histopathology of broiler chickens fed GBM with dietary enzyme.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Unit of the Department of Animal Science, Ahmadu Bello University, Nigeria located within the Northern Guinea Zone at latitude 11º 12' N and longitude 7º 33' E and altitude of 610 m.a.s.l. The climate is sub-humid with a mean annual rainfall of 1050 mm (Oluwasemire, 1999). A total of 270 broiler chicks were allocated to six experimental diets (0, 15 and 30% Ginger By-product Meal (GBM); 0 and 0.01% enzyme) in a completely randomized design (3 x 2 factorial) for eight weeks. The diets formulated for the finisher phase according to NRC requirements are shown on Table 1.

Carcass Study

At the end of the experiment, nine birds of average weight (1.8 kg) fasted overnight were randomly selected from each treatment, slaughtered and divided into standard cuts, weighed and expressed as a percent of dressed weight. The length and weight of the GIT and the weight of the organs were expressed as percent of the live weight of the corresponding broiler, resulting in a relative length and weight measurement.

Histological Study

Tissue samples (intestine, liver, spleen and kidney) were collected from two sacrificed birds per treatment at the end of the experiment and trimmed to about 0.5 – 1 cm thick and about 1 -2 cm long, and fixed in buffered neutral 10% formalin for at least 48 hours. The fixed tissues were dehydrated in graded concentrations of alcohol (70, 80, 90, 95 and 100 %) at intervals of one hour. The tissues were cleared with xylene for about two hours, infiltrated with molten paraffin wax at 50 ºC to 60 ºC for about two hours, embedded in the molten paraffin and labelled appropriately. Sections 5µ thick were cut from the embedded tissues using a microtome knife attached to a microtome. The sectioned tissues were mounted on grease free, clean glass slides dried at room temperature and stained with Haematoxylin and Eosin (H and E) stain. The slides were studied using light microscope x 400 magnifications. Photomicrographs of the slides were taken using a digital camera, transferred to a computer and appropriately labelled.

Statistical Analysis

All data obtained from the trial were subjected to analysis of variance test using the SAS (2002) general linear model procedure and treatment means were compared for significance using Tukey's studentized range test. In order to compensate for variance heterogeneity, measured values for spleen, kidney, intestinal length were log (10) transformed.

RESULTS AND DISCUSSIONS

Effect of Dietary Levels of GBM/Enzyme on Morphometry of Broiler Chickens

Table 2 shows that the inclusion of GBM had a significant effect ($p<0.05$) on the weights of the intestine, gizzard, intestinal length and abdominal fat. The increase observed in the weight of the gizzard could be attributed to improved digestive organ development with increase in the level of fibre in the diet (Mateos *et al*., 2012). Olorede and Longe (2000) also observed increase ($p<0.05$) in the size of the gizzard with increasing dietary fibre. The decrease in abdominal fat with the inclusion of GBM is an indication that it reduced the fat content of the carcass and can be included in diets to produce healthier meat. Ademola *et al*. (2009) also reported that ginger had hypolipidemic effect on abdominal fat deposits.

There was also an increase in the length and weight of the intestine due to increase in dietary fibre with increase in the level of inclusion of GBM. This result agrees with the finding of Pond *et al*. (1981) who reported that the relative weight (percentage of body weight) of the intestine increased with high level of fibre in the diet and Stagniogias and Pearce (1985) reported that the increase in the length of the gastrointestinal tract (GIT) is an indication of the hypertrophic response of the GIT to high fibre diet. Also, Savoury and Gentle (1976) reported that changes in the gut size could be due to additional bulk and greater volume of digesta staying in the gastrointestinal tract during enzymatic digestion.

Enzyme supplementation brought about a decrease ($p<0.05$) in the weight of the breast and an increase ($p<0.05$) in the weight and length of the intestine. The effect of enzyme on the breast and intestine may be attributed to the enzyme not improving the digestion of the fibre in GBM. The observed increase in intestinal length agrees with the report of Zarghi and Golian (2009) who observed an increase in the length of the large intestine with the inclusion of enzyme. This finding disagrees with Khan *et al*. (2006) who observed a decrease in the length of the GIT with the inclusion of enzyme, suggesting that the enzyme did not break down much of the fibre in the GBM due to its non specificity. The weights of the liver and kidney were similar ($p>0.05$) for all the birds suggesting that GBM and enzyme had no toxic effect on the birds and the microscopic investigation of the organs showed normal histology for most birds.

Figure 1 shows interaction between 30% GBM and enzyme, breast weight was better when enzyme was not included. Figure 2 shows interaction between 30% GBM and 0.01% enzyme, an increase was observed in the intestinal length which makes feed stay longer in the digestive tract thus bringing about absorption of more feed.

Effect of Dietary Levels of GBM and Enzyme on Histology of Organs of Broiler Chickens

Light microscopic examination showed normal liver structure and size for chickens fed 15 and 30 % GBM with or without enzyme supplementation, an indication that the feed had no toxic effect on the organ. The feeding of 0% GBM with or without enzyme supplementation caused widening of the liver sinusoidal spaces of birds which could be due to the non inclusion of GBM which has antioxidant activity. Light microscopic examination showed normal kidney structures for chickens fed 0, 15 and 30% GBM diets with and without enzyme supplementation. Ginger has been reported to have nephroprotective antioxidant effect.

The histology of the spleen of chickens fed 0 and 15% GBM diets without enzyme supplementation and 0 % GBM diet with enzyme supplementation were normal. Microscopic investigation showed the presence of hemosiderin particles in the spleen of birds fed 30% GBM diet without enzyme supplementation; birds fed 0, 15 and 30% GBM diets with enzyme supplementation also had hemosiderin particles in their spleen which is normal. Sheppard and Dierenfeld (2002) reported that the deposition of iron, in the form of hemosiderin, is normal in the liver, spleen, marrow and reticulocytes and iron load never reaches levels that cause health problems. The similarity in the weights of the spleen also suggests that the presence of hemosiderin particles did not cause health problems.

Changes were observed in the intestinal structure of birds fed 0, 15 and 30% GBM diet with or without enzyme supplementation. This was indicated by sloughing of the villi which increased at higher inclusion level of GBM and with the inclusion of enzyme (Plates 1 and 2). This is contrary to the report of Kanduri *et al*. (2009) that feeding of ginger and garlic increases height of villus of small intestine and activates the absorption process. The sloughing of the intestinal villi of the broiler chickens is an indication of reduced absorptive surface and limited utilization of digested feed (Ganong, 2005). Bennett (2002) reported that emptying of the gut stops blood flow to the intestinal lining since feed is not being absorbed and the villi start sloughing off, therefore the withdrawal of feed for about 14 hours before slaughtering could possibly account partially for the sloughing observed. The higher intestinal sloughing observed in birds fed GBM could be attributed to the level of GBM utilized. Duru (2010) reported erosion of the intestinal villi when 10 g of enzyme was added to the control diet but this does not corroborate the finding of this research since sloughing was observed in all the diets. The sloughing observed in this study could also be attributed to the enzyme acting negatively on the intestinal villi.

Table 1: Composition of Broiler Finisher Diet.

	GBM Level (%)					
	0		15		30	
	Enzyme Level (%)					
Ingredients	0	0.01	0	0.01	0	0.01
Maize	48.70	48.69	32.00	31.49	14.50	14.49
Enzyme	0.00	0.01	0.00	0.01	0.00	0.01
Soya bean (full fat)	39.00	39.00	39.00	39.00	39.00	39.00
Groundnut cake	0.30	0.30	2.00	2.50	4.50	4.50
Maize offal	8.00	8.00	8.00	8.00	8.00	8.00
Ginger By-product Meal	0.00	0.00	15.00	15.00	30.00	30.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.40	0.40	0.40	0.40	0.40	0.40
*Premix	0.30	0.30	0.30	0.30	0.30	0.30
Total (Kg)	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis						
ME (MJ/kg)	13	13	13	13	13	13
Crude Protein (%)	20.10	20.10	19.93	20.11	20.04	20.04
Crude Fibre (%)	3.85	3.85	5.07	5.11	6.36	6.36
Ether Extract (%)	9.26	4.19	3.96	3.98	3.76	3.76
Calcium (%)	1.09	1.09	1.69	1.69	2.30	2.30
Available P (%)	0.83	0.83	1.20	1.20	1.58	1.58
Lysine (%)	1.19	1.19	1.18	1.19	1.18	1.18
Meth. (%)	0.74	0.74	0.72	0.73	0.71	0.71
Met.+Cys.(%)	0.88	1.98	2.14	2.57	2.32	0.85
Cost (₦/Kg)	70.68	71.04	67.98	68.34	65.28	65.64

Table 2: Effect of Dietary Levels of GBM/Enzyme on Carcass and Organ Weights of Broiler Chickens.

	GBM Levels (%)					Enzyme Levels (%)			
	0	15	30	SEM	P	0	0.01	SEM	P
Dressing percent	76.26	77.83	77.41	3.25	0.88	78.87	75.46	2.66	0.22
Shank (%)	5.34	5.97	5.87	0.48	0.39	5.63	5.82	0.38	0.63
Wing (%)	10.71	10.61	10.85	0.53	0.90	10.65	10.8	0.43	0.74
Back (%)	15.94	14.46	15.12	0.77	0.18	15.51	14.83	0.63	0.29
Breast (%)	20.10	18.63	19.29	0.96	0.32	20.30^{a}	18.38^{b}	0.78	0.02
Thigh (%)	24.14	22.23	23.02	1.09	0.23	23.84	22.41	0.89	0.12
Spleen (%)	0.16	0.17	0.18	0.03	0.58	0.18	0.16	0.02	0.32
Liver (%)	2.35	2.67	2.63	0.18	0.16	2.51	2.59	0.14	0.59
Intestinal weight (%)	4.70^{b}	6.03^{a}	6.06^{a}	0.29	0.0001	5.27^{b}	5.92^{a}	0.24	0.01
Gizzard (%)	3.13^{b}	3.48^{b}	4.53^{a}	0.36	0.002	3.43	4.01	0.29	0.059
Kidney (%)	0.91	0.75	1.02	0.18	0.17	0.91	0.88	0.15	0.42
Heart (%)	0.57	0.58	0.58	0.04	0.98	3.37	3.71	0.03	0.61
Intestinal length (%)	14.48^{b}	18.76^{a}	21.05^{a}	1.42	0.0003	16.1^{b}	20.09^{a}	1.16	0.002
Abdominal fat (%)	2.64^{a}	1.16^{b}	1.41^{ab}	0.62	0.05	2.02	1.46	0.50	0.27

[ab] Means within rows with different superscripts are significantly different ($p<0.05$); GBM- Ginger By-product Meal

CONCLUSION

GBM can replace 70% of maize in broiler finisher diets in areas where it is an industrial waste for comparable carcass weights. Diets of GBM (15 and 30 %) without or with enzyme supplementation (0.01 %) did not induce histological changes in the tissues of the liver and kidney but sloughing of the intestinal villi was observed which is associated with dysfunction.

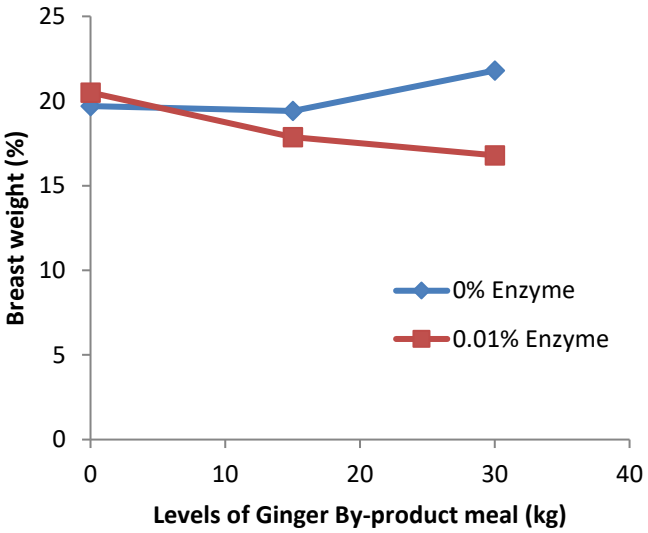

Figure 1. Effect of interaction of Ginger By-product meal and enzyme on weight of breast (%)

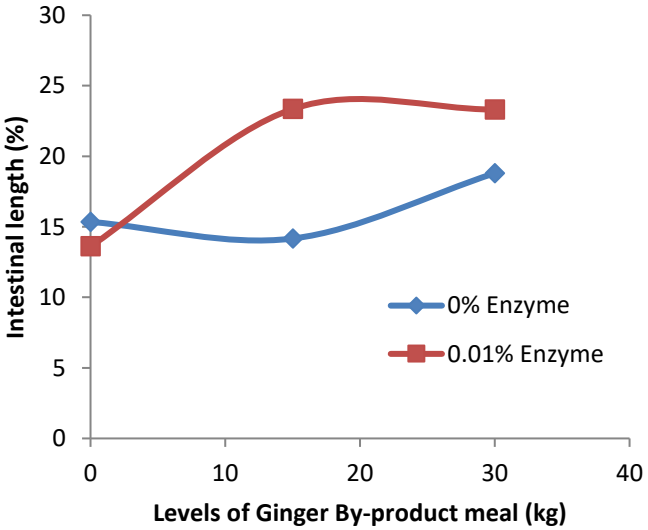

Figure 2. Effect of interaction of Ginger By-product meal and enzyme on Intestinal length (%)

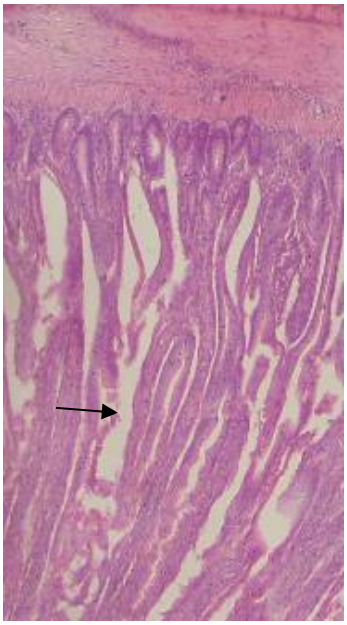

Plate 1a. Photomicrograph of a section of the intestine of a broiler chicken fed 0% GBM and 0% enzyme showing mild sloughing (H and E stained, x400)

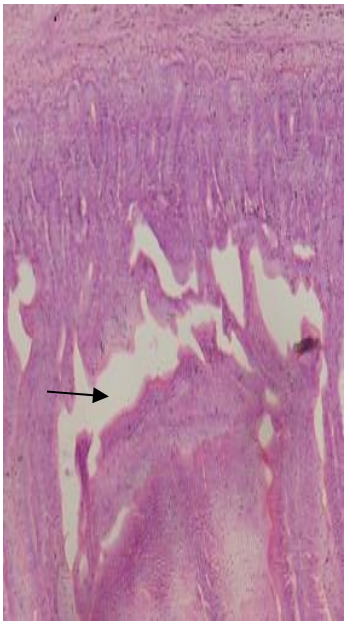

Plate 1b. Photomicrograph of a section of the intestine of a broiler chicken fed 15% GBM and 0% enzyme showing severe sloughing (H and E stained, x400)

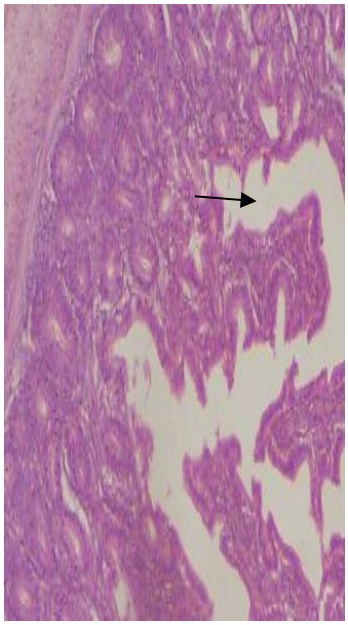

Plate 1c. Photomicrograph of a section of the intestine of a broiler chicken fed 30% GBM and 0% enzyme showing severe sloughing (H and E stained, x400)

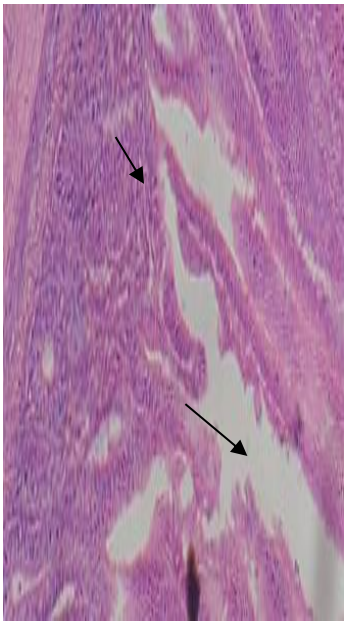

Plate 2a. Photomicrograph of a section of the intestine of a broiler chicken fed 0% GBM and 0.01% enzyme showing severe sloughing (H and E stained, x400)

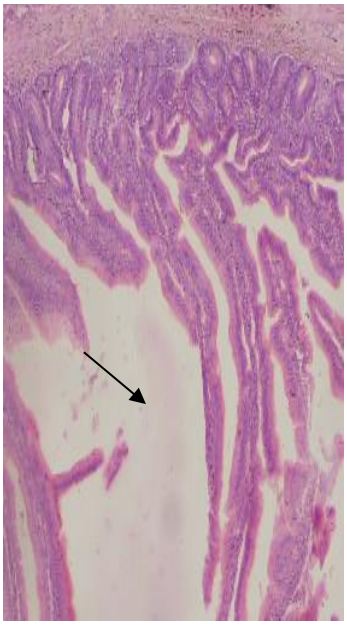

Plate 2b. Photomicrograph of a section of the intestine of a broiler chicken fed 15% GBM and 0.01% enzyme showing severe sloughing (H and E stained, x400)

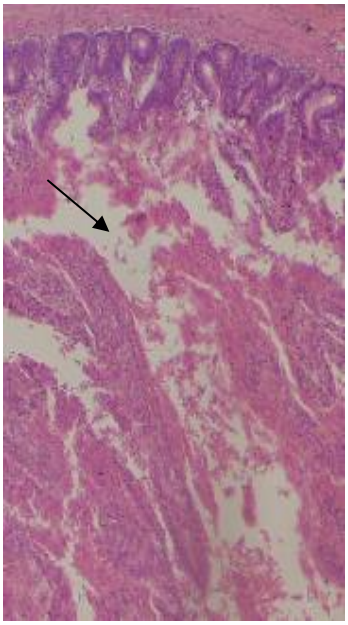

Plate 2c. Photomicrograph of a section of the intestine of a broiler chicken fed 30% GBM and 0.01% enzyme showing severe sloughing (H and E stained, x400)

Conflict of interest. The authors confirm that there are no known conflicts of interest associated with this publication.

Data availability. Data are available with the corresponding author (remidaudu@yahoo.com) upon reasonable request**.**

REFERENCES

Acamovic, T. 2001. Commercial application of enzymes technology for poultry production. World's Poultry Science Journal, 57: 225-235. https://www.cambridge.org/core/journals/world-s-poultry-science-journal/article/commercial-application-of-enzyme-technology-for-poultry-production/FABBB3F7028FAFF2F1C0A4E9A52A439E

Ademola, S. G. Farinu, G. O. and Babatunde, G. M. 2009. Serum Lipid, Growth and Haematological Parameters of Broilers Fed Garlic, Ginger and Their Mixture. World Journal of Agricultural Sciences, 5 (1): 99-104. Doi:https://pdfs.semanticscholar.org/133d/8aeb7e82b7309a61d8d59c8d2d68dbd4dc56.pdf

Attia, Y. A., Qota, E. M. A., Aggoor, F.A.M., and Kies, A.K. 2003. Value of rice bran, its maximal utilization and upgrading by phytase and other enzymes and dietformulation based on available amino acid for broiler chicks. Archiv fur Geflugelkunde, 67: 157-166. Doi: https://www.european-poultry-science.com/.../s-157 - 166_NzgwNQ.PDF

Bedford, M. 1996. The effect of enzyme on digestion. Journal of Applied Poultry Research, 5: 370-377.

Bennett, C. 2002. Feed withdrawal for broiler chickens. https://www.gov.mb.ca/agriculture/livestock/poultry/.../bba 01s 28.pdf

Bone F. J. 1979. Evaluation of two herbal spices as feed Additives for finisher broilers. In: Biotechnology in Animal Husbandry, 26 (5-6): 383-392.

Brenes, A., Smith, M., Guenter, W. and Marquardt, R. R. 1993. Effects of Enzyme Supplementation on Performance, Nutrient Digestibility, Gastrointestinal Morphology, and Volatile Fatty Acid Profiles in the Hindgut of Broilers Fed Wheat-based Diets. Poultry Science, 84: 875–881.

Duru, S. 2010. Evaluation of three types of rice offal in broiler diets. Unpublished PhD. Thesis submitted to the Department of Animal Science, Ahmadu Bello University, Zaria.

Ganong, W.F. 2005. Review of Medical Physiology. 22nd Edition. The McGraw-Hill Companies, Inc.

Khan, S.H., Sardar, R. and Siddique, B. 2006. Influence of enzymes on performance of broilers fed sunflower-corn based diets. Pakistan Veterinary Journal, 26(3): 109-114.

Kanduri, A. B., Pawar, A.A., Kokane, S.S. and Deshmukh, G.B. 2009. Effect of Ginger and Garlic Feeding in Organic Broiler Production. Poultry line, 9(7):5

Mateos G.G., Jimenez-Moreno, E., Serrano, M.P. and Lazaro, R.P. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. The Journal of Applied Poultry Research, 21(1): 156-174. https://www. science direct.com/ science /article/pii/S2405654518301069

Ngxumeshe, A.M. and Gous, R.M. 2009. Effects of varying levels of thermostable xylanase, amylase and protease (TXAP) composite enzyme supplement on body growth of broiler chickens. South African Journal of Animal Science, 39 (Supplement 1). Pp. 312-315. www.scielo.org.za/scielo.php? script=sci...1589 2009 000500072

Olorede, B.R. and Longe, O.G. 2000. Effect of replacing PKC with shea butter cake in egg quality characteristics. Nigerian Journal of Animal Production, 27: 9-23. https://pdfs.semanticscholar.org/5fd0/6a210bdac9537408d3d1049a720dd29e0dd3.pdf

Oluwasemire, K.O. 1999. Some aspects of rainfall trends and their implications on soil and crop management practices in north western Nigeria. In: Management of the soil resources of Nigeria for sustainable agricultural production in the 21st Century. Proceedings of the 25th

Annual Conference of Soil Science Society of Nigeria, pp.244-254.

Panda, A.K., Rao, R., Raju, S.V., Reddy, M.R. and Praharaj, N.K. 2011. The Role of Feed Enzymes in Poultry Nutrition Project Directorate on Poultry, Hyderabad. Poulvet.com accessed 30/07/2011.

Pond, W.G., Yen, J.T., Lind-Wall, R.N. and Itill, D. 1981. Dietary alfalfa meal for genetically obese and lean pigs. Effect on body weight gain, carcass and gastro-intestinal tract measurements and blood metabolite. Journal of Animal Science, 51:367-373.

Savoury, C.J. and Gentle, M.J. 1976. Effects of dietary dilution with fibre on the food intake and gut dimensiom of Japanese quail. British Poultry Science, 17:561-570.

Sheppard, C. and Dierenfeld, E. 2002. Iron Storage Disease in Birds: Speculation on Etiology, Implications for Captive Husbandry. www.nashvillezoo.org/piciformes/pdf/iron storage in birds.pdf

Stagniogias, G. and Pearce, G.R. 1985. The digestion of fibre by pigs. The effect of amount and type of fibre on apparent digestibility, nitrogen balance and rate of passage.British Journal of Nutrition, 53: 513-530. https://www.cambridge.org/core/ journals / british - journal - of-nutrition /article/

Statistical Analysis System (SAS) 2002. Users guide statistics, Version 9 Edition, SAS institute Inc. Cary. North Carolina, U.S.A.

Zarghi, H. And Golian, A. 2009. Effect of Triticale Replacement and Enzyme Supplementation on Performance and Blood Chemistry of Broiler Chickens. Journal of Animal and Veterinary Advances, 8 (7): doi: 1316-1321. https://www.medwell journals .com/abstract/?doi=javaa. 2009. 1316.1321