Serum biochemistry profile and liver function indices of broiler chickens served dietary inclusion of probiotics (*Saccharomyces cerevisiae*) and enzyme

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Abstract

*As the use of antibiotics as growth promoters have been banned in different countries, researchers have been working hard to find alternatives to antibiotics, and also come up with other substances that can promote growth, health and productivity in broiler chickens. The study was conducted to evaluate the serum biochemistry of broiler chickens served dietary inclusion of probiotics (*Saccharomyces cerevisiae*) and enzyme. One hundred and twenty, one day-old broiler chicks Ross strain were used for the study. The birds were randomly allotted to four dietary treatments. Each treatment contained 30 birds replicated three times to contain 10 birds per replicate in a completely randomized design experiment. The four dietary treatments were T1 Control (no probiotics and no enzyme), T2 (both probiotics and enzyme), T3 (Probiotics only) and T4 (Enzyme only). The probiotics and enzyme was included at 0.8% and 0.02%, respectively. The birds were given feed and water ad-libitum. The experiment lasted for eight weeks. At the end of the experimental period, three birds were randomly chosen from each replicate for serum biochemistry profiling and liver function test. The results showed that inclusion of probiotics and enzyme had significant effect ($p < 0.05$) on the serum biochemistry profile and liver function indices. Low density lipoprotein was reduced from 3.83 ± 0.60 g/l in the control group to 2.18 ± 0.12 g/l in T2 (Probiotic and Enzyme) group. Lymphocytes values ranged from 26.00% to 38.00% in the treated groups, while the control had 45.67%. The dietary inclusion of probiotics and enzyme reduced total cholesterol from 5.07 g/l to 4.22 g/l and low density lipoprotein from 3.83 g/l to 2.18 g/l in the (probiotics and enzyme) treated group and also improved liver function indices of the broiler chickens. Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase and total bilirubin had range values of 114.67 to 150.67 μ l, (21.00 to 28.67 μ l, 10.33 to 17.00 μ l and 1.30 to 1.47 mg/dl, respectively. It was therefore, concluded that combination of probiotics and enzyme should be included in broiler diets for immunity improvement, good liver function and reduction in cholesterol levels.*

Keywords: Broilers, probiotics, enzyme, serum biochemistry, liver function and diets

Le profil biochimique sérique et les indices de la fonction hépatique des poulets de chair ont servi à l'inclusion alimentaire de probiotiques (*Saccharomyces cerevisiae*) et d'enzymes

Résumé

*Depuis l'interdit de l'utilisation d'antibiotiques comme stimulateur de croissance dans différents pays, les chercheurs ont travaillé dur pour trouver des alternatives aux antibiotiques, ainsi que d'autres substances susceptibles de favoriser la croissance, la santé et la productivité des poulets de chair. L'étude a été menée pour évaluer la biochimie sérique des poulets de chair a servi d'inclusion alimentaire de probiotiques (*Saccharomyces cerevisiae*) et d'enzymes. Des poussins de chair Ross de cent vingt et un jours ont été utilisés*

pour l'étude. Les oiseaux ont été attribués au hasard à quatre traitements diététiques. Chaque traitement contenait 30 oiseaux répliqués trois fois pour contenir 10 oiseaux par réplica dans une expérience de conception complètement aléatoire. Les quatre traitements diététiques étaient le contrôle T1 (sans probiotiques et sans enzyme), T2 (probiotiques et enzyme), T3 (probiotiques uniquement) et T4 (enzyme uniquement). Les probiotiques et l'enzyme ont été inclus à 0,8% et 0,02%, respectivement. Les oiseaux ont reçu de la nourriture et de l'eau ad-libitum. L'expérience a duré huit semaines. À la fin de la période expérimentale, trois oiseaux ont été choisis au hasard dans chaque réplique pour le profilage de la biochimie sérique et le test de la fonction hépatique. Les résultats ont montré que l'inclusion de probiotiques et d'enzymes avait un effet significatif ($p < 0,05$) sur le profil biochimique sérique et les indices de la fonction hépatique. Les lipoprotéines de basse densité ont été réduites de $3,83 \pm 0,60$ g/l dans le groupe témoin à $2,18 \pm 0,12$ g/l dans le groupe T2 (probiotique et enzyme). Les valeurs des lymphocytes variaient de 26,00% à 38,00% dans les groupes traités, tandis que le témoin en avait 45,67%. L'inclusion alimentaire de probiotiques et d'enzymes a réduit le cholestérol total de 5,07 g/l à 4,22 g/l et les lipoprotéines de basse densité de 3,83 g/l à 2,18 g/l dans le groupe traité (probiotiques et enzyme) et a également amélioré les indices de la fonction hépatique des poulets de chair. L'aspartate aminotransférase, l'alanine aminotransférase, la phosphatase alcaline et la bilirubine totale avaient des valeurs comprises entre 114,67 et 150,67 μ l, (21,00 à 28,67 μ l, 10,33 à 17,00 μ l et 1,30 à 1,47 mg/dl, respectivement. Il a donc été conclu que l'association de probiotiques et d'enzyme doivent être inclus dans les régimes alimentaires pour poulets de chair pour l'amélioration de l'immunité, la bonne fonction hépatique et la réduction du taux de cholestérol.

Mots clés : poulets de chair, probiotiques, enzyme, biochimie sérique, fonction hépatique et alimentation

Introduction

Antibiotics as growth promoters have been extensively used in poultry feed for more than 50 years (Haque *et al.*, 2017), playing a crucial role in promoting chicken growth and lowering enteric diseases. However, the deleterious effect of this product on the health of the consumers cannot be overemphasized. In Europe and South Korea, growth promoting antibiotics have been banned since 2006 and 2012, respectively, and these bans are expected to affect the rest of the world (Mohamed *et al.*, 2019; Aude *et al.*, 2015). Hence, tremendous research has been geared towards finding alternatives to antibiotics that would maintain low mortality rate, good level of animal yield while preserving environment and consumer health (Mehdi *et al.*, 2018). Several alternative strategies have been proposed with some success that

mimics the function of antibiotics as growth promoters and these include the use of probiotics, prebiotics, organic acids, and exogenous enzyme, among others (Yadav and Jha, 2019). Probiotics are living microorganisms that confer health benefits to the host when administered in adequate amounts (Julio *et al.*, 2019). The most important advantage of probiotics is that they neither have any residues in animal products nor exerts any antibiotic resistance on consumption while having good impact on the poultry performance (Koenen *et al.*, 2004 and Mountzouris *et al.*, 2007). The use of probiotics in poultry production is a recent practice which has progressed from the transplantation of feces from healthy adult hens into neonatal chicks to a more mature technology focused on developing probiotics formulation to address specific, wide-ranging issues relevant to today's

modern poultry industry (Wolfenden and Hargis, 2014). In broiler nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida* and *Saccharomyces* have some beneficial effects on broiler performance, modulation of intestinal flora, intestinal histological changes, immunomodulation, in addition, have an impact on specific haemato-biochemical parameters and improving microbiological meat quality of broilers (Matsuzak and Chin, 2000; Islam *et al.*, 2004; Matsuzaki *et al.*, 2007; Apata, 2008 and Ashayerizadeh *et al.*, 2009).

The role of exogenous enzymes is to fulfill the absence of endogenous enzymes, to counter the anti-nutritional factors present in conventional and unconventional poultry diet (Yadav and Jha, 2019). Probiotics and exogenous enzymes can also enhance broiler productivity and likely immunity too, that may represent an alternative to the use of sub-therapeutic doses of antibiotics (Talebi *et al.* 2008), meeting the growing public concerns on the contribution of this practice to the increasing antibiotic resistance (Yadav and Jha, 2019). A combination of lactobacilli and enzymes increases the efficacy of the digestion of different ingredients in feed (Chen *et al.* 2006); hence, it could improve feed conversion rate, increase the release of essential nutrients and reduce faecal losses and thus, lower manure production, as well as less phosphorus and nitrogen excretion (Yang *et al.* 2009; Wang *et al.*, 2011). As information on the effect of a mixture of probiotic microorganisms and enzymes on the immunity of broilers are very limited, this study was designed to determine the effect of different dietary inclusion levels of probiotics (*Saccharomyces cereviasae*) and

enzyme in broiler chickens on their immune response, lipid profile and liver function indices.

Materials and methods

Study location

The experiment was conducted at the Poultry Unit, Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Enugu State, Nigeria. Nsukka is in Enugu state, Nigeria. Nsukka lies within longitude 6°45'E and 71E and latitude 7°12.5'N (Ofomata, 1975) and on the altitude 447m above sea level. The climate of the study area is typically tropical, with relative humidity ranging from 65 – 80% and mean daily temperature of 26.8°C (Agbagha *et al.*, 2000). The rainy season is between April and October while dry season is between November and March with annual rainfall range of 1680 – 1700mm (Breinholt *et al.*, 1981).

Experimental design, animals and management

The study was conducted to evaluate the serum biochemistry profiles of broiler birds served dietary inclusion of Probiotics (*Saccharomyces cereviasae*) and enzyme (Bio-enzyme). One hundred and twenty day-old broiler chicks of Ross strain were used for the study. The birds were randomly divided into four groups and assigned to the four dietary treatments. Each treatment contained 30 birds replicated three times to contain 10 birds per replicate in a completely randomized design. The four dietary treatments were T1 Control (no probiotics and enzyme), T2 (both probiotics and enzyme), T3 (probiotics only) and T4 (Enzyme only). The probiotics and enzyme was included at 0.8% and 0.02%, respectively. Gross composition of the diet is shown in Table 1.

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Table 1: The percentage composition of the experimental diets of the broiler chickens

Parameters	Treatments			
	T1 (Control)	T2 (Probiotics and Enzyme)	T3 (Enzyme only)	T4 (Probiotics only)
Maize	53.00	53.00	53.00	53.00
Wheat offal	10.00	10.00	10.00	11.00
Palm kernel cake	5.50	5.50	5.50	5.50
Groundnut cake	15.00	15.00	15.00	15.00
Soya bean meal	10.00	10.00	10.00	10.00
Fish meal	1.50	1.50	1.50	1.50
Bone meal	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vit-Premix	0.25	0.25	0.25	0.25
Probiotics	-	0.80	-	0.80
Enzyme	-	0.02	0.02	-
Total	100.00	100.00	100.00	100.00
Calculated composition				
CP%	20.12	20.01	20.11	20.04
Energy (MJME/kg)	3.68	3.81	3.75	3.71

The birds were given feed and water *ad-libitum* and the quantity of feed consumed by the birds each day were determined through weigh back techniques, (i.e. measuring the amount of feed served using weighing scale and also measuring the left-over feed each day, subtracting the amount feed left-over from the initial amount served was the daily feed intake). The birds were weighed weekly throughout the experimental period and the experiment lasted for eight weeks. Vaccination and medications were provided as when due and other routine management practices were provided accordingly. At the end of the experimental period, three birds were randomly chosen from each replicate for serum biochemistry profiling. The biochemical analyses carried out included: serum cholesterol status, differential count and liver function tests. Blood samples were collected into plain containers (no anticoagulant) with the aid of sterile syringes and needles. The blood was collected from the wing web vein and taken to the laboratory for analysis. Proximate

analysis of feeds was done according to AOAC (1990).

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, 2013) version 19.00. Significant different means were separated using Duncan's new multiple range test (Duncan, 1955).

Results and discussion

The effect of probiotics (*Saccharomyces cerevisiae*) and enzyme (bio-enzyme) inclusion on the lipid profile of broiler birds is presented in Table 2. The serum total cholesterol, low density lipoprotein (LDL) and triglyceride concentrations were significantly ($p < 0.05$) reduced in broiler chickens fed diets supplemented with combination of probiotics and bio-enzyme, while HDL value increased. The value of total cholesterol reduced from 5.07g/l to 4.22g/l, Triglyceride reduced from 1.90g/l to 1.79g/l and LDL reduced from 3.83g/l to 2.18g/l in T2 (Probiotics and enzyme) inclusion diet. The values of total

cholesterol (4.22g/l to 4.49g/l and Triglyceride (1.61g/l to 1.79g/l obtained in this work were in line with the findings of Haque *et al.* (2017), and they also, reported

higher total cholesterol and Triglyceride levels in the control group compared to all other groups supplemented with either antibiotics or probiotics.

Table 2: Effect of probiotics and enzyme inclusion on the lipid profile of broiler birds

Parameters	T1 (Control)	T2 (Prob. & Enz.)	T3 (Enzyme only)	T4 (Prob. Only)
Tot. Chol. (g/l)	5.07±0.07 ^a	4.22±0.02 ^c	4.40±0.06 ^b	4.49±0.01 ^d
Trig. (g/l)	1.90±0.00 ^a	1.79±0.02 ^b	1.89±0.00 ^a	1.61±0.02 ^c
HDL (g/l)	1.30±0.03 ^a	1.50±0.00 ^c	1.40±0.01 ^b	0.79±0.05 ^d
LDL (g/l)	3.83±0.60 ^a	2.18±0.12 ^c	2.78±0.10 ^b	2.92±0.02 ^b

Tot. Chol = Total Cholesterol, Trig. = Triglyceride, HDL = High density lipoprotein, LDL = Low density lipoprotein, Prob. = Probiotic, Enz. = Enzyme.

Similarly, the result concurred with the result of Sho kryazdan *et al.* (2017) who observed that the supplementation of the three *L. salivarius* strains to broilers significantly reduced their serum LDL, but did not affect HDL levels at 21 and 42 days of age. This result is also in agreement with Saleh *et al.* (2012) who reported that the inclusion of *Aspergillus awamori* and *Saccharomyces cerevisiae* in chicken feed reduced blood saturated fatty acids and increased the polyunsaturated fatty acid. The effect of probiotics (*Saccharomyces cerevisiae*) and enzyme inclusion on the differential count of broiler birds is presented in Table 3. Neutrophil and lymphocyte were lower in treatment groups with dietary inclusion of probiotics. However, the neutrophil and lymphocyte counts was significantly ($p<0.05$) higher in the control treatment groups, except in T3 where neutrophil was highest (70.33%).

Monocyte and eosinophil count were significantly ($p<0.05$) higher in treatment group with a mixture of probiotics and enzyme with the value 2.33% and 3.67% for monocyte and eosinophil respectively. There were no significant differences in the basophil count across the treatment groups. This result perfectly agrees with the work of Mohamed *et al.* (2019) who reported a significant reduction in lymphocyte percentage by treatment groups with dietary inclusion of probiotics. This implies that probiotics improves the immune system of broiler chickens. This improvement is achieved by reducing intestinal pH, intestinal bacteria composition and digestive activity. Probiotic bacteria produce molecules with antimicrobial activities such as bacteriocins which inhibits toxins' production and pathogens' adhesion (Matsuzak and Chin, 2000; Ashayerizadeh *et al.*, 2009).

Table 3: Effect of probiotics and enzyme inclusion on the differential count of broiler birds

Parameters	T1 (Control)	T2 (Prob. & Enz.)	T3 (Enzyme only)	T4 (Prob. Only)
Neut. (%)	68.33±0.33 ^a	56.00±2.00 ^b	70.33±1.53 ^a	52.00±0.00 ^c
Lymph (%)	45.67±0.33 ^a	38.00±1.16 ^b	26.00±1.16 ^d	30.67±0.33 ^c
Eosi (%)	2.33±0.33 ^b	3.67±0.33 ^a	2.00±0.00 ^b	2.33±0.33 ^b
Mono (%)	0.00±0.00 ^b	2.33±0.03 ^a	0.00±0.00 ^b	0.00±0.00 ^b
Baso (%)	0.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00

Neut. = Neutrophil, Lymph. = Lymphocyte, Eosi. Eosinophil, Mono. = Monophil, Baso. = Basophil, Prob. = Probiotic, Enz. = Enzyme.

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On the other hand, probiotics stimulated the immune response and increase resistance to colonization of bacteria (Hassanein and Soliman, 2010). The effect of probiotics (*Saccharomyces cerevisiae*) and enzyme inclusion on the liver function profile of broiler birds is presented in Table 4. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were significantly ($p<0.05$) higher in

treatment groups with bio-enzyme compared to the control groups and the treatment group with only probiotics, except *Alkaline phosphatase* (ALP) which had lower value 10.33ul in the control group. The activities of Total bilirubin were lower in treatment groups than the control group, while the activities of Double bilirubin were lower in the control group compared to the treatment groups.

Table 4: Effect of probiotics and enzyme inclusion on the liver function profile of broiler birds

Parameters	T1 (Control)	T2 (Prob. & Enz.)	T3 (Enzyme only)	T4 (Prob. Only)
AST (μ l)	124.33 $\pm$ 1.45 ^c	150.67 $\pm$ 1.45 ^a	132.00 $\pm$ 1.16 ^b	114.67 $\pm$ 0.33 ^d
ALT (μ l)	27.17 $\pm$ 0.33 ^c	29.03 $\pm$ 0.03 ^b	21.00 $\pm$ 0.29 ^d	28.67 $\pm$ 0.67 ^a
ALP (μ l)	10.33 $\pm$ 0.33 ^d	15.67 $\pm$ 0.03 ^b	17.00 $\pm$ 0.00 ^a	12.00 $\pm$ 0.58 ^c
Tot. Bili (mg/dl)	1.47 $\pm$ 0.03 ^a	1.43 $\pm$ 0.03 ^a	1.30 $\pm$ 0.06 ^b	1.30 $\pm$ 0.00 ^b
D. Bili (mg/dl)	0.30 $\pm$ 0.03 ^d	0.41 $\pm$ 0.01 ^c	0.87 $\pm$ 0.03 ^a	0.70 $\pm$ 0.03 ^b

AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, Prob. = Probiotic, Enz. = Enzyme, Tot. Bili = Total Bilirubin, D. Bili = Double Bilirubin

The decrease in AST values in the probiotics treated group perfectly aligns with the work of Haque *et al.* (2017) who measured highest value of AST in the control treatments and least value in probiotic supplemented group. These findings concur with the fact that the supplementation of probiotics lowers AST and ALT level. The values of AST (114.67 to 150.67 μ l), ALT (21.00 to 29.03 μ l) and ALP (10.33 to 17.00 μ l) obtained in this study were within the ranges as reported by Adriana *et al.* (2018); Ezeonwumelu and Omeodu (2019). Furthermore, Hassanein and Soliman (2010) in agreement had earlier opined that mechanisms of action of probiotics included stimulation of endogenous enzymes, reduction of metabolic reactions that produce toxic substances, and production of vitamins or antimicrobial substances.

Conclusion

Dietary inclusion of probiotics and enzyme significantly lowered blood cholesterol,

improved immunity and liver function indices in broiler which are beneficial parameters to enhance broiler production. Thus, combination of probiotics and enzyme should be included in the diet of broiler chickens for quality meat production and reduction in mortality.

References

- Adriana, J. A., Leite, L. S., Eckhardt, L. A., Albernaz, A. P., Teixeira, A. B. and Torres, K. A. A. 2018. Serum biochemical profile of emus (*Dromaius novaehollandiae*) reared in captivity. *Journal of Applied Animal Research*; 46: 1, 5 9 3 - 5 9 8 , DOI:10.1080/09712119.2017.1367686
- Agbagha, F. M., Ezema, F. U. and Omeke, B. C. O. 2000. Studies of Mangament Effects on Fertility of Purbred and Crossbred Exotic Gilts in Two Breeding Farms at Nsukka. *Nigeria Journal of Animal*

- Production*. 28(1): 20-25.
- AOAC, 1990.** Association of Official Analytical Chemists international (AOAC) Official methods of analysis, 17th edition AOAC International, Gaithersburg, MD, USA
- Apata, D. F. 2008.** Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. *Journal of the Science of Food and Agriculture*, 88: 1253-1258.
- Ashayerizadeh, A., Dabiri, N., Ashayerizadeh, O., Mirzadeh, K. H., Roshanfekar, H. and Mamooee, M 2009.** Effect of dietary antibiotic, probiotic and prebiotic as growth promoters, on growth performance, carcass characteristics and hematological indices of broiler chickens. *Pakistan Journal of Biological Sciences*; 12 (1): 52-57.
- Aude, T., Ramanan, L. and Thomas V. 2015.** The Economic Costs of Withdrawing Antimicrobial Growth Promoters from the Livestock Sector. Report number: 78 Affiliation: OECD Food, Agriculture and Fisheries Papers DOI: 10.1787/5js64kst5wv1-en
- Breinholt, K. A., Gowen, F. A. and Nwosu, C. C. 1981.** Influence of Environmental and Animal Factors on Day and Night Grazing Activity of Imported Holstein Freisian Cows in the Humid Lowland Tropics of Nigeria. *Tropical. Animal Production*; 6 (4): 300-307.
- Chen Y.J., Min B.J., Cho J.H., Kwon O.S., Son K.S., Kim H.J. 2006.** Effects of Dietary *Bacillus*-based probiotic on growth performance nutrients digestibility blood characteristics and fecal noxious gas content in finishing pigs. *Asian Australas J Anim Sci.*; 19(4):587-592.
- Duncan D.B. 1955.** New Multiple Range Test. *Biometrics*. 11: 1-42.
- Ezeonwumelu, E. C. and Omeodu, S. I. 2019.** Serum Lipid Profile, Liver Function Indices and Electrolyte Levels in Diabetics and Subjects with Hepatic Impairment in the University of Port Harcourt Teaching Hospital. *J. Appl. Sci. Environ. Manage.*; 23 (2) 261-268.
- Haque, M. I., Ahmad, N. and Miah, M. A. 2017.** Comparative analysis of body weight and serum biochemistry in broilers supplemented with some selected probiotics and antibiotic growth promoters. *Journal of Advanced Veterinary and Animal Research* 4(3):288-294.
- Hassanein, S. M. and Soliman, N. K. 2010.** Effect of probiotic (*Saccharomyces Cerevisiae*) adding to diets on intestinal microflora and performance of hy-line layers hens. *Journal of American Science*. 6(11):159-169
- Islam, M. W., Rahman, M. M., Kabir, S. M. L., Kamruzzaman, S. M. and Islam, M. N. 2004.** Effect of probiotics supplementation on growth performance and certain haematobiochemical parameters in broiler chickens. *Bangladesh Journal of Veterinary Medicine*; 2(1): 39-43.
- Julio, P., Francisco, J. R., Mercedes, G. and Angel, G. 2019.** Mechanisms of Action of Probiotics. *Adv. Nutr*; 10 (Suppl 1): S49-S66, <https://doi.org/10.1093/advances/nmy063>.
- Koenen, M. E, Kramer, J., van der Hulst,**

- R., Heres, L., Jeurissen, S. H and Boersma, W. J. 2004. Immunomodulation by probiotic lactobacilli in layer and meat-type chickens. *Br. Poult. Sci.*; 45:355–366.
- Matsuzaki, T., and Chin, J. 2000. Modulating immune responses with probiotic bacteria. *Immunology & Cell Biology*; 78: 67–73.
- Matsuzaki, T., Takagi, A., Ikemura, H., Matsuguchi, T., and Yokokura, T. 2007. Intestinal microflora: probiotics and autoimmunity. *The Journal of Nutrition Supplement*; 137: 798S-802S.
- Mehdi, Y., Letourneau-Montminy, M., Gaucher, M., Chorfi, Y., Rouissi, T., Brar, S.K., Cote, C., Ramirez, A.A. and Godbout, S. 2019. Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition*; 4:170-178.
- Mohamed, F. M., Thabet, M. H. and Ali, M. F. 2019. The use of probiotics to enhance immunity of broiler chicken against some intestinal infection pathogens. *SVU-International Journal of Veterinary Sciences*; 2 (1): 1-19.
- Mountzouris K C, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G and Fegeros K. 2007. Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poult Sci.*, 86(2): 309-317.
- Ofomata, G. E. K. 1975. Nigeria in Maps. Eastern States, Ethiopie Publishing Co. Ltd. Benin.
- Saleh, F., Mumu, S. J., Ara, F., Begum, H. A. and Ali, L. 2012. Knowledge and self-care practices regarding diabetes among newly diagnosed type 2 diabetics in Bangladesh: a cross-sectional study. *BMC Public Health*, 12, Article number: 1112 (2012)
- Shokryazdan, P., Faseleh, J.M., Navidshad, B. and Liang, J. B. 2017. Effects of prebiotics on immune system and cytokine expression. *Med Microbiol Immunol*; 206(1):1-9. doi: 10.1007/s00430-016-0481-y.
- SPSS. 2013. SPSS 16.0.1 for Windows. SPSS Inc., Chicago, IL, USA.
- Talebi, A., Amirzadeh, B., Mokhtari, B. and Gahri, H. 2008. Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathol.*, 37(5): 509-12.
- Wang, J., Ji, H., Zhang, D., Liu, H., Wang, S. and Shan, D. 2011. Assessment of probiotic properties of *Lactobacillus plantarum* ZLP001 isolated from gastrointestinal tract of weaning pigs. *Afr. J. Biotech.*;10(54):11303–11308.
- Wolfenden, R. E. and Hargis, B. M. 2014. Poultry probiotics getting specific. *Feed stuff*. <https://www.feedstuffs.com/story-poultry-probiotics-getting-specific-53-109997> Retrieved 28th January, 2020.
- Yadav, S. and Jha, R. 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and

health of poultry. *Journal of Animal Science and Biotechnology*; 10(2): 1-11. DOI: 10.1186/s40104-018-0310-9

Yang, Y., Iji P. A. and Choct, M. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Science Journal*; 65:97-114.

Received: 10th August, 2020

Accepted: 20th December, 2020