

Histopathology of gastrointestinal tract and organoleptic attributes of broiler chickens administered with different probiotic formulations

Femi Gabriel Oyeniyi*¹ and Florence Ebunoluwa Ajayi¹

*corresponding author: femigabriel382@gmail.com

¹*School of Agriculture and Agricultural Technology, Department of Agricultural Technology, Ekiti State Polytechnic, Isan-Ekiti, Nigeria.*

ABSTRACT

Probiotics were prepared in the biological laboratory of Ekiti State Polytechnic in three separate 5 Litre capacity air-tight plastic kegs each having maize, sorghum and rice as substrates. They wholly replaced Tetracin® (a feed-grade antibiotic) in the diet of Arbor acre broiler chickens (corresponding to each treatment) from week 2-8 of rearing. There were two control diets - positive and negative (Treatments 1 and 2 respectively) that had inclusion of Tetracin® at the recommended rate and no inclusion of Tetracin®. At the end of the feeding trial, 20 birds per treatment were humanely sacrificed defeathered, eviscerated with primal cuts made and used for sensory evaluation. The total bacterial count in maize-based probiotic was 8.50CFU/mL(x103), Sorghum was 12.55 CFU/mL(x103) and Rice-9.25CFU/mL(x103). Fungal count in maize was 5.50SFU/mL(x103), sorghum-4.35SFU/mL(x103) and Rice-11.70SFU/mL(x103). Histopathological evaluation showed that birds in Treatment 1 had moderate portal congestion while those in 2 had mild cellular infiltration. No visible lesion was seen in liver samples of birds in Treatment 3 while those in 4 had severe portal hypoplasia. Liver samples of birds in Treatment 5 had no visible lesions. Overall, results showed that birds administered sorghum and rice-based probiotics fared better than those provided with maize-based probiotic.

Keywords: broiler chickens, histopathology, meat, assay

INTRODUCTION

Though there was a ban in the European Union in 2006 for the use of antibiotics to promote growth in poultry and other livestock species, they are still being used to prevent diseases, and improve feed efficiency in broiler chickens (Ekpo et al. 2023, Van et al. 2020, Mehdi et al, 2018). The trend has

the potential to assume a dangerous dimension in Low- and Middle-Income Countries (LMIC) over the coming years due to overuse and misuse of antibiotics. This is due to the projected rise in demand for meat products as a result of human population growth and rise in levels of income among other things. Already, there is a rise in intensification of poultry farms in Nigeria (Herrero, 2020) and a previous study reported the indiscriminate use of antibiotics in broiler chickens (Oyeniyi et al. 2024). Among other things, this may contribute to the emergence of antibiotic-resistant bacteria, posing significant threats to human health and animal welfare. In response, there is a growing interest in exploring alternative strategies to maintain gut health and prevent disease in poultry (Abd El-Hack et al. 2022).

Little attention has been paid to the impact of probiotics on the sensory attributes of broiler chicken meat. Sensory characteristics such as texture, colour, flavour and aroma play a crucial role in determining consumer acceptance and preference. Probiotics are live microorganisms that confer health benefits when administered in adequate amounts and they have shown promise as potential replacements for antibiotics in poultry production (Jha et al. 2020). Local probiotics, in particular, offer a unique opportunity to identify and harness the beneficial properties of indigenous microorganisms, reducing reliance on imported products and promoting sustainable agriculture methods (Boranbayeva et al. 2025; Amenyogbe, 2023).

This study was geared at investigating the effects of local probiotics on gut health in finisher broiler chickens, with a focus on histopathological changes in the gut tissue. Through evaluating the efficacy of local probiotics as alternatives to antibiotics, this research seeks to contribute to the development of more sustainable and health-conscious poultry production practices. In addition, it is expected that understanding by broiler chicken farmers of the effects of local probiotics on the sensory quality of broiler chicken meat will inform the development of more sustainable and consumer-friendly poultry production systems.

MATERIALS AND METHODS

A total number of 240 Arbor-Acre Broiler chicks were transported humanely to the experimental site at Isan-Ekiti under conducive weather conditions. The location is roughly between latitudes 15° 39' 15" and 15° 36' 19" N and longitudes 70° 12' 33" and 70° 10' 40"E at a height of around 544 metres above mean sea level. It is situated in the rainforest agroecological zone, Southwestern district of Nigeria.

The birds (of mixed sexes) had an average initial body weight of 40.2 ± 1.56 g and were brooded together for a period of 14 days following the standard conditions. They were then randomly assigned to five treatments in a completely randomized design containing 48 birds each with 12 birds per

replicate. Raw grains of maize, brown rice and sorghum (500g each) were purchased from the open market. A total of 200g of each grain was weighed in the laboratory using a digital weighing laboratory scale. Each grain was carefully emptied into separate 5kg pre-washed kegs (3 each). Subsequently, 50cl of molasses, 1 tablespoon of black salt and 3 litres of water were all added. The keg's opening was sealed with a nylon, before covering with the lid. This was done so as to provide an extra layer of firmness as air-tight condition is necessary for the culture of the micro-organisms through fermentation. The keg was shaken 9 times before being placed in the laboratory cupboard. This shaking procedure was repeated for three consecutive days (at 9:00 a.m., corresponding to the time of preparation of the mixture). After confirmation that fermentation had taken place – 5 days after preparation of the mixtures - (evidenced by physical expansion of the keg as well as the smell associated with fermentation), a volume of 35cl of each probiotic was collected in separate bottles and taken for microbial assay. The antimicrobial properties of each probiotic were subsequently evaluated. Another 35cl of each probiotic sample was collected in a different set of bottles for proximate analysis. Birds in Treatment 1 were administered with conventional doses of Tetracin®, those in Treatment 2 had no Tetracin® nor probiotic while Treatments 3, 4 and 5 respectively had oral probiotic formulations prepared with rice, maize and sorghum correspondingly as substrates. All the birds were provided with iso-caloric and iso-nitrogenous feed in a manner that met their nutritional needs, along with clean water. Their average initial body weight was determined as well as weekly weights throughout the experiment's duration. After an eight-week period post arrival and rearing of the chicks, they were placed on fasting metabolism and one bird per replicate (for each treatment) was randomly selected, the final body weight determined and each, humanely sacrificed by severing the jugular vein.

At the end of the experiment, the gastrointestinal tract (GIT) was collected from two birds per treatment and then transferred into specimen bottles containing 10% formalin where normal Haematoxylin and Eosin standard staining procedures were performed according to the methods of Purwanti (2018). Small specimens of the GIT were taken from each experimental group, fixed in normal buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%) cleared in zylene and embedded in paraffin according to the method described by Khenenou et al. (2017). Sections of 4-6 µm thickness were prepared and stained with haematoxylin and eosin according to the method described by Khenenou et al. (2013). The prepared slides were viewed under a microscope and photomicrograph captured using a motic camera and the photomicrograph were transferred to the computer for further pathologic reading.

Meat preparation was done using a wet cooking method. The samples were wrapped in impervious polythene pouches which could not be destroyed by cooking process. In the process, the meat samples were cooked in boiling water for 20 minutes using water bath with no spices added to the meat. The meat was then served to a 20-member taste panel drawn from faculty and students in the Department of Agricultural Technology, Ekiti State Polytechnic, Isan-Ekiti. The trained panelists evaluated the samples for colour, flavour, juiciness, tenderness and general acceptability. The assessment was based on a 9-point hedonic scale. The score was arranged in a descending order with a maximum score of 9 given to extremely like condition while the lowest score of 1 was assigned for the poorest condition. All data obtained were processed and subjected to a one-way analysis of variance (ANOVA) using statistical analysis software (SPSS version 21). Significantly different means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Plates 1-5 reveals the histopathological sections of the liver of finisher broiler chickens used in this study (positive and negative control as well as the different probiotic formulations). The liver sample in Plate 1 shows that there is a moderate portal congestion.

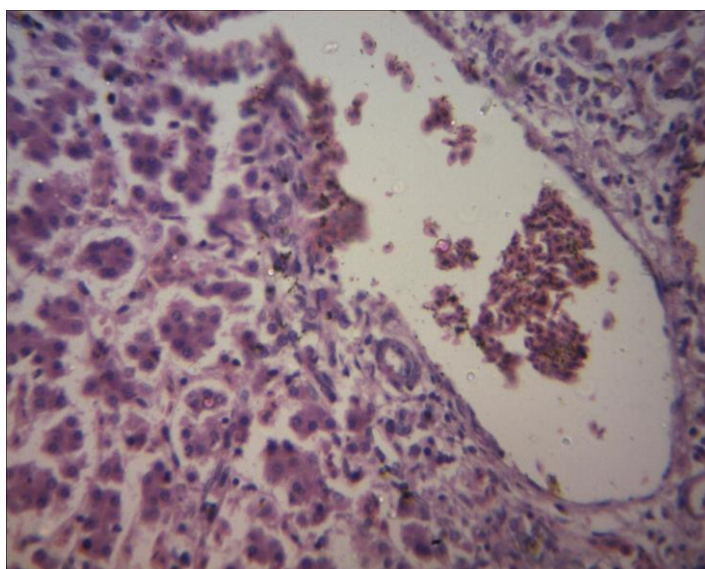


Plate 1. Section of liver, excised from Birds in Treatment 1 (Birds provided with conventional dose of Tetracin®)

In Plate 2, there was mild extensive cellular infiltration that is non-specific.

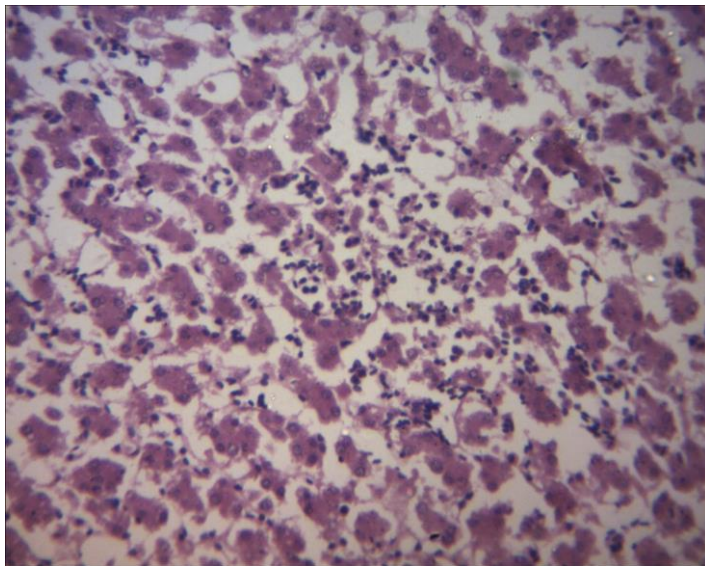


Plate 2. Section of liver, excised from Birds in Treatment 2 (Birds not provided Tetracin®)

Plate 3 and 5 reveals that there are no visible lesions seen. In Plate 4, there is severe portal fibroplasia with severe periportal cellular infiltration.

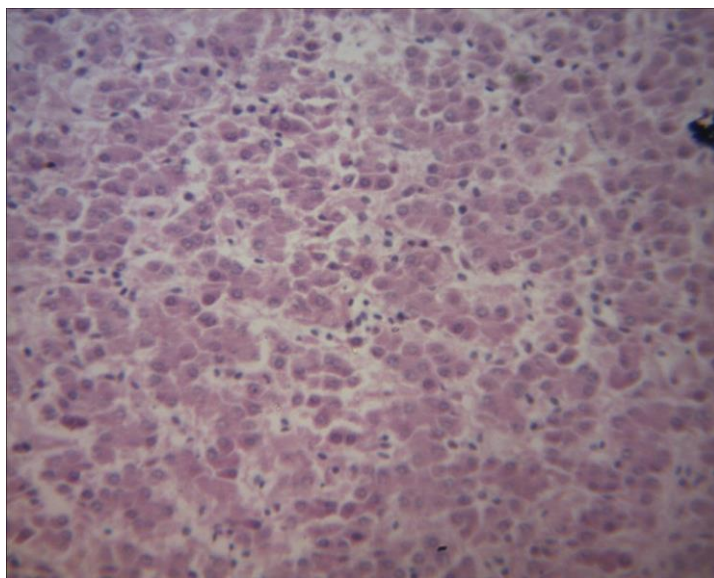


Plate 3. Section of liver, excised from Birds in Treatment 3 (Birds provided with sorghum-based probiotic)

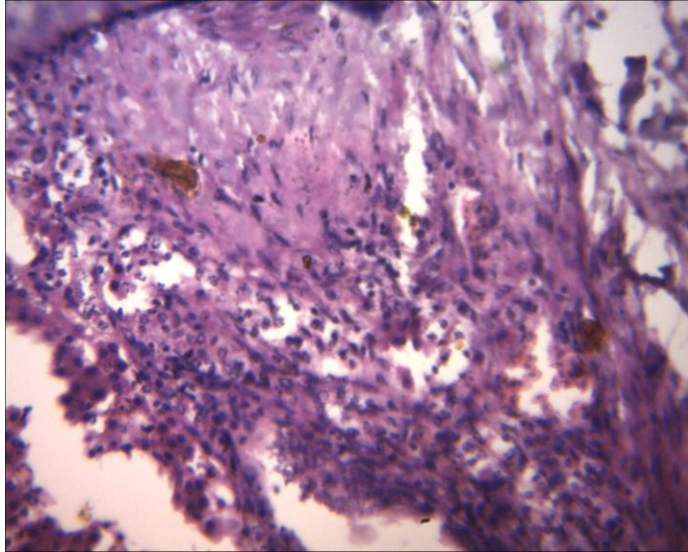


Plate 4. Section of liver, excised from Birds in Treatment 4 (Birds provided with Maize-based probiotic)

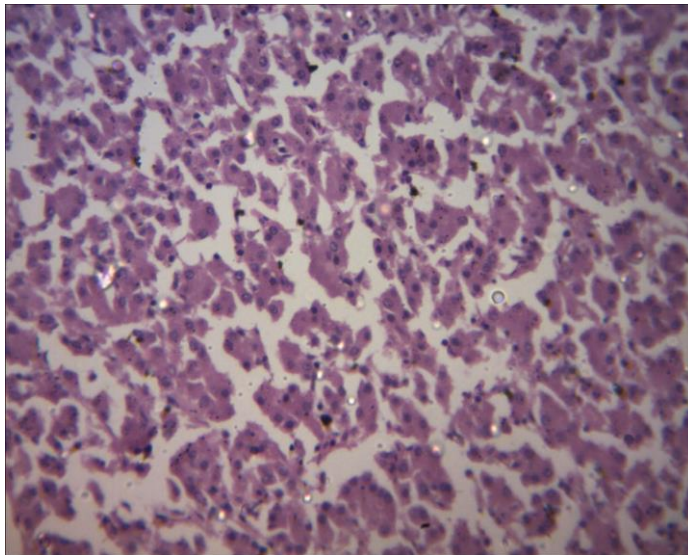


Plate 5. Section of liver, excised from Birds in Treatment 5 (Birds provided with Rice-based probiotic)

Results presented in Table 1 show that NFE is significantly ($P<0.05$) lower in Rice (4.57%) than Sorghum (9.01%) and Maize (10.02%) respectively. Total Bacterial Count (CFU/mL X103) in Sorghum (12.55) was significantly ($P<0.05$) higher than that of Maize (8.50) and Rice (9.25) respectively as presented in Table 2.

Table 1. Proximate composition of the Probiotic formulations

Probiotic Substrates/ Proximate Fractions	Moisture (%)	Ash (%)	Ether Extract (%)	Crude Fibre (%)	Crude Protein (%)	NFE (%)
Maize	86.50	0.71	0.21	1.38	1.10	10.02 ^a
Rice	91.30	0.59	0.37	1.74	1.41	4.57 ^a
Sorghum	85.42	0.41	0.65	1.81	2.67	9.01 ^b

^{a, b} Means along the same row with different superscripts differ significantly ($P < 0.05$);

Note: NFE – Nitrogen Free Extractives.

Table 2. Microbial load in Probiotics

Probiotic Substrate	TBC (CFU/mL) x (10 ³)	TFC(SFU/mL) x (10 ³)
Maize	8.50 ^b	5.50 ^b
Sorghum	12.55 ^a	4.35 ^b
Rice	9.25 ^b	11.70 ^a

^{a, b} Means along the same row with different superscripts differ significantly ($P < 0.05$);

TBC = Total Bacteria Count; TFC = Total Fungal Count; CFU = Colony forming unit; SFU= Spore forming unit.

In Table 3, the probable organisms identified after various tests were carried out on the probiotics are as follows: *L. acidophilus*, *M. luteus*, *B. subtilis* and *S. boulardii*.

Table 3. Biochemical assay on isolated organisms

Sample Codes	Sugar Ferment	Gram Rxn	Catalase	Oxidase	Citrate	Motility	Indole	Met hyl Red	Coagulase	Urease	Probable Organisms
A	+	+	-	-	-	-	-	+	-	-	<i>L. acidophilus</i>
B	-	+	+	-	-	-	-	-	-	+	<i>M. luteus</i>
C	+	+	+	-	+	+	-	-	-	-	<i>B. subtilis</i>
D	+	-	+	-	-	-	-	-	-	-	<i>S. boulardii</i>

Note: + = Positive, - = Negative

The level of abundance of these microbes in the various probiotic samples is presented in Table 4. Maize based probiotic had very large quantities of *L. acidophilus* and *M. luteus* while sorghum had large quantities of these as well in addition to *B. subtilis* (that was not however present in Maize based

probiotic). In the rice based probiotic, very large quantities of *L. acidophilus* and *S. boulardii* were present while there was no trace of *M. luteus* and *B. subtilis* respectively in it.

Table 4. Micro-organisms identified in the probiotic formulations

Probiotic Substrate	<i>Lactobacillus acidophilus</i>	<i>Saccharomyces boulardii</i>	<i>Micrococcus luteus</i>	<i>Bacillus subtilis</i>
Maize	V	v	V	X
Sorghum	V	v	V	V
Rice	V	V	X	X

Note: V = Present (in large quantities), v = Present (in small quantities), X = Not present

Tables 5a, 5b and 5c respectively showed that as the concentration of probiotic samples increased, the zones of inhibition of activities of micro-organisms increased correspondingly.

Table 5a. Anti-microbial properties of Maize-based probiotic

Concentration (mg/L)	100	150	200
<i>Salmonella spp.</i>	5.5	12.4	12.8
<i>Klebsiella aerogenes</i>	2.0	3.6	4.0
<i>Escherichia coli</i>	7.8	10.6	13.7
<i>Staphylococcus aureus</i>	3.0	3.5	4.2

Table 5b. Anti-microbial properties of Sorghum-based probiotic

Concentration (mg/L)	100	150	200
<i>Salmonella spp.</i>	8.2	16.1	16.5
<i>Klebsiella aerogenes</i>	5.6	6.4	7.2
<i>Escherichia coli</i>	11.5	12.4	13.5
<i>Staphylococcus aureus</i>	1.0	2.2	3.0

The effects of different probiotic formulations on the organoleptic properties of broiler chickens are presented in Table 6. Parameters evaluated include appearance, flavour, taste, texture, juiciness, and overall acceptability. Significant ($P < 0.05$) differences were observed among the parameters measured except taste and juiciness.

Table 5c. Anti-microbial properties of Rice-based probiotic

Concentration (mg/L)	100	150	200
<i>Salmonella spp.</i>	3.7	5.3	15.6
<i>Klebsiella aerogenes</i>	3.0	3.4	4.4
<i>Escherichia coli</i>	7.4	9.6	13.2
<i>Staphylococcus aureus</i>	1.0	1.5	1.6

Note: Zone of inhibition is in mm and concentration is in mg/L

Table 6. Organoleptic properties of broiler chickens administered with different probiotic formulations

Parameters	T1	T2	T3	T4	T5	SEM
Appearance	7.45 ^a	6.60 ^b	6.60 ^b	6.70 ^{ab}	7.00 ^{ab}	0.12
Flavour	6.53 ^{ab}	5.80 ^b	6.65 ^a	6.20 ^{ab}	6.85 ^a	0.12
Taste	5.88	5.65	6.20	6.38	6.40	0.11
Texture	6.28 ^{ab}	6.25 ^{ab}	5.98 ^b	6.25 ^{ab}	6.85 ^a	0.13
Juiciness	6.20	6.05	6.48	6.35	6.35	0.14
Overall Acceptability	7.73	7.20	7.50	7.35	7.48	0.11

^{a, b} Means along the same row with different superscripts differ significantly ($P < 0.05$); SEM – Standard Error of Means.

Broiler chickens from T1 (Tetracin®) had the highest appearance score (7.45), the result is statistically similar to those on T5 (7.00). These values were significantly higher than T2 and T3 (6.60), which had the lowest value. Treatments 5 and 3 had the highest flavour enhancement of 6.85 and 6.65 respectively, while the least was seen from treatment 2, as 5.80. Meat samples from broiler chickens in T5 were also proven to have the highest (6.85) texture. However, the result is statistically similar to T1. (6.28).

In birds, the liver is a multipurpose organ that plays vital roles in detoxification, protein, lipid, and carbohydrate synthesis and metabolism, muscle and egg production, blood proteins, enzymes, and immunological components (Maria, 2021). In order to ensure proper fat digestion, it also contributes to the formation and release of bile. Therefore, any substance that alters the physiology of the liver will have a detrimental effect on the final weight and feed conversion of broiler chickens. It will also make them more susceptible to illnesses, which will raise the overall mortality rate and treatment expenses. As a result of their impact on agricultural production and their potential to compromise food safety due to their presence in animal-source foods, hepatotoxic compounds are therefore extremely dangerous.

It can be deduced that the condition of liver of birds in Treatment 1 may stem from various factors, including respiratory infections, heart issues, or liver damage. Respiratory diseases like infectious bronchitis or mycoplasma infections can lead to inflammation and congestion in the respiratory system, potentially affecting the portal circulation (Liu et al. 2025). Additionally,

conditions like ascites, often linked to pulmonary hypertension and right ventricular failure, can cause fluid buildup in the abdomen, including the portal area. Liver damage, potentially due to toxins or other diseases, can also contribute to portal congestion. Stressful conditions like overcrowding or poor management can weaken the immune system and make chickens more vulnerable to diseases that affect the respiratory system or liver, leading to congestion. The above conditions mirror some observations noted by Mahjoor and Hadipoor (2020) and corroborated by Islam et al. (2021) are indicative of ascites syndrome. The primary cause of ascites in meat-type chickens is right ventricular failure (RVF) as a result of increased pulmonary (lung) arterial pressure. Increased pulmonary arterial pressure can be produced in birds by lack of oxygen. When the oxygen supply is insufficient, the heart must circulate blood more rapidly to provide the same amount of oxygen to the body. In meat-type chickens, the lack of oxygen causes a marked increase in the number of red blood cells that makes the blood more viscid and difficult to pump through the lung, causing pulmonary hypertension (PH). The right side of the heart enlarges in response to the increased workload, and, if the heart has to continue working harder than normal, the result is right ventricular valve insufficiency, volume overload, right ventricular dilatation and RVF. As a result, venous blood pressure increases, and fluid leaks out of the veins and accumulates in peritoneal cavities, resulting in ascites. The causes of insufficient oxygen supply and subsequent development of ascites in broilers are complex. Predisposing factors that increase the amount of oxygen required, reduce oxygen-carrying capacity of the blood, increase blood volume or interfere with blood flow through the lung may result in flock outbreaks of ascites. The lungs of chickens are rigid and moulded into the thoracic cavity and they cannot expand like mammalian lungs. The capillaries can expand only a little to allow for increased blood flow. The lungs of chickens grow less rapidly than the rest of the body, and lung capacity does not keep up with the very rapid growth of muscle in fast-growing broiler chickens. In addition to high oxygen demands associated with their fast growth rate, the fast metabolism of the modern broiler chicken increases the production of reactive oxygen types that increase oxidative stress, causing damages to pulmonary vessels (Kalmar et al. 2013). Cold is a common cause of outbreaks of ascites in backyard flocks because of enhanced blood flow through the lungs of birds in an effort to produce endogenous heat (Kalmar et al. 2013). Though the average daily ambient temperature was maintained at 31.7°C, the period was about the coldest season of the year in Nigeria. This may explain in part a key predisposing factor of birds to ascites.

According to Visnuvinayagam et al., 2019, the pathomorphological changes in Marek's disease include enlargement of liver and lymphoid cell infiltration. Mild, extensive, and non-specific cellular infiltration in the liver of broiler chickens can be caused by various factors, including bacterial

infections like *Clostridium perfringens* and *Campylobacter*, viral infections such as fowl adenovirus (FAdV), and toxins like aflatoxin B1. Nutritional imbalances and stress, including heat stress, can also contribute to this type of liver pathology. *Clostridium perfringens* in broiler chickens primarily causes Necrotic Enteritis, but can also lead to liver issues like cholangiohepatitis and ascites. The bacteria, normally present in the gut, overgrows due to predisposing factors like intestinal microflora imbalances caused by *Eimeria* infection or certain diets (Hargis and Swayne, 2024). These factors allow *Clostridium perfringens* to multiply, release toxins, and cause intestinal damage, which can then affect the liver. It is worth emphasising that birds in Treatment 2 served as negative control i.e. received no antibiotic nor probiotic. It thus may account for this condition in the liver sample. A liver sample from another bird in this treatment (not provided in this paper) shows that there was mild periportal cellular infiltration. The results of a study by Lovland and Kaldhusdal (1999) suggest that the occurrence of necrotic enteritis in broiler flocks may be monitored by using meat inspection data on liver lesions.

As for birds in Treatment 4, severe portal fibroplasia in broiler chickens, characterized by excessive fibrous tissue formation in the liver's portal areas, is primarily caused by *Clostridium perfringens* infection and subsequent cholangiohepatitis. This condition can also be exacerbated by factors like aflatoxin contamination, which leads to liver damage and bile duct proliferation (Dinev, 2024). It is worthy of note that a maize-based probiotic was used for birds in Treatment 4 and the source was from the open market. There could have been contamination of the grains with aflatoxin as a result of improper drying.

Severe portal fibroplasia, also known as idiopathic portal hypertension or non-cirrhotic portal fibrosis, in human subjects in Nigeria is a complex condition with several proposed causes, though the exact aetiology remains unclear. Potential causes may include infections like schistosomiasis, exposure to toxins, and immunological abnormalities (Garzali et al. 2024). It presupposes that there may be a correlation of sorts between this condition in the liver of humans and avians.

The better visual appeal of T1 and T5 could be attributed to their antimicrobial properties, which may reduce spoilage and enhance visual appeal (Fadiji et al., 2023). The sorghum-based probiotic (T5) demonstrated comparable appearance to the antibiotic-treated group, suggesting its potential to replace antibiotics, which could have residual effect on the consumer.

It appears that maize- and sorghum-based probiotics enhanced meat flavour by modulating the gut microbiota and reducing off-flavour compounds from microbial metabolites (Çapan, and Bağdatlı, 2022). The lower flavour score in T2 augment the importance of dietary additives in

sensory improvement. The texture improvement in T5 suggests that sorghum-based probiotics may influence muscle fiber development, possibly through better protein utilization and reduced stress in broilers (Oyeagu et al., 2023).

CONCLUSION AND RECOMMENDATION

It appears that birds administered sorghum-based probiotic as well as rice-based probiotic fared better compared to those supplied with maize. In addition, they seemed to out-perform the positive and negative control respectively (Treatments 1 and 2). It thus corroborates the results from an earlier report by the same authors (Oyeniyi and Ajayi, 2025). This suggests that they hold the potential to replace antibiotic use in broiler chickens. There is however a need for further validation of this study before wide adoption by farmers. In particular, it is recommended that such studies should be conducted on other broiler strains commonly reared in Nigeria and at different seasons of the year as well as across multiple locations in the country. Also, aflatoxin-free grains are recommended for use as probiotics so as to ensure food safety ultimately.

ACKNOWLEDGEMENT

The authors declare that there is no conflict of interest and hereby extend profound gratitude to Tertiary Education Trust Fund (TETFund) for the funds provided that enabled the conduct and completion of this investigation. Also, profound gratitude is hereby expressed to Mr. Ogungbaibi who availed us the use of a space on his farm for conduct of the experiment.

REFERENCES

- Abd El-Hack, M. E., El-Saadony, M. T., Salem, H. M., El-Tahan, A.M., Soliman, M. M., Youssef, G. B. A., Taha, A. E., Soliman, S. M., Ahmed, A. E., El-Kott, A. F., Al Syaad, K. M., Swelum, A. A. (2022). Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poultry Science* 101 (4): 5-6.
- Amenyogbe, E. (2023). Application of probiotics for sustainable and environment-friendly aquaculture management -A review. *Cogent Food & Agriculture* 9:1-15. <https://doi.org/10.1080/23311932.2023.2226425>.
- Boranbayeva, G., Tekebayeva, Z., Temirkhanov, A., Temirbekova, A., Yevneyeva, D., Abilkhadirov, A., Mkilima, T. and Abzhalelov, A. (2025). Probiotic consortium from poultry strains for supporting gut immunity against pathogens. *Microbial Pathogenesis* 204, 107584.

- Çapan, B., and Bağdatlı, A. (2022). Usage of probiotics in the poultry industry and effects on meat quality. *Selcuk Journal of Agriculture and Food Sciences*, 36(1), 120-126.
- Dinev, I. (2024). Diseases of Poultry. The Poultry Site. <https://www.thepoultrysite.com/publications/diseases-of-poultry/185/cholangiohepatitis-in-broiler-chickens>.
- Eko, P. M., Ekpo, U. E., Afolabi, K. D. and Idio, A. D. (2023). Assessment of Antibiotic Use in Broiler Management: Unnoticed Effects and Alternative Strategies for Sustainable Poultry Farming – A Review. IN: Ayandele, I. A.; Udom, G. N.; Effiong, E. O.; Etuk, U. R.; Ekpo, I. E.; Inyang, U. G.; Edet, G. E.; and Moffat, I. (Editors). Contemporary Discourse on Nigeria's Economic Profile, A Festschrift in honour of Prof. Nyaudoh, U.
- Ndaeyo. A Publication of University of Uyo, Uyo, Akwa Ibom State, pp. 632-640.
- Fadiji, T., Rashvand, M., Daramola, M. O., and Iwarere, S. A. (2023). A review on antimicrobial packaging for extending the shelf life of food. *Processes*, 11(2), 590.
- Garzali, I. U., Shehshe, A. A., Suleiman, I. E., El-Yakub, A. I. (2024). Early Experience with Surgical Management of Non-Cirrhotic Portal Hypertension in Nigeria: Report from a Single Centre. *J. West. Afr Coll. Surg.* 15(2):176–180. doi: 10.4103/jwas.jwas_10_24.
- Hargis, B. M., and Swayne, D. E. (2024). Necrotic Enteritis in Poultry. MSD Veterinary Manual.
- Herrero, M. (2020). Factsheet on poultry production in Nigeria. <https://research.csiro.au/livegaps/wpcontent/uploads/sites/37/2021/03/1.-LiveGAPS-factsheet-Poultry-production-in-Nigeria-22-April-2020.pdf>.
- Islam, S.J., Rajkhowa, T.K., Arya, R.S., Singh, Y.D., Dutta, T.K. and Kalita, P.C. (2021). Studies on ascites syndrome in poultry population of Mizoram. *Indian J Vet Pathol* 45(1): 56-58.
- Jha, R., Das, R., Oak, S., and Mishra, P. (2020). Probiotics (Direct-Fed Microbials) in Poultry Nutrition and Their Effects on Nutrient Utilization, Growth and Laying Performance, and Gut Health: A Systematic Review. *Animals* 10(10):1863. doi: 10.3390/ani10101863.
- Kalmar, I. D., Vanrompay, D., Janssens, G. P. J. (2013). Broiler ascites syndrome: Collateral damage from efficient feed to meat conversion. *The Veterinary Journal* 197 (2) : 169-174.
- Khenenou, T., Boughrara, M., Melizi, M., Lamraoui, R. (2017). Histomorphological Study of the Bursae of Fabricius of Broiler Chickens during Gumboro Disease in Algeria Area. *Global Vet.* 18 (2):132-136.

- Khenenou, T., Melizi M., Benzaoui H., Bennoune, O., Ibrir, M. (2013). Histological Changes in Liver and Pectoral Muscles of Broiler Chickens Slaughtered with and Without Naming of Allah. *Int. J. Poult. Sci.* 12(9): 550-552. *Science Alert*. <https://doi.org/10.3923/ijps.2013.550.552>.
- Liu, H., Pan, S., Wang, C., Yang, W., Wey, X., He, Y., Xu, T., Shi, K., and Si, H. (2025). Review of respiratory syndromes in poultry: pathogens, prevention and control measures. *Veterinary Research* 56 101 (2025) <https://doi.org/10.1186/s13567-025-01506-y>.
- Lovland, and Kaldhusdal (1999). Liver lesions seen at slaughter as an indicator of necrotic enteritis in broiler flocks. *FEMS Immunology & Medical Microbiology*, 24(3):345-351,
- Mahjoor, A. A., and Hadipoor, M. M. (2021). Clinical, Gross and Histopathological Studies on Natural Ascites Syndrome in Broiler Chickens. *Journal of Alternative Veterinary Medicine*, Islamic Azad University, 3 (6): 333-342.
- Maria, S. (2021). The Liver, a key organ in Poultry Production. Engormix factsheet. https://en.engormix.com/poultry-industry/metabolic-nutritional-diseases-poultry/the-liver-key-organ_a47819/.
- Marra, F., Aleffi, S and Provenzano, S. (2009). Mononuclear cells in the liver fibrosis. *Immunology*, 31(3): 345-358.
- Mehdi, Y., Letourneau-Montminy, M-P., Gaucher, M-L., Chorf, Y., Suresh, G., Rouissi, T., Brar, S. K., Cote, C., Ramirez, A. A., Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition* 4 (2): 170-178.
- Oyeagu, C. E., MLambo, V., & Lewu, F. B. (2023). Histomorphometric traits, microbiota, nutrient digestibility, growth performance, carcass traits and meat quality parameters of chickens fed diets supplemented with different levels of *Bacillus protease*. *Journal of Applied Animal Research*, 51(1), 137-155.
- Oyeniyi, F. G., and Ajayi, F. E. (2025). Carcass characteristics of broiler chickens provided with locally made probiotics as replacement for antibiotics. *Proceedings of the 50th Annual Conference of the Nigerian Society for Animal Production* held between March 16-20, 2025 at Federal University Nassarawa.
- Oyeniyi, F. G., Dada, O., Popoola, I. F., Olowoyo, E., Oyeniyi, T. O., Fadairo, O., Popoola, L. (2024). A review of contract poultry farming in Nigeria. *Proceedings of the Nigerian Academy of Science* 17 (1): 141-159.
- Purwanti. S. (2018). Physical and Sensory Quality of Broiler Meat as Influenced by Dietary Supplementation of Turmeric (*Curcuma longa*), Garlic (*Allium sativum*) and in Combinations as a Feed DOI: 10.20884/1.jap.2018.20.1.633

-
- Van, T.T.H., Yidana, Z., Smooker, P. M., Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *Journal of Global Antimicrobial Resistance* 20 (2020) 170–177.
- Visnuvinayagam, S., Dash, B. B., Barathidasan, R., Mayilkumar, K. and Selvaraju, G. (2019). Anatomopathological and molecular studies of Mareks disease virus in India. *Brazilian Journal of Veterinary Pathology* 12(2): 33-40.