INFLUENCE OF DOSED BASED PROBIOTICS (*Lactobacillus)* ON BLOOD AND MICROBIAL INDICES OF BROILERS

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Abstract. This study was carried to evaluate the effect of probiotics (*Lactobacillus*) on the blood parameters and microbial load of one hundred and twenty day old unsexed Abor Acres+ for a period of eight weeks. The birds were randomly allocated into four equally major groups (T_1, T_2, T_3 and T_4) each having two replicates with ten birds each. T_1 is the control, while, the test ingredient was given at different levels of inclusion T_2 (5ml), T_3 (10ml) and T_4 (15ml) at starter phase and T_2 (15ml), T_3 (20ml) and T_4 (25ml) at the finisher phase respectively. On the 8th week of the experiment, blood and caecal samples droppings were collected and analyzed for blood and microbial load parameters. The results showed that haemoglobin was significantly higher in T2 (8.85±0.37) and lowest in T3 (8.22±0.47). White blood cell was highest in T3 (4.55±1.21) and significantly lowest (P<0.05) in T2 (1.00±0.89). High density lipoprotein was highest in T2 (1.80±0.37) and significantly lowest (P<0.05) in T1 (1.47±0.16) and T4 (1.47±0.13). Low density lipoprotein was highest in T4 (2.60±0.04) and significantly lowest (P<0.05) in T2 (2.03±0.26). Very low density lipoprotein was highest in T4 (0.82±0.02) and significantly lowest (P<0.05) in T2 (0.56±0.41). Total heterotrophic bacteria was highest in T4 (113.33±24.50) and significantly lowest (P<0.05) in T3 (75.33±11.38). Total coli form count was highest in T4 (77.59±25.91) and significantly lowest (P<0.05) in T2 (49.17±15.06). The utilization of probiotics had a beneficial effect on the gut health and blood indices of the broiler chicken.

Keywords: Haematology, microbiota, poultry, probiotics, serum biochemistry

INTRODUCTION

The poultry industry is blooming yearly globally due to an increase in demand for the consumption of meat. The demand for poultry meat over red meat is higher because of its superiority in terms of human health. Chicken contains unsaturated fatty acids, oleic acid and linoleic acids and reduced amounts of low-density lipoproteins and cholesterol, which are harmful to humans (Zhang *et al.*, 2021). Hence, in order to meet the large demand for broilers in the market, there is an excessive use of antibiotics in broiler diet to promote the growth of broilers. This abuse in the use of antibiotics has led to issues such as drug resistance in animals and drug residues in livestock products. This further threatens the sustainable development of both humans and the livestock sector, and has emerged as a severe food security issue (Rana *et al.*, 2019). Also, there is instability of the beneficial intestinal flora and the appearance of resistant bacteria (Popova, 2017). Thus, several countries have legislated and banned the application of antibiotics as growth promoters in feeds (Vieco-Saiz *et al.*, 2019).

Researchers are in constant search for the selection of alternative additives to antibiotics to promote growth rate and prevent intestinal infections. Thus, probiotics are being considered to stem the tide and several farmers are using probiotics as alternative to antibiotics in poultry production (Onabanjo *et al.*, 2018; Onunkwo *et al.*,

2022; Sam *et al.*, 2023). The utilization of probiotics in poultry production will increase in the future as a result of the restriction of antibiotics as growth promoters and also because some consumers will avoid products produced using antibiotics. Furthermore, both the positive results obtained and the economic nature revealed that the use of probiotics, at least at a certain level in broiler diet, will continue both in the short and long terms. Probiotic microorganisms that are commonly used in poultry production include *Bifidobacterium, Lactococcus, Lactobacillus, Bacillus, Streptococcus* (Park *et al.*, 2016; Çapan and Bağdali, 2022).

Probiotics are live bacteria, fungi, or yeasts that supplement the gastrointestinal flora and help to maintain a healthy digestive system, thereby promoting the growth performance and overall health of poultry (Jha *et al.*, 2020). The study of the normal blood indices of broilers are essential tools in diagnosing the various pathological and metabolic disorders. Blood indices have been reported to provide valuable information on assessing the immune status of animals (Upah *et al.*, 2024).

The action of probiotics in broiler diet varies with the dose used. Hence, more investigation is required to assess the application of various doses on the overall assessment of the blood indices and microbial load. Therefore, the objective of this study was to evaluate the influence of different levels of probiotics on the blood indices and microbial load of broiler chickens.

MATERIALS AND METHODS

Experimental Site

The study was carried out in the poultry unit of the University of Port Harcourt Teaching, Research and Demonstration farm, Choba, Obio-Akpor Local Government Area of Rivers State in Niger Delta area of Nigeria. The poultry unit is situated at latitude 4º44'49" North and longitude 7º2'23" East of the equator. It falls within the humid rain forest zone of West Africa with a long duration of rainfall (March-November) and a very quiet dry season. Temperature ranges from 25-28°C and a very high relative humidity above 80% (Olalere *et al.*, 2023).

Experimental Animals and Management

The poultry house was disinfected for a week prior to the arrival of the day-old chicks. Wood shavings and saw dust were used as litter materials over a concrete floor. A total of one hundred and twenty day old unsexed Abor Acres + (AA+) chicks with an average weight of 35 to 45g were procured from a known hatchery. Electric bulbs were used as a source of heat. On arrival, the birds were given vitamins and vitamin supplement to ease the stress during transportation. The birds were weighed and randomly assigned to various treatments. Fresh water was provided on daily basis.

Experimental test ingredients

The probiotics (*Lactobacillus*) was purchased from a certified vendor. Feeding of the test ingredient started after three days of the arrival of the chicks until the termination of the research. The test ingredient was incorporated in the drinking water at inclusion level of 5ml, 10ml and 15ml at starter stage and 15ml, 20ml and 25ml at finisher stage.

Experimental Design and Experimental Procedure

The experiment lasted for the period of eight weeks (8weeks). The experimental design used in this study was completely randomized design. The birds were distributed randomly into four (T_1, T_2, T_3 and T_4) different treatments with each treatment replicated three (3) times with each replicate having ten (10) birds in it. The T_1 is the control, while the test ingredient was given to other treatments at different level of inclusion T_2 (5ml), T_3 (10ml) and T_4 (15ml) at starter stage and T_2 (15ml), T_3 (20ml) and T_4 (25ml) at the finisher stage.

Haematological analyses and procedures

Blood sample (2ml) was collected from two (2) birds per replicate into container. Blood samples that was collected were analyzed for the packed cell volume (PVC), hemoglobin,(Hb), red blood cell (RBC), white blood cell (WBC), platelet, neutrophil, lymphocytes, monocytes (M) and eosinophil using the Auto Analyzer method. While, total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), very low density lipoprotein (VLDL), and cholesterol using the calorimetric end point method as described by Ritchie et al. (1994).

Microbial Load

On the 8th week of the experiment, 2 cecal samples droppings was collected from each replicate and analyzed for microbial load. At the termination of the experiment, the content of the trachea and the large intestine for microbial culture was collected to determine the microbial load and the type of bacteria count present using standard microbiological methods as described by Lorch et al. (1995).

Data Analysis

All data obtained were analyzed with statistical analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS). Treatment means was compared using Duncan multiple range test of the same software.

Statistical model

$Yij = Ti + Eij$

Where

Yij= Individual observation

Ti= Treatment Effects

Eij= Experiment error

Table 1. Composition of broiler starter diet

INGREDIENTS (%)	**$T_{1(CONTROL)}$**	**T_2**	**T_3**	**T_4**
Maize	50	50	50	50
Palm kernel cake	8	8	8	8
Wheat bran	8	8	8	8
Soya bean meal	23	23	23	23
Fish meal	5	5	5	5
Palm oil	2.5	2.5	2.5	2.5
Bone meal	2.97	2.97	2.97	2.97
Methionine	0.06	0.06	0.06	0.06
Lysine	0.02	0.02	0.02	0.02
Vitamin Premix	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20
Total	100	100	100	100

Table 2. Composition of broiler finisher diet

INGREDIENTS	$T_{1(CONTROL)}$	T_2	T_3	T_4
Maize	55	55	55	55
Palm kernel cake	9	9	9	9
Wheat bran	9	9	9	9
Soya bean meal	15.5	15.5	15.5	15.5
Fish meal	5	5	5	5
Palm oil	2.5	2.5	2.5	2.5
Bone meal	3	3	3	3
Methionine	0.06	0.06	0.06	0.06
Lysine	0.02	0.02	0.02	0.02
Vitamin Premix	0.25	0.25	0.25	0.25
Salt	0.67	0.67	0.67	0.67
Total	100	100	100	100

RESULTS AND DISCUSSION

The result from the present study showed the influence of Lactobacillus on the hematological parameters of broilers chicken. The results showed that packed cell volume (PVC) concentration were similar between T1 (26.50 ± 1.73), T2 (26.50 ± 1.12) and T4 (26.33 ± 0.92) the values were significantly ($P<0.05$) higher than T3 (24.67 ± 1.41). Haemoglobin was significantly higher in T2 (8.85 ± 0.37) and lowest in T3 (8.22 ± 0.47). Red blood cell concentration were similar between T2 (4.33 ± 0.17) and T4 (4.33 ± 0.13) and the values were significantly ($P<0.05$) higher than T3 (4.13 ± 0.26) and T1 (4.27 ± 0.24). White blood cell (WBC) was highest in T3 (4.55 ± 1.21) and significantly lowest ($P<0.05$) in T2 (1.00 ± 0.89). Platelet was highest in T2 (184.33 ± 9.39) and significantly lowest ($P<0.05$) in T3 (159.50 ± 10.34). Neutrophil was highest in T4 (49.17 ± 3.09) and significantly lowest ($P<0.05$) in T2 (41.83 ± 3.52). Lymphocytes was highest in T1 (54.83 ± 2.50) and significantly lowest ($P<0.05$) in T4 (41.00 ± 3.03). Monocytes was highest in T1 (6.83 ± 0.48) and significantly lowest ($P<0.05$) in T2 (5.67 ± 0.67). Eosinophil was highest in T4 (3.50 ± 0.43) and significantly lowest ($P<0.05$) in T2 (2.83 ± 0.48). Blood indices are used to assess the state of the physiological, pathological and nutritional status of broilers. The indices are compared to normal values in order to interpret the metabolic state of an animal. The values obtained for some haematological parameters were within the normal ranges of 24-35% (PCV), 2.5–4.84 x 10^{12}/L (RBC), 7–13 g/dl (Haemoglobin), 1.0 - 9.5×10^9 u/L (WBC) in chickens as reported by several authors (Mitruka and Rawnsley, 1977; Wakenel, 2010; Campbell, 2013; Banerjee, 2014; Onabanjo *et al.*, 2018; Lakurbe *et al.*, 2018). The values for monocyte fall within the normal range of 3%–10% (Mangaonkar *et al.*, 2021). The platelet and neutrophil values were within the normal values reported by Auta et al. (2024) and Ekine et al. (2019) respectively. The findings in the total lymphocyte count in broiler chickens agree with those of Kamruzzaman et al. (2005) and Owosibo et al. (2013). The range of values obtained for eosinophil falls within the normal range of 1-4% reported by Mitruka and Rawnsley (1977). Since, the values of the haematological parameters in this study are normal, it

shows that the animals are physiologically healthy and there is no negative effect of the action of probiotics. The value of lymphocytes count falls within 31-72% recommended by Scholtz et al. (2009).

Table 3. Influence of *Lactobacillus* on hematology of broiler chicken

PARAMETERS	T_1	T_2	T_3	T_4
PVC (%)	26.50 ± 1.73^{a}	26.50 ± 1.12^{a}	24.67 ± 1.41^{b}	26.33 ± 0.92^{a}
HB (g/dl)	8.83 ± 0.58^{ab}	8.85 ± 0.37^{a}	8.22 ± 0.47^{bc}	8.83 ± 0.29^{ab}
RBC ($x10^{12}$/L)	4.27 ± 0.24^{b}	4.33 ± 0.17^{a}	4.13 ± 0.26^{c}	4.33 ± 0.13^{a}
WBC ($x10^{9}$)	1.33 ± 0.57^{c}	1.00 ± 0.89^{d}	4.55 ± 1.21^{a}	1.81 ± 1.50^{b}
Platelet ($x10^{9}$/L)	170.67 ± 11.50^{c}	184.33 ± 9.39^{a}	159.50 ± 10.34^{d}	175.50 ± 7.90^{b}
Neutrophil (%)	44.00 ± 2.31^{c}	41.83 ± 3.52^{d}	45.83 ± 1.70^{b}	49.17 ± 3.09^{a}
Lymphocytes (%)	54.83 ± 2.50^{a}	49.67 ± 3.52^{b}	45.00 ± 1.97^{c}	41.00 ± 3.03^{d}
Eosinophil (%)	3.33 ± 0.42^{b}	2.83 ± 0.48^{d}	3.17 ± 0.31^{c}	3.50 ± 0.43^{a}
Monocyte (%)	6.83 ± 0.48^{a}	5.67 ± 0.67^{d}	6.00 ± 0.52^{c}	6.33 ± 0.84^{b}

^{a,b,c,d} Means showing distinct values across rows that were significantly($P<0.05$) different among the treatments.PVC-Packed Cell Volume, HB-Hemoglobin, RBC-Red Blood Cell, WBC-White Blood Cell

Result from the study shows the influence of *Lactobacillus* on serum biochemistry of broilers chicken in Table 4. Results showed that total cholesterol concentration were similar between T1 (3.17 ± 1.11), T3 (3.17 ± 1.25) and T4 (3.17 ± 1.17) and the values were significantly ($P<0.05$) lower than and T2 (3.38 ± 1.14). High density lipoprotein was highest in T2 (1.80 ± 0.37) and significantly lowest ($P<0.05$) in T1 (1.47 ± 0.16) and T4 (1.47 ± 0.13). Triglycerides (TG) was highest in T4 (1.80 ± 0.05) and significantly lowest ($P<0.05$) in T2 (1.24 ± 0.89). Low density lipoprotein (LDL) was highest in T4 (2.60 ± 0.04) and significantly lowest ($P<0.05$) in T2 (2.03 ± 0.26). Very low density lipoprotein (VLDL) was highest in T4 (0.82 ± 0.02) and significantly lowest ($P<0.05$) in T2 (0.56 ± 0.41). The values of total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein are in tandem with the findings of Panda et al. (2006) in broilers fed diets supplemented with a probiotic containing *Lactobacillus sporogenes*, which may be associated with utilization of cholesterol by the probiotic bacteria for their own metabolism.

Table 4. Influence of *Lactobacillus* on serum biochemistry of broiler chicken

PARAMETERS	T1	T2	T3	T4
TC (mmol/L)	3.17 ± 1.11^{b}	3.38 ± 1.14^{a}	3.17 ± 1.25^{b}	3.17 ± 1.17^{b}
TG (g/l)	1.63 ± 0.69^{b}	1.24 ± 0.89^{d}	1.41 ± 0.15^{c}	1.80 ± 0.05^{a}
HDL (mmol/L)	1.47 ± 0.16^{c}	1.80 ± 0.37^{a}	1.78 ± 0.88^{b}	1.47 ± 0.13^{c}
LDL (mmol/L)	2.44 ± 0.10^{b}	2.14 ± 0.17^{c}	2.03 ± 0.26^{d}	2.60 ± 0.04^{a}
VLDL (mmol/L)	0.74 ± 0.03^{b}	0.56 ± 0.41^{d}	0.64 ± 69^{c}	0.82 ± 0.02^{a}

^{a,b,c,d} Means showing distinct values across rows that were significantly($P<0.05$) different among the treatment, TC-Total Cholesterol, TG-Triglycerides, HDL-High density lipoprotein, LDL-Low density lipoprotein, VLDL-Very low density lipoprotein

The result of microbial load from the trachea and the colon of broiler chicken fed *Lactobacillus* are shown in table 5. The result revealed that total heterotrophic bacteria (THB) was highest in T4 (113.33±24.50) and significantly lowest (P<0.05) in T3 (75.33±11.38). Total coli form count (TCC) was highest in T4 (77.59±25.91) and significantly lowest (P<0.05) in T2 (49.17±15.06). Microbial changes in the cecal environment of chickens are studied because the microbial system in the cecum is the primary culprit for food fermentation and produces some useful substances for birds such as organic acids (Elbaz and El-sheikh, 2020). Dibner and Richards, (2005) reported that there is a strong interaction between probiotics and the intestinal micro flora. The utilization of probiotics in this study altered the microbiota in terms of the numbers of total heterotrophic bacteria and total coli form count and thus likely controlled gut microorganisms (Sherief *et al.*, 2012; Chen and Yu, 2020). The results of the present study and those of other studies on the effect of probiotics on enteric microbiota reveals the microorganismal diversity and indicate that changes in the enterobacterial population depend on multiple factors such as the handling of litter, feed offered, type and composition of the probiotics, method of enterobacterial determination, and intestinal location for the enteric microbiota count. He et al. (2019) and Ramlucken et al. (2020) reported that the probiotic composition is important because certain bacteria are aerobic and provide a suitable environment for lactobacilli under anaerobic conditions, which subsequently produce acids and thus might prevent the growth of pathogenic microorganisms. These results are in harmony with those of previous researchers Panda *et al.* (2006) and Song (2014) who reported that probiotic mixture containing *Bacillus licheniformis* and *Bacillus subtilis* decreased viable counts of Coliforms and Clostridium.

Table 5. Influence of *Lactobacillus* on microbial load of broiler chicken

PARAMETERS	T1	T2	T3	T4
THB	95.50 ± 24.00^{b}	83.67 ± 22.08^{c}	75.33 ± 11.38^{d}	113.33 ± 24.50^{a}
TCC	62.67 ± 13.61^{b}	49.17 ± 15.06^{c}	48.83 ± 9.09^{d}	77.59 ± 25.91^{a}

^{a,b,c,d} Means showing distinct values across rows that were significantly(P<0.05) different among the treatment THB: Total Heterotrophic bacteria; TCC: Total coli form count

CONCLUSION

The microbial load, haematological and serum biochemical indices obtained in this study revealed that the use of probiotics (*Lactobacillus*) in broiler production is an effective strategy with beneficial impacts and without adverse effect on the health and intestinal microbial balance of the broiler chickens. Supplementing the diet of broiler chickens with probiotic (*Lactobacillus*) led to a decreased pathogenic population of total coli form counts especially in T3 and T2 respectively compared to other experimental groups. T3 and T2 could be adopted by poultry farmers.

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