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Full Length Research Article

THE EFFECT OF PROBIOTIC CONTAINING *LACTOBACILLUS FERMENTUM*, *LACTOBACILLUS PLANTENRUN* AND *WEISSALLA CIBERIA* ON SOME BLOOD PARAMETERS OF BROILER CHICKEN

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ABSTRACT
The research was aimed at studying the effect of *Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia* on lipid profiles, blood glucose concentration, total protein and total immunoglobulin of broiler chicken. This is targeted at finding an alternative to antibiotics in broiler production. The study was carried out at the Department of microbiology, faculty of sciences Kaduna State University, Kaduna between January to April 2018. A total of twenty day-old broiler chicks were administered probiotics (*Lactobacillus fermentum, Lactobacillus plantenrun and Weissalla ciberia*) in water at 10^8 cells/milliliters/isolates/birds/day for six weeks. Blood glucose concentration, total protein, lipid profiles and Total immunoglobulins (IgY) were determined from blood sample at the ages of 14 and 42 days. The results shows that there was no significance difference between the mean of the treatment on blood glucose concentration, total protein and other parameters of lipid profiles except for triglycerides that a significant difference was observed P=0.0009. Significant difference was also observed between mean of the treatment on IgY at day 14 and 42 P=0.0001. The probiotic lactic acid bacteria have showed an increased level of IgY in broiler chickens which clearly indicates the superiority of probiotics over antibiotics to immune responses.

Keywords: Probiotics, total, immunoglobulin, proteins, lipid, profiles.

INTRODUCTION

Lactic acid bacteria are generally, group of Gram-positive bacteria preferring anaerobic conditions, fastidious, acid-tolerant and strictly fermentative (Gatesoupe and Lesel, 1998). They are catalase, oxidase, indole, methyl red, voges-proskauer and citrate negative (Dhanasekaran *et al*., 2010). Lactic acid bacteria provide numerous benefits to mankind by producing metabolites that inhibit the growth of pathogenic and nonpathogenic microorganisms (Fernandez *et al.,* 2011). Among different genera of LAB; Lactobacilli produce various organic acids like lactic acid, acetic acid and propionic acid exhibiting anti-microbial activity (Arokiyamary and Sivakumar 2011; Oluwafemi and Adetunji 2011).

Probiotics are Preparations of viable microorganisms that is consumed by humans or other animals with the aim of inducing beneficial effects by qualitatively or quantitatively influencing their gut microflora and/or modifying their immune status (Fuller, 2004). In addition, citing Food and Agriculture Organization and World Health Organization (FAO/WHO 2017), look at probiotics as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host.

Antibiotic resistance has been the thing of concern for scientist and poultry farmers alike. The needs for supplement that can effectively take the place of antibiotics in broiler production have become very imperative. A variety of different supplements, as the alternatives to antimicrobial growth promoters, have been explored to maintain growth performance of broilers (Ghadban, 2002; Biggs and Parsons, 2008; Chowdhury *et al.*, 2009). The consumption of oral probiotics acts to modify the intestinal microflora balance in a beneficial "rebalancing" manner, and thus helps the digestive health of the consumer. Probiotics are also known for their established benefits in improving gut disorders, such as ulcerative colitis, Crohn's disease and irritable bowel syndrome. They are also beneficial in other conditions, such as heart disease, autism and allergies (Savini *et al.*, 2010). The probiotic properties of lactic acid bacteria have been widely studied, demonstrating that their capability of adhering to mucus and epithelial cells is one of the potential mechanisms of providing a competitive advantage in the intestinal microbiota (Ghadban, 2002) and consequently inhibiting the in vitro growth of Salmonella enteritidis. Gao *et al.*, (2008) and Higgins *et al.*, (2008) reported that intestinal immunity was increased in chickens fed diets supplemented with yeast product and *Lactobacillus*-based probiotic culture, respectively.

Previous study on probiotics products incorporating *Lactobacillus fermentum* and *Saccharomyces cerevisiae* indicated that they improved the intestinal balance of the diverse microflora species in the rectum of broiler chickens (Lei *et al*., 2009).

Some natural microorganisms in human intestine are beneficial in terms of lowering serum cholesterol (Fukushima *et al*., 1999). The lactic acid bacteria (LAB), Lactobacillus and Bifidobacterium spp. in particular, have the ability to metabolize cholesterol. Many LAB could adjust blood lipid and lower cholesterol, which can also prevent some of the diseases by stimulating antioxidant enzymes (Jain *et al.,* 2009, Koiche and Dilmi 2010). These strains have the ability to tolerate both acid and bile concentrations typically found in the upper gastrointestinal tract of humans.

This research was aimed at studying the effect of *Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia* on lipid profiles, blood glucose concentration, total protein and total immunoglobulin of broiler chicken.

MATERIALS AND METHODS

Standardization of Pure Isolates of LAB

The Pure isolate of *Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia* was obtained from microbial

bank of the department of Microbiology, Kaduna State University with accession numbers: NC010610.1, MF428738.1 and N2CP012873.1 respectively. The standardization was done using 0.5 McFarland turbidity standards adopted by Ebu *et al* (2018). One milliliters (ml) of concentrated H_2SO_4 was added to 99 of distilled water in a conical flask and mix well. A 1.0 % v/v solution of H_2SO_4 is prepared. Then 0.5 g of dihydrate barium Chloride salt ($BaCl_2$. $2H_2O$) was dissolved in 50 ml of distilled water. In this way, a 1 % w/v of $BaCl_2$ was prepared. This is followed by adding 0.6 ml of $BaCl_2$ solution to 99.4 ml of H_2SO_4 solution to make up to 100 ml. The solution was then mixed well. This is the stock solution of the 0.5 McFarland turbidity standards. Exactly 2ml of the solution was transferred into capped tubes and store at room temperature until ready for use.

Experimental Design

A total of 60, one-day old broiler chicken was used in this research work. Out of which 20 was fed with probiotic LAB, 20 were administered with antibiotics and 20 were used as control without antibiotic or probiotic. The standardized lactic acid bacteria (10^8cells/milliliters/isolates/birds/day) was administered in 200ml of drinking water at day 6, 7, 8, 21, 22, and 23 (Brzoska, *et al* 2012). The birds were administered vaccine against Gumboro virus at week 1 and 3. Then Lasota vaccine (newscastle disease) was administered to the birds at week 2 and 4. Hybrid feed (Nigeria) was used to feed the birds which were provided in marsh form in two phases (starter phase 0 to 3 weeks and finisher phase 4 to 6 weeks). Ethical approval was obtained from Kaduna State Ministry of Agriculture, Kaduna.

Evaluation of Blood Glucose Concentration

At the ages of 21 days and 42 days, two broilers per treatment were randomly selected and blood samples were collected from the wing vein of the birds in heparinized tubes (Mountzouris *et al*., 2010). Fresh plasma sample obtained from the blood collected from the wing vein of the broilers was used to analyze blood glucose concentration using SD Codefree glucometer (Republic of Korea). The test strip was removed from the container and inserted into the test strip slot on the glucometer until the machine turn on automatically. A drop of fresh blood was then put on the edge of the strip until the yellow window on the strip is completely filled with the blood. The display now counts down from 5 to 1 seconds and the result appears on the screen in just five seconds and was recorded. The strip was then removed and discarded.

Determination of Total Protein

At the age of 14 and 42 days, two broilers per treatment were randomly selected and blood samples were collected from the wing vein of the birds in heparinized tubes. Blood samples were subsequently stored in ice, centrifuged at 2,500 × *g* for 10 min at 4°C, and the plasma was stored at −80°C (Mountzouris *et al.,* 2010) until Total protein was analysed. The spectrophotometric method as described by Zaia, *et al.* (2005) was used. A 50 µl aliquot of blood plasma was transferred to a test tube and the volume was made up to 2.0 mL with distilled water. A 50 µl aliquot of this solution was transferred to a second test tube and the volume was adjusted to 2.0 ml with 0.2mol L^{-1} of acetic acid. Standard curve was prepared by taking 0.0, 20.0, 40.0, 60.0, 80.0, 100.0 and 120.0 µl of standard solution of BSA (1.5 g L^{-1}), a calibration curve with the concentrations from 0.0 to 84.0 µg mL^{-1} was obtained. The volumes were adjusted to 2.0 ml with acetic acid (0.2 mol L^{-1}). Then 100 µl of TBPEE (0.005% m/v) was added, shaken and incubated at 37ºC for 10 minutes. The tubes were then cooled to room temperature and, after 30 minutes the absorbance was measured using Jenway 6305 spectrophotometer (Bibby Scientific Ltd, United Kingdom) at 610 nm against the blank (0.0 µg mL^{-1}).

Evaluation of Total Protein and Lipid Profiles of the Broiler Chickens

At the age of 14 and 42 d, 2 broilers per treatment were randomly selected and blood samples were collected from the wing vein of the birds in heparinized tubes. Blood samples were subsequently stored in ice, centrifuged at 2,500 × *g* for 10 min at 4°C, and the plasma was stored at −80°C (Mountzouris *et al* 2010) until Triglycerides (T), cholesterol and high density lipoprotein (HDL) were analysed Triglycerides, cholesterol and high density lipoprotein were determined using kits (Sigma, USA). The absorbance of the sample and of the standard were measured against Reagent Blank using Jenway 6305 spectrophotometer (Bibby Scientific Ltd, United Kingdom) at 546nm wavelength. The low density lipoprotein (LDL) was determined using the formula; LDL-c = 3/4 (TC - HDL-c) as describe by De Cordova and Mauricio (2013).

Determination of Total Immunoglobulin (IgY) of the Broiler Chicken

At the age of 14 and 42 d, 2 broilers per treatment were randomly selected and blood samples were collected from the wing vein of the birds in heparinized tubes. Blood samples were subsequently stored in ice, centrifuged at 2,500 × *g* for 10 min at 4°C, and the plasma was stored at −80°C until antibody analyses (Mountzouris *et al* 2010). The IgY concentrations were determined in appropriately diluted samples by sandwich ELISA using microtiter plates and chicken-specific IgY ELISA quantitation kits (Immunology Consultant Labolatory Inc USA). The ELISA procedure was carried out according to the protocol of the manufacturer and absorbance was measured at 450 nm. The concentrations of IgY was determined using standard curves constructed from IgY standards run on the assay microtiter plate and were expressed as milligrams of IgY per milliliter of plasma.

Data Analysis

The data were analyzed using one way analysis of variance with the aid of graph pad prism (USA) version 6. Statistically significant effects were further analyzed and means were compared using Duncan's multiple range test. Statistical significance was determined at $P \leq 0.05$.

RESULTS

Table 1: Effects of Lactic Acid Bacteria on Blood Glucose Concentration and Total Protein

	Experimental Treatment			
Tests	**A**	**B**	**C**	**P VALUE**
3rd Week				
Total Protein	26.90	32.30	29.8	0.5704
Blood Glucose	316	309	305	0.6707
6th Week				
Total Protein	35.20	37.60	36.55	0.7591
Blood Glucose	269	266	342	0.5766

Results are mean of duplicate value

Key:
A= Probiotics Group
B= Antibiotics group
C= Control group
*Significant value, * P < 0.05*

Table 2: Effects of Lactic Acid Bacteria on Lipid Profiles

Experimental Design				
Tests	**A**	**B**	**C**	**P VALUE**
3rd Week				
Total Cholesterol	2.91	3.20	3.32	0.2519
HDL	1.93	2.07	2.19	0.2171
LDL	0.65	0.63	0.57	0.3617
TRIG	0.91	0.94	1.10	0.0009
6th Week				
Total Cholesterol	3.05	3.31	2.89	0.6578
HDL	1.96	2.1	1.89	0.8176
LDL	0.62	0.71	0.61	0.6794
TRIG	1.20	0.78	0.49	0.6198

Results are mean of duplicate value

Key:
A= Probiotics Group
B= Antibiotics group
C= Control group
*Significant value, * P < 0.05*

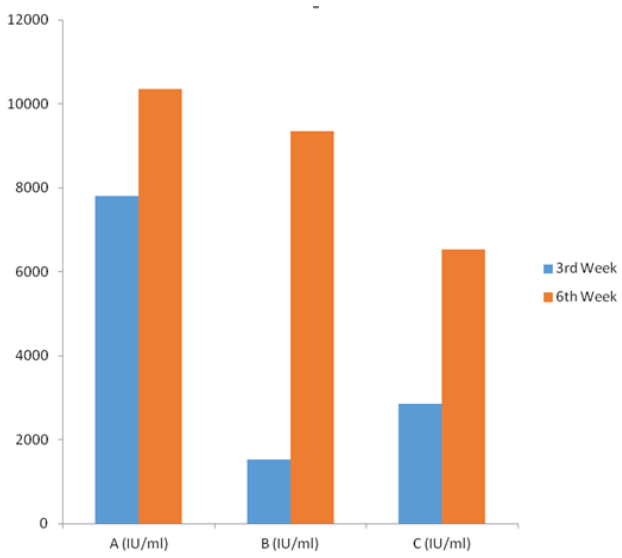

Figure I: Effect of Lactic Acid Bacteria on IgY of Chicken

Key:
A= Probiotics Group
B= Antibiotics group
C= Control group

DISCUSSION

The effect of the experimental treatment on blood glucose concentration and total protein is shown in Table I. The results indicates that there was no significant difference (*P>0.05*) between the means of treatments. This finding is similar to the earlier works of Brozka *et al.* (2012) who also reported no significant differences in plasma levels of glucose, total protein and total cholesterol in different groups of chickens that was administered *Lactococcus lactis, Lactobacillus delbruecki* and *Lactobacillus plantarun*. In a separate study, Owosibo *et al.* (2013) further confirmed that, there was no significant difference (*P>0.05*) in all the serum biochemical indices examined except for the cholesterol which was influenced by the dietary treatment (probiotics).

The effects of experimental treatment on lipid profiles are shown in table II. The result indicates that there was no significant difference between the mean of the treatment of all the parameters of lipid profiles at both the starter and finisher stage except for triglyceride at starter stage P=0.0009. The lactic acid bacteria group recorded the lowest level of triglyceride. In agreement with this finding, Yalcinkaya *et al*. (2008) reported that the use of MOS in broilers diet could not significantly reduce the serum cholesterol and triglycerides levels as compared with the control group. In contrast, the authors (Panda *et al*., 2001; Kalavathy *et al*., 2003; Ashayerizadeh *et al.* 2011) reported that the probiotic supplementation significantly reduces the serum cholesterol level of the chickens. Kannan *et al.* (2005) have reported that the use of 0.5 g kg-1 mannanoligosaccharide obtained from yeast in the ration of broiler chickens, significantly reduced the serum cholesterol level on day 35 as compared with the control. Even though from this research, probiotics group recorded the lowest means of cholesterol, HDL and Triglycerides. The use of probiotics can disintegrate bile salts and de-conjugate production of enzymes by the activity of lactic acid bacteria, as well as reduction of the pH in the intestinal tract can be effective in reducing the cholesterol concentration. Solvability of non-conjugate bile acids is lowered at a low pH and consequently, they are absorbed less from the intestine and are excreted more in the feaces (Ashayerizadeh *et al*., 2011).

The effect of probiotic LAB on IgY of chicken is shown in figure I. This shows the evident effect of probiotic LAB on IgY, as it clearly indicates that it improves the IgY concentration of the broiler chicken. The concentration of IgY increases as the experiment moves from starter to finisher stage. Generally immunoglobulin is used to evaluate the immune status of animals due to their important roles in immune function Bostami *et al* (2015). Dietary supplementation of lactic acid bacteria showed increase trend of serum total immunoglobulin in broilers in this study which is in contrast with the work of Mountzouris *et al*. (2010) who reported that the concentration of IgA, IgM, IgG and Total Ig (IgY) increase with broiler age but there was no significant differences between the treatment. However, it agrees with the work of the authors (Huang *et al* 2004; Koenen *et al.* 2004; Kabir *et al* 2004) who reported that probiotics administration resulted in enhancement of broiler humoral immune responses. Salim *et al*. (2013) reported that Multi-microbe probiotic resulted in enhanced broiler humoral response and due to supplementation with probiotics may lead to modulation of mucosal and systemic immune activity (Fedorak and Madsen, 2004).

Conclusion

The use of probiotic containing *Lactobacillus fermentum*, *Lactobacillus plantenrun* and *Weissalla ciberia* shows that there was no significance difference between the mean of the treatment on blood glucose concentration, total protein and other parameters of lipid profiles except for triglycerides that a significant difference was observed (P=0.0009). Significant difference was also observed on IgY at day 14 and 42 (P=0.0001). The probiotic lactic acid bacteria have showed an increased level of IgY in broiler chickens fed with probiotic combination which clearly indicates the superiority of probiotics over antibiotics to immune responses.

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