Haematological and serum indices of broiler chicken fed graded dietary levels of immunnowall® prebiotic additive

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Abstract
The Haematology and serum indices of ninety-six (96) day-old Agric desk (ROSS 308 strain) broiler birds fed diets containing immunowall® prebiotic additive were evaluated. The birds were randomly allotted to four (4) treatments of graded dietary levels (0, 1.5, 3.0 and 4.5) g of immunowall® prebiotic additive denoted as; T_1, T_2, T_3, andT_4, respectively. Each treatment with 24 birds, reared on deep litter system was also replicated three times with 8 birds per replicate in a completely randomized design for 56 days. Haematological parameters evaluated include; Packed cell volume (%), Haemoglobin (g/dl), Red blood cell ($x10^6/mm^3$), White blood cell ($x10^3/mm^3$), Neutrophils (%), Lymphophiles(%), Eosinophils (%), Monocytes, Basophils (%), Mean Corpuscular Volume (MCV) (fl), Means Corpuscular Haemoglobin, (MCH) (pg) , Means Corpuscular Haemoglobin Concentration (MCHC) (g/dl). Serum parameters evaluated were Total Protein (g/dl), Albumin (g/dl), Globulin (g/dl), Cholesterol (mg/dl) and Alkaline Phosphatase (μ/l). Results showed that immunowall® prebiotic additive significantly ($P<0.05$) improved the haematological and serum parameters within the normal range values with exception of eosinophils for T_2 (4.33%), T_3 (3.67%) and T_4 (5.33%). This is an indication that broiler chickens fed diets containing 1.50 - 3.00 g of immunowall® prebiotic additive enhanced good physiological state of broiler chickens. Further research is suggested in other avian species available in warm wet regions of Nigeria.

Keywords: Immonowall® prebiotic, broiler birds, haematology, serum indices

Introduction

Through genetic improvements, the productivity of broilers has improved significantly (Claudia, 2008, Al-khalaifa *et al.*, 2019). While this is a good development for poultry industry, increased rearing density has concentrated and increased diseased challenges making birds more susceptible to various pathogens especially enteropathic microbes such as *Escherichia coli*, *Salmonella spp*, *Clostridium perfringens* and *Campylobacter spp.* (Alonge *et al.*, 2017; Tavemello *et al.*, 2018). This increased susceptibility has resulted in the use of antimicrobial growth promoters which are primarily used to enhanced gut health and control sub- clinical challenges (Alonge *et al.*, 2017, Tavemello *et al.*, 2018). The use of antimicrobial growth promoters such as prebiotics have been examined and can potentially reduce enteric diseases in poultry and enhance their productivity (Bindels *et al.*, 2015, Ducatelle *et al.*, 2015). One of such commercially available growth promoters is immunowall® commonly refers to as hardcore yeast cell wall (Ekachai *et al.*, 2019, Melina *et*

al., 2020). Immunowall® is composed of *Saccharomyces cerevisiae* cell wall and contains a high concentration of *β - glucans*(>35%) Mannan-oligosaccharides, Mos (~20%) (Ekachai *et al*., 2019, Melina *et al.,* 2020; JA FARMS, 2021).

Due to the processing conditions to which cells are subjected, the wall become denser compared to other yeast cell walls in the market. This yeast cell wall is resistant to degradation by digestive tract enzymes and bacteria, and its resistance to digestion in the gastrointestinal tract and to fermentation in the large intestine is the main criteria for its use as prebiotics (Osfar *et al.,* 2019, Daniel, 2019, Ekachai *et al.,* 2019). Prebiotics are considered excellent contributors to animal health because they stimulate the immune system and contribute to intestinal mucosa integrity, prevent adhesion of enteropathogenic microorganisms, and have the ability to bind and inactivate mycotoxins in the intestinal lumen (Ajuwon, 2015; Lee, 2016; Alonge *et al.,* 2017).

Mannan-oligosaccharides (MOS) are known for its ability to agglutinate pathogens (Ekachai *et al.,* 2019, Melina *et al.,* 2020). By providing a binding site for harmful bacteria present in the intestinal tract that have type 1 and 4 fimbriae, MOS prevents colonization of pathogens in the intestine. Since *β-glucans* are not digesting, "trapped" bacteria are excreted with fecal material (Ekachai *et al.,* 2019, Melina *et al.,* 2020).

More importantly, to achieve full functionality, yeast cell wall must have low digestibility in the intestine. *β -glucans* constitute the indigestible portion of the yeast cell wall, so that the higher its concentration, the lower yeast cell wall digestibility (Alonge *et al*., 2017). Therefore, this current study was initiated to investigate the effect of immunowall® prebiotic additive on haematology and serum indices of broilers reared in warm wet climate.

Materials and Methods

Experimental site, birds and their management

This study was conducted at the Poultry Unit, Teaching and Research Farm of the Department of Animal Science, Akwa Ibom State University, Obio Akpa campus, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria. Obio Akpa is located on Latitude $5^{o}17^{1}$ and$5^{o}27^{1}$ North of the equator and the Longitude$7^{o}21^{1}$ and$7^{o}58^{1}$ East with the temperature ranging between 24-25^{o}c, average annual rainfall range of 3500mm-5000mm and relative humidity of between 60-90% (Udo *et al*., 2020)

Ninety-six (96) day-old broiler chicks, *ROSS 380* strain were reared on deep litter system. All vaccines and drugs were administered as at when due. The formulated broiler straight or single phase diet mash and water were provided *ad libitum*.The experiment lasted for 56 days.

Experimental Design

96 day-old broiler birds were randomly allotted to four (4) dietary treatments of 24 birds. Each treatment was replicated three (3) times with eight (8) birds per replicate in a completely randomized design (CRD).

Feed Additive

Immunowall® (Hardcore yeast cell wall) prebiotic additive was obtained from ICC Industrial ComercioExportacaoeImportacao Ltd, Sao Paulo SP, Brazil. Immunowall® is a prebiotic additive that is rich in β-glucans and mannanoligosaccharides (MOS) derived from the Saccharomyces cerevisiae yeast cell wall, made in Brazil. The features of the prebiotic include: light brown in colour, 1000 mesh particle size and pleasant in odour. The prebiotic was supplemented at 0.00g (T_1), 1.50g (T_2), 3.00g ($T_{3)}$ and 4.50g ($T_{4)}$ per 100kg of poultry feed, respectively.

Blood Collection and Carcass Analysis

The blood samples were collected from three birds randomly selected from each treatment on the last day of the experiment and used for haematological and serum biochemical analyses. 2.5mls blood samples taken from the veins of the selected birds were emptied into samples bottles containing an anticoagulant EDTA (Ethylenediamine-tetra acetic acid), to determine haematological indices as reported by Mitruka and Rawnsley, 1997. Blood samples for serum biochemical analysis was collected and emptied into samples bottles without anticoagulant. Total protein, albumin, globulin, cholesterol and alkaline phosphatase were determined according to the method according to the method described by Reinhold (1953), Duomas and Briggs (1977); Schaefar*et al.*, (1990) and Basdson (1965). Globulin levels were determined by subtraction of albumin values from total protein values.

Statistical Analysis

Data obtained were subjected to one way analysis of variance (ANOVA) according to Steel and Torrie (1990) and the means were separated using Duncan Multiple Range Test (Duncan, 1995).

Results and discussion

The haematological parameters of broiler chickens fed diets containing different levels of immunowall® prebiotic additives is as shown in Table 2. There were significant ($P < 0.05$) differences between packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), lymphophils and eosinophils observed among the treatments. The haematological values obtained for broiler birds fed immunowall® prebiotic additives in $T_{2,}$ T_3 and T_4 recorded a significantly lower ($P<0.05$) values for PCV (26.00%, 27.33% and 29.33%), Hb (7.63g/dl, 8.00g/dl and 8.60g/dl), RBC ($2.90x10^6/mm^3$, $3.04\ x10^6/mm^3$ and $3.25\ x10^6/mm^3$), WBC ($20.52\ x10^3/mm^3$, and $22.31\ x10^3/mm^3$) and lymphophils (50.00% and

50.33%) than broiler birds fed control diet with PCV (30.33%), Hb (8.90g/dl), RBC ($3.36x10^6/mm^3$), WBC ($25.50\ x10^3/mm^3$) and lymphophils (51.00%).

However, values obtained for the eosinophils indicated that broiler birds fed different levels of fed immunowall® prebiotic additive in T_2 and T_4 had a significantly higher ($P < 0.05$) values (4.33%, 3.67% and 5.33%) than broiler birds fed control diets (3.00%). The haematological parameters obtained from the study were within the normal range of broiler chickens (Red blood cell = 2.0 – 4.0 $x10^6$/mm, packed cell volume = 25.0 – 45.0%, haemoglobin = 7.0 – 13.0g/dl, white blood cells = 9 – 13.0 $x10^3$/mm, neutrophils = 10 – 40%, lymphophils = 45 – 80.0%, basophils = 1 – 3.0%, eosinophils = 1 – 3.0%, monocytes = 0 – 5.0%, means corpuscular haemoglobin = 33 – 47%, means corpuscular haemoglobin concentration = 26 – 35% and means corpuscular volume = 90 – 140%) as reported by Mitruka and Rawnsley (1977); Howlett (2000) and Banarjee (2007) except for eosinophils values which were above the normal range for T_2, T_3 and T_4, respectively. This positive immune status response may be due to the immunomodulatory effect of fed immunowall® prebiotic additive (Mohamed *et al.,* 2013; Ekachai *et al.,* 2019; Melina *et al.,* 2020). Beta- glucans which are the constituent portion of the yeast cell wall, and are considered immunomodulators that improve immune response effectiveness and agility in animals as reported by Ekachai *et al.* (2019) and Melina *et al.* (2020). These polysaccharides are natural and effective stimulants of the innate immune system. When phagocytic cells are in contact with beta-glucans, these cells are stimulated, and cytokines are produced. The production of cytokines triggers a chain reaction, inducing a higher immune status in animals, making them better able to resist opportunistic infections. One of these immune system reactions is the increased number of goblets cells responsible for mucus production. With increased production and release in the intestinal lumen, the mucosa (villus protection barrier and the medium that allows the action of various enzymes) increases, providing greater protection to intestinal cells and the villi. The normal ranges for PCV, Hb, RBC, WBC lymphophils and eosinophils in broiler chickens had been put at 25-45%, 7-13g/dl, 2- 4x106/mm^3, 9-31x103/mm^3, 45-80% and 1-3% respectively (Mitruka and Rawnsley, 1977; Howlett, 2000 and Banarjee, 2007). The abnormally observed for eosinophils for treatments T_2, T_3 and T_4, respectively could probably to bacterial infection arising from unfavourable restricted environmental conditions. This parameter is important effector cells in allergy and host defence response particularly against parasitic infections (Ibu, 2005; Owai *et al.,* 2010). Increase in eosinophil counts occurs in conditions of acute parasitic infections and

allergic conditions (Ibu, 2005). Eosinophils are neither markedly motile nor phagocytic like the neutrophils but their granules contain many substances such as eosinophil peroxidase enzymes that is capable of destroying helminthes, bacteria and tumor cells.

The Serum biochemistry of broiler birds fed different levels of immunowall® prebiotic additive is as shown in Table 3. There were significant ($P < 0.05$) differences observed for total protein and cholesterol values only. According to Ibu, (2005) total protein measurement indicates the total amount of protein in the blood. Its values obtained in the study revealed that T_3 (Basal diet + 3.00g immunowall®) had an increased value (3.79g/dl) that was significantly ($P < 0.05$) higher than other treatments. From the result obtained, all the birds fed different levels of immunowall® prebiotic additive fell within the normal range (2.5 - 5.5g/dl) reported by Mitruka and Rawnsley (1977); Howlett (2000); Thrall (2007) and Monika *et al.,* (2012). Cholesterol plays vital roles in many animals' body processes, including building cellular membranes, making hormones, metabolizing vitamin D in the skin and producing bile acids to digest fatty feeds (Jenifer, 2018). The cholesterol values obtained the in present study for T_1 (117.90mg/dl), T_2 (115.30mg/dl), T_3 (118.30mg/dl) and T_4 (118.20mg/dl), respectively fell within the normal physiological range (82 - 211mg/dl) reported by Aiello and Mays (1999).

Conclusions and Recommendation

Feeding broiler chicken with diets that contain 1.50g and 3.00g immunowall® prebiotic additive induced better haematological and serum parameters which has promoted the positive effects of immunowall® prebiotic additive in poultry nutrition. Besides, given the promising status of immunowall® prebiotic additive as a non-toxic and natural feed additive, it is suggested that further research be undertaken on other avain species available in the warm wet region as well as devising efficient storage methods and conditions for making immunowall® prebiotic additive available all year round for its use in research.

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Table 1. Ingredient composition (%) of experimental diet

Ingredients	**Percentage Composition**
Maize	50.00
Soybean meal	21.00
Groundnut cake	8.00
Fish meal	4.00
Wheat offal	8.30
Palm kernel meal	3.00
Bone meal	5.00
Salt	0.25
Vitamin premix	0.25
Lysine	0.10
Methionine	0.10
	100.00

* Vitamins premix per kg of diet: Vitamin A, D3, E, K, B1, B2, B6, B12, Naicin, Pathotheric Acid, Folic Acid, Biolin, Choline, Chloride, Manganese, Zinc, Iron, Copper, Iodine, Selenium, Cobalt and Antioxidants

Table 2: Haematological Parameters of broiler birds fed diets with different levels of immunowall® prebiotic additive

Parameters	T_1 (0g)	T_2(1.5)	T_3(3.0g)	T_4(4.5g)	SEM
Packed cell volume (%)	30.33^{a}	26.00^{c}	27.33^{b}	29.33^{a}	0.55
Haemoglobin (g/dl)	8.90^{a}	7.63^{c}	8.00^{b}	8.60^{c}	0.14
Red blood cell ($x10^6/mm^3$)	3.36^{a}	2.90^{d}	3.04^{c}	3.35^{b}	0.04
White blood cell ($x10^3/mm^3$)	25.50^{b}	20.52^{d}	22.31^{c}	28.30^{a}	0.38
Neutrophils (%)	39.67	39.33	38.67	39.00	1.41
Lymphophils (%)	51.00^{ab}	50.00^{b}	52.67^{a}	50.33^{b}	0.88
Eosinophils (%)	3.00^{b}	4.33^{ab}	3.67^{b}	5.33^{a}	0.58
Monocytes	4.33	4.67	4.33	5.00	0.41
Basophils (%)	0.00	0.00	0.00	0.00	0.00
MCV (fl)	91.04	91.97	90.81	91.68	0.68
MCH (pg)	26.52	29.38	29.27	29.32	0.32
MCHC (g/dl)	29.34	29.38	29.27	29.32	0.50

The values a, b, c, d mean in a row with different superscripts are significantly ($P < 0.05$) different. MCV=Means Corpuscular Volume, MCH=Means Corpuscular Haemoglobin, MCHC=Means Corpuscular Haemoglobin Concentration

Table 3: Serum Biochemistry Values of Broilers Birds fed different levels of immunowall® prebiotic additive

Parameters	T_1 (0g)	T_2(1.5)	T_3(3.0g)	T_4 (4.5g)	SEM
Total Protein (g/dl)	3.65^{b}	3.54^{c}	3.79^{a}	3.56^{c}	0.03
Albumin (g/dl)	2.27	2.62	2.30	2.20	0.16
Globulin (g/dl)	1.38	1.28	1.49	1.36	0.15
Cholesterol (mg/dl)	117.90^{a}	115.30^{b}	118.30^{a}	118.20^{a}	0.35
Alkaline Phosphatase (µ/l)	56.93	60.27	59.40	56.20	1.74

The values a, b, c means in a row with different superscripts are significantly ($P < 0.05$) different