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HAEMATOLOGICAL AND SEROLOGICAL RESPONSE OF BROILER CHICKENS FED VARYING LEVELS OF DIRECT FED MICROBES AS FEED ADDITIVE

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ABSTRACT
A study was conducted using one hundred and twenty day-old Ross 308 broiler chicks to evaluate blood and serum chemistry response of broiler chickens fed direct fed microbes The birds were obtained from a reputable hatchery and randomly assigned to four dietary treatments, each with three replicates of ten birds per treatment. The treatments were T1, T2, T3 and T4, the levels of inclusion of direct fed microbes (DFM) were 0%, 2.5%, 5.0% and 7.5% respectively. The birds were assigned to these treatment diets, feed and water was given ad libitum throughout the duration of the experiment which lasted for 56 days. The experimental design was Completely Randomized Design (CRD). At the end of the experiment blood samples were collected from the broilers and taken to the laboratory for hematological and serum chemistry analysis. The hematological characteristics of the broiler shows that there was no significant ($P>0.05$) in haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC). The WBC portrayed decreasing trend as the level of DFM increased, also the Hb, MCV and MCH portrayed an increasing trend as the DFM increased in the treatment diet up to the 5% inclusion level. The serum chemistry result shows that there was a significant difference ($P<0.05$) in total serum protein and globulin whereas no significant difference ($P>0.05$) was observed in serum albumin, glucose, urea and creatinine. From the result it could be concluded that birds fed diet 2 performed better as they compete favourably with the control. Birds fed diet 2 also had a balanced physiological system.

Keyword: Direct Fed Microbe, Growth Performance and Broiler birds

Introduction

The ultimate aim of any livestock industry is the attainment of sustainable livestock production in the shortest time possible in order to produce animal protein with minimum cost (Doumba, 2002). Feed additives have been the major intervention used to improve performance and profitability of commercial poultry enterprise (Mandel *et a*l., 2000). The additives in use are the antibiotics which are used at sub-therapeutic levels to improve growth, health, feed efficiency and subsequent economic improvement benefit (Brorsen *et al*., 2002). However, concern about the routine use of antibiotics and anti-microbe resistance development and transference gene from animal to human microbiota make it unsafe for use (4). The seriousness of the problem has led to withdrawal of approval from antibiotics as growth promoters in the European Union (EU) since January 1, 2006 due to negative human health issue of antibiotic resistance (Castanon, 2007). The antibiotic resistance development and the subsequent ban in Europe and United States have resulted to an increased interest in finding alternatives for poultry and livestock production by nutritionists and production managers (Ahmad, 2006). The use of probiotics otherwise known as direct fed microbes (DFM) (Ashayerzadeh *et al*., 2011) are one of the approaches that have a potential to replace

antibiotics as a result of the direct fed microbe ability to prevent internal colonization of entero pathogenic enzymes (Sieo *et al*., 2005), stimulate intestinal immunity of birds (Fuller, 2000) and also reduce stress in animals (Line *et al*., 1997). The advantages of using probiotics over the tradition antibiotics are: no withdrawal time, no residual effect and no causes of microbial mutation (Ghally *et al*., 2007). Probiotics have been defined as a live microorganism when administered through the digestive tract has a positive impact on the host health through its direct nutritional effect (Banday and Risam, 2002). Also, according to FAO/WHO (FAO/WHO, 2002), probiotics is seen as "live microorganisms which when administered in adequate amount confer a benefit to the host health". The probiotics is a product that contains a dynastic vital microorganism with enough number to have an ability to change a number of flora (formation of colonies) inside the host which alters hygienic imported trails in the host (Schrezenmier and Vrese, 2001). The search for safe and natural alternative to reduce over-dependence of the use of antibiotics (growth promoters) has led to the evaluation of dietary inclusion of direct fed microbes on the blood constituents and serum chemistry of broiler birds.

Materials and Methods

Experimental Site

The experiment was conducted at the poultry unit of the teaching and research farm of Michael Okpara University of Agriculture Umudike, Abia State. The area is located on latitude 05°27 North, longitude 07°32 East, with an altitude of 123m above sea level. Umudike has an ambient temperature of 22°C – 36°C with annual rainfall of 2177mm and relative humidity of above 50-90% (NRCRI, 2017).

Test Material

The microbes used in this study were L. acidophilus, Bifidobacterium thermophilus and Enterococcus faecium which are mainly beneficial bacteria

Experimental Birds and Management

A total of one hundred and twenty (120) Ross broiler strain chicks were used. They were divided into four (4) treatments, with each treatment containing thirty (30) birds. Each treatment as replicated three (3) times while each replicate contained ten birds.

A week before the birds were introduced, the poultry house was fumigated, washed, disinfected and allowed to dry for seven days. The birds were vaccinated against Newcastle disease (NCD 1/0). They were also given anti-stress preparations to enable the chicks recover from stress they may have passed through during transportations from the hatchery to the site of the experiment. Infections bursa disease vaccine was administered at the 10^{th} and 28^{th} day respectively. Antibiotics and anti-coccidial drug recommended by a veterinarian was also administered as at when due.

Heat and light was supplied to the birds with the aid of kerosene stove and lamps respectively. The litter was always replaced with wet to discourage the growth of pathogens. Biosecurity and other important routine management practices were observed. The feeding trial lasted for eight weeks (56 days). Feed and clean water was given *ad libitum* to the birds.

Experimental Diets

The experimental diet contained the adequate level of nutrients for broilers as recommended by the National Research Council (NRC, 1994). The test-ingredient, direct fed microbes was supplied from United States of America (USA). Four experimental diets were formulated with direct fed microbes. The composition of the experimental diets is shown in Table 1 and 2 below.

The treatment one (T1) which is the control did share as follows:

T1 = 0% (control)
T2 = 2.5% inclusion level
T3 = 5% inclusion level
T4 = 7.5% inclusion level

Table 1: Percentage composition of broiler starter diets supplemented with direct for microbes

Ingredients	T1	T2	T3	T4
Maize (yellow)	50.00	50.00	50.00	50.00
Soya bean meal	25.00	25.00	25.00	25.00
Wheat offal	8.00	8.00	8.00	8.00
Fish meal	3.00	3.00	3.00	3.00
Palm kernel cake	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Vit./mineral premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
DFM (%)	0.00	2.50	5.00	7.50
Crude protein	22.95	22.93	22.91	22.89
ME (Kcal/kg)	2945	2930	2945	2950

DFM: Direct fed microbes

Experimental Design

The experimental design used was completely randomized design (CRD). The statistical model for completely randomized design in given below:

Yij = μ+Ti+T eij

Where

Yij = single observation

μ = overall mean

Ti = effect of the treatment

Eij = experimental error

Table 2: Percentage composition of broiler finisher diet supplemented with direct fed microbes

Ingredients	T1	T2	T3	T4
Maize (yellow)	52.00	52.00	52.00	52.00
Soya bean meal	20.00	20.00	20.00	20.00
Wheat offal	10.00	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00	3.00
Palm kernel cake	11.00	11.00	11.00	11.00
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Vt/mineral premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
DFM (%)	0.00	2.50	5.00	7.50
Crude protein	20.86	20.53	20.34	20.22
ME (Kcal/kg)	3050	3062	3068	3071

DFM: Direct fed microbes

Data Collection

At the end of the experiment, six broilers from each treatment were selected and blood samples collected. A sterile needle was used to collect 10ml of blood from the web of the wing of the bird, 2ml kept in a container containing Ethylene Diamine Tetra acetic Acid (EDTA), an anti-coagulant to prevent blood clothing. The remaining 8mls was allowed to coagulate to generate blood sera for blood serum biochemistry analysis. The blood samples collected was taken to the laboratory for haematological assessment such as packed cell volume (PCV), red blood cell (erythrocyte) count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), haemoglobin (Hb), and white blood cell (leukocyte) count. For serum biochemistry, the

following was determined, serum albumin, serum protein, serum globulin, serum urea, serum glucose and serum creatinine.

Results and Discussion

The haematological characteristics of broiler chicken fed diets containing different levels of direct fed microbes are presented in Table 3. No significant differences (P>0.05) were observed in haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), meal cell hemoglobin concentration (MCHC). The WBC portrayed a decreasing trend as the level of DFM increased. The Hb, MCV and MCH portrayed an increasing trend as the level of DFM increased in the diet up to the 5% level. A lot of researchers have reported normal ranges for hematological parameters in avian species. Campbell, (2013) recorded normal range of hematological parameters of PCV (35.9-41;0%), Hb (11.60-13.68g/dl), RBC (4.21-4.84 X 10^6/ml, WBC (4.07-4.32 X 10^3/ml, MCV (81.60-89.10FI), MCH (27.20-28.90pg) and MCHC (32.41-33.37%) in broiler chickens. The PCV, Hb, RBC and MCHC obtained in this study were lower whereas, the WBC and MCV were higher than the normal reference values reported by this author. The MCH values in this study however, compared with the normal reference range given by the author. This reference values obtained by Jain, (1993) for Hb (7.00-13.0), RBC (2.5-3.5), PCV (22.0-35.0), WBC (1.2-3.0) agree with the values reported in this study for Hb, RBC and PCV but lower than the values reported for WBC. Merck, (2012) reported normal means values of blood parameters for PCV (32%), Hb (12 g/dl), WBC (5.5 x 10^3µl), MCV (174 FI), MCH (61 Pg), MCHC (33 g/dl) which are higher than the values reported for PCV, Hb, MCV, MCH and MCHC but lower than the value reported for WBC in this study. The variability in results will depend on multitude of factors among which includes the fact that most normal reference values are established in temperate countries, whose data may not effectively reflect tropical animal characteristics due to the differences in environmental conditions as well as genetic variability. The WBC showed a declining trend as the level of the DFM increased, which though, did not produce any significant difference among the treatments. Shareef, (2009) and Cetin *et al*. (2005) did not obtain a significant increase in the number of white blood cells (WBC) (P<0.05) with probiotic treatment which corroborates the present findings. The WBC of the trial diets T3 and T4 were numerically lower than that of the control, whereas, T2 was higher than the control and thus produced the highest effect on immunity. Increase in white blood cells count was observed in T2, represents stimulation of the host's immune system (Al-Kassi *et al*., 2009). However, it has been shown that probiotics stimulate different subsets of immune system cells to produce cytokines, which in turn play a role in the induction and regulation of the immune response (Christensen, 2002). The RBC did not vary between the trial diets and the control suggesting that the supplementation of the DFM did not disrupt its normal function. The RBC obtained in this study compares with the values (2.7-3.1 x 10^6) reported by Beski and Al-Sardary (2015) in dietary supplementation of probiotic in broiler chicken and fall within the range reported by Clement *et al*. (2010). Djouvinov *et al*. (2005) found that the probiotic supplementation did not affect the RBC of birds which agrees with the present findings.

Table 3: Hematological characteristics of broiler chicken fed diets containing different levels of direct fed microbes

Parameter	T1 (0%)	T2 (2.5%)	T3 (5%)	T4 (7.5%)	SEM
Hb (g/dl)	7.13	7.54	7.81	7.40	0.17
PCV (%)	27.17	28.00	29.00	28.00	0.64
RBC x 10^6/ml	2.90	2.82	2.68	2.86	0.13
WBC x 10^3/ml	9.58	9.85	7.73	6.23	0.79
MCV (FI)	98.03	100.21	111.26	97.70	4.82
MCH (Pg)	25.64	26.95	30.06	25.83	1.31
MCHC (%)	26.25	26.93	27.16	26.38	0.51

Means did not differ significantly p>0.05; SEM = Standard error of the mean

The PCV values obtained in this study compared with the range of values reported by Beski and Al-Sardary, (2015) and Bonus *et al*. (2012) in dietary supplementation of probiotic in broiler chicken. Al-Saad *et al*. (2012) observed significant increase in haematocrit values (HCT) in probiotic treatment compared with the other treatments in broiler chicken, whereas, there was no significant increase in this present study. Saied *et al*. (2011) reported that dietary probiotics had no effects on PCV when introduced into broilers diet which corroborates with the present findings. The MCV values compared with that reported by Clement *et al*. (2010). The MCV value in this study however, was higher than the reference value 8-89 FI given by Campbell, (2013). A high MCV may indicate anaemia due to nutritional deficiencies, bone marrow abnormalities, liver disease, alcoholism, chronic lung disease, or therapy with certain medications (Jaime and Howlette, 2008). Since the result of the control diet (T_1) did not vary significantly from that of the trial diets T_2, T_3 and T_4, therefore, there are no such cases of either microcytic or macrocytic anaemia in this study. The result of MCH and MCHC compared closely to the result obtained by Duwa *et al*. (2013). However, this present finding did not show any significant difference between the control and the trial diets, suggesting no abnormality with the supplementation of the probiotic in broilers diet. The similarity of MCHC values between the control and the trial diets again shows that the MCHC values were not affected by the supplementation of probiotics in broilers diet. In other poultry species, Strompfova *et al*. (2005) showed no significant differences among the treatments in RBC, leucocyte count, PCV and hemoglobin concentration in Japanese quail fed *Lactobacillus fermentum* which agrees with the present findings for broiler chicken.

The serum characteristics of broiler chicken fed diets containing different levels of direct fed microbes (DFM) is presented in Table 4. Significant differences ($P<0.05$) were observed in total serum protein and globulin, whereas, no significant differences ($P>0.05$) were observed in serum albumin, glucose, urea and creatinine. The 2.5% DFM recorded the most significant value in total serum protein. The trial diets (T_2, T_3 and T_4) generally recorded higher serum protein value than the control diet (T_1). This shows that inclusion of DFM increases the serum protein in broiler chicken. Again, it was observed that the trial diets portrayed higher values for serum globulin than the control diet. The 5% DFM-based diet recorded the most significant values for serum globulin which however was inconsistent as the level of inclusion of DFM increases. The control diet recorded the last serum globulin. It can therefore be stated that the inclusion of DFM equally increased the serum level of broiler chicken in this study. Beski and Al-Sardary, (2015) recorded higher serum glucose value (227.7-232.0) in broiler chicken diets supplemented with probiotics, whereas, Duwa *et al*. (2013) obtained lower glucose in comparison to the result of this study. Al-Saad *et al*. (2014) and Adil, (2010) observed no significant effect of treatments on glucose levels in broiler chicken which corroborates the result of this present study. The serum glucose in this study concurs with the normal reference values (197-299) given by Clinical Diagnostic Division (1990) for serum glucose. This shows that adequate energy was derived for metabolic functions by the broiler chicken fed the DFM based diets. The similarity of albumin in the control and the trial diet suggests that the function of serum albumin in regulating colloidal pressure in the body was not affected by the dietary supplementation of DFM in this study. The albumin levels in this study fall within the range established by Merck (2012) which is 1.4 g/dl.

Paryad and Mahmoudi, (2008) noticed that dietary probiotics increases serum concentrations of protein and globulin which agrees with the increases observed in this study for serum globulin and serum protein. The increase in serum globulin of the trial diets in this study may suggest increased immunity which would predispose the animals for better performance. Stimulation of immune response by host is among the mechanisms proposed for improvements in animal performance when fed a DFM (Keeney and Finlay, 2011). Duwa *et al*. (2013) and Beski and Al-Sardary, (2015) who fed dietary probiotics, obtained higher serum protein values in broiler chicken compared to the values established in this study. However, Duwa *et al*. (2013) obtained lower globulin in broiler chicken in comparison to the result of this study. Serum total protein and albumin have been reported to be directly responsive to protein intake and quality (Eggum, 1989). The significant increases

in the serum protein of the diets supplemented with probiotics in this study suggest that the beneficial microbes in the probiotics increased protein digestion and utilization in the broiler chicken fed these diets than that of the control. However, no significant effect was observed in the serum albumin as earlier reported. This suggests that probably the strains and concentrations of the DFM probiotic used in this study did not affect the serum albumin. In other words, the function of serum albumin in maintaining colloidal osmotic pressure between the blood and body fluid was not affected. The creatinine levels of this study fall within the normal range established by Merck (2012) which is between 0.1-0.4 mg/dl. Owosibo *et al*. (2013) obtained higher serum creatinine values for broiler chicks fed diets containing probiotics, Duwa *et al*. (2013) also obtained higher creatinine in broiler chicken in comparison to the result of this study.

Table 4: Serum characteristics of broiler chicken fed diets containing different levels of direct fed microbes

Parameter	T1 0%	T2 2.5%	T3 5%	T4 7.5%	SEM
Total protein	2.06^{b}	2.66^{a}	2.55^{ab}	2.41^{ab}	0.09l
Albumin	0.96	1.15	0.96	1.09	0.04
Globulin	1.10^{b}	1.51^{ab}	1.59^{a}	1.32^{ab}	0.08
Glucose	189.33	201.67	205.33	181.67	6.40
Urea	2.45	1.90	1.01	3.30	0.55
Creatinine	0.28	0.20	0.33	0.23	0.03

a.b. means differed significantly at P<0.05); SEM = Standard error of the mean

The creatinine level, though it did not vary significantly in the treatment diets, portrayed higher values (0.33) in the 5% (T_3) DFM supplementation than the control; but was inconsistent as the level of DFM increased. This suggests that other factors other than the DFM used might have affected the result of this study. The serum urea did not differ significantly between the control and trial diets and fall within the normal reference values (19.-12.5) given by Clinical Diagnostic Division (1990) for uric acid. The urea value was numerically lower in T_2 and T_3 compared to the control diet. A low urea suggests more efficient metabolism cum proper renal and hepatic function (Adeyemo and Sani, 2013). This suggests that the 2.5% and 5% level of the DFM probiotic mixture used in this study had the most efficient metabolism in the broiler chicken. Generally, the differences in results may be due to the microbes species, strain, concentration or dosage, production techniques, storage condition, management practices, diets and other environmental conditions among the experiments (Paryad and Mahmoudi, (2008) and probably sex and breed differences according to Ladokun *et al*. (2008) who opined that there is an influence of sex and breed in total protein and uric acid content of the serum.

Conclusion

This present study investigated the effect of direct fed microbes in the health status of broiler chicken as is often revealed in their hematological and biochemical characteristics. The direct fed microbes used in this study did not produce any significant effect between the control and trial diets of the broiler chicken used in this study. It was thus concluded that T_2 produced the highest immune response compared to the other trial diets and the control diet. It is apparent from this study that T_2 which revealed the highest serum protein, lower urea value compared to the control and highest immune response based on its highest white blood cell count, could be considered for adoption by farmers in the diets of broiler chicken.

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