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EFFECT OF DIETARY FEED ADDITIVES ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS OF BROILER CHICKENS

Emmanuel Olakunle ALONGE[1], Daisy ERUVBETINE[1], Olusegun Mark Obafemi IDOWU[1], Adewale Olusegun OBADINA[3] and Oladapo Oluwaseye OLUKOMAIYA[2]✉

[1]Department of Animal Nutrition, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria
[2]Department of Animal Production and Health, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria
[3]Department of Food Science and Technology, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria
✉Email: oladapooluwaseye@ymail.com

ABSTRACT: The effect of dietary feed additives on haematological and serum biochemical parameters of broiler chickens was evaluated. 180 day-old Arbor acre broiler chicks were weighed and randomly allotted to five dietary treatments with 3 replicates of 12 birds each. Broiler starter diet (2855.7 kcal/kg ME; 23.01%) and finisher diet (2911 kcal/kg; 20.71% CP) were formulated. Dietary treatments were control diet (basal diet without additives), OXYT diet (basal diet with oxytetracycline at 600 ppm as antibiotic, GRO-UP diet (basal diet with probiotic at 500 ppm), MOS-500 diet (basal diet with mannan oligosaccharide at 500 ppm) and MOS-1000 diet (basal diet with mannan oligosaccharide at 1000 ppm). Feed and water were supplied ad libitum. At the end of weeks 4 and 8, blood samples were collected and analyzed. The haematological and serum biochemical parameters of broiler chickens fed diets containing feed additives at the starter phase were not statistically significant (P>0.05). At the finisher phase, there were no significant (P>0.05) differences in all the parameters measured except in the heterophils and eosinophils where birds fed the control diets had the lowest value among all treatments. Serum globulin values were significantly (P<0.05) different as birds fed diets containing OXYT (antibiotics) recorded the lowest value among all treatments. The inclusion of prebiotics and probiotics in the diets of broiler chickens elicited no adverse effect on haematological and serum biochemical parameters, thus, they can be used as replacement for antibiotics.

Keywords: Haematology, Serum Biochemistry, Broiler Chickens, Prebiotics, Probiotics

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INTRODUCTION

The benefits of broiler chicken production cannot be over emphasized in the face of increasing demand for animal protein in many developing countries of the world. To improve production, poultry birds must be free from infections as well as being fed with suitable diets needed for optimal production (Tannock, 1988). Antibiotics are widely being used in animal feed to enhance animal performance and production. Tetracyclines are arguably the most commonly-used therapeutic antibiotics in food animal production (Fairchild et al., 2005). Oxytetracycline (OTC) is a broad spectrum antibiotic developed to enhance the control of bacterial infections (Alam, 2000). The dietary inclusion of 50 mg/kg OTC during 21-42 days of age increased body weight gain of broiler chicks (Zulkifli et al., 2000). Talabi et al. (2013) also stated that the use of dietary oxytetracycline antibiotic powder at 0.05 g/kg promoted the growth of broiler chicks under different feeding regimes.

However, at low levels of antibiotic administration, resistant microbial cells survive and produce resistance. Thus, the use of antibiotics in animal feeds has been limited in the European Union since January 2006 (Toghyani et al., 2011). As a result, other feed additives such as prebiotics and probiotics have been proposed to livestock producers. They are being increasingly adopted as replacements for antibiotics in improving growth and gut health in poultry and swine (Higgins et al. 2008; Markovic et al. 2009; Zhang and Kim, 2013). Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its intestinal health (Fuller, 1989). Prebiotics are non-digestible food ingredients that beneficially affect a host animal by selectively stimulating the growth and/or activity of one or more bacterial population in the colon (Gibson and Roberfroid, 1995). Due to the fact that more information is still needed on the haematology and serum biochemistry of broiler chickens as far as animal health is concerned, considerable attention is still being drawn towards testing the potency of dietary prebiotics and probiotics as antibiotic substitutes.

Therefore, the present study was carried out to investigate the effect of dietary feed additives on haematological and serum biochemical parameters of broiler chickens.

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MATERIALS AND METHODS

The experiment was conducted at the Poultry Unit of Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The area lies within the rainforest zone of South-Western Nigeria at longitude 7°10', 37'N, latitude 3° 26' 58'E and altitude 173 m above sea level. The climate is humid with a mean annual rainfall of 1037 mm. The mean annual temperature and humidity are 34.7°C and 82%, respectively (Google Earth, 2013). 180 day-old Arbor acre broiler chicks raised on formulated broiler starter and finisher diets were used. They were weighed and randomly divided into 5 groups with 3 replicates of 12 birds each in a completely randomized design. They were brooded for two weeks in a deep litter system. Water and feed were supplied *ad libitum*. Routine medications were administered to the birds accordingly. The test ingredients used were Kepro® Oxytetracycline (antibiotics) - feed grade oxytetracycline (OXYT) was obtained from a reputable veterinary pharmacy shop in Abeokuta having the composition (per g) consists of oxytetracycline hydrochloride 1000 mg. GRO-UP™ (probiotics) was supplied by Bio Ingredients Ltd., Lagos, Nigeria with the composition (per kg) of *Saccharomyces cerevisiae*: 1.5x10^{11} cfu, *Lactobacillus sporogenes*: 30 million cfu and fortified with phytase phosphorus, proteins, calcium, carbohydrates and vitamins. Oligomanno® (Mannan Oligosaccharides) (prebiotics) was supplied by Yonichi Chemical Institute Co., Ltd. Machikita 9-25, Moriyama-Ku, Nagoya, Japan. Composition: hydrolyzed Guar gum fiber (Mannan Oligosaccharide). Diet 1 was the control without feed additive, diet 2 had the inclusion of OXYT at 600 ppm, diet 3 had the inclusion of GRO-UP at 500 ppm while diets 4 and 5 had the inclusion of MOS at 500 ppm and 1000 ppm respectively as presented in Tables 1 and 2.

Table 1 - Ingredient composition of starter experimental diet (0-4 weeks)

Ingredients (%) \ Diets	1	2	3	4	5
Maize	50.66	50.66	50.66	50.66	50.66
Wheat offal	5.00	5.00	5.00	5.00	5.00
Fish meal (72%)	3.00	3.00	3.00	3.00	3.00
Soybean meal	24.24	24.24	24.24	24.24	24.24
Groundnut cake	10.00	10.00	10.00	10.00	10.00
Palm kernel cake	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.25	0.25	0.25	0.25	0.25
*Premix	0.50	0.50	0.50	0.50	0.50
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25
[1]OXYT®	-	+	-	-	-
[2]GRO-UP®	-	-	+	-	-
[3]MOS®	-	-	-	+	++
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Crude Protein (%)	23.01	23.01	23.01	23.01	23.01
ME (Kcal/kg)	2855.7	2855.7	2855.7	2855.7	2855.7

*A kilogramme premix contains Vit. A: 10000000 IU, Vit. D_3: 2500000 IU, Vit. E: 20000 mg, Vit. K_3: 3000 mg, Vit. B: 30000 mg, Vit. B_3: 3000 mg, Vit. B_2: 7000 mg, Vit. B_6: 5000 mg, Vit. B_{12}: 25mg, Panthotenic acid: 10000mg, Folic acid: 800 mg, Biotin: 50mg, Manganese: 80000 mg, Iron: 40000 mg, Zinc: 60000 mg, Copper: 8000 mg, Cobalt: 250 mg, Iodine: 1000 mg, Selenium (1%), 150 mg, Choline: 200000 mg and Antioxidant: 100000 mg.
-: no additive; +: 500 ppm of MOS; ++: 1000 ppm of MOS
[1]OXYT® (antibiotics) at 600 mg/kg inclusion; [2]GRO-UP® (probiotics) at 500 mg/kg inclusion and [3]Mannan oligosaccharides® (prebiotics) at levels of 500 mg/kg and 1000 mg/kg respectively.

At end of weeks 4 and 8, blood samples were drawn from the wing (brachial vein) of two birds per replicate. 2 ml of blood was collected from two birds per replicate into the tube containing Ethylene Diamine Tetra Acetate (EDTA) as anti-coagulant and another 2 ml was collected for serum analysis. The haematological analysis of RBC, WBC and its differentials, MCH, MCHC, MCV, PCV and Haemoglobin concentration (Hb) were done according to standard methods (Schalm, 1986). The second set of bottles without EDTA was centrifuged in a macro centrifuge to obtain serum for biochemical analysis. Serum glucose was determined colorimetrically using GOD/PAD reagent method (Trinder, 1969). Serum uric acid was determined using enzymatic colorimetric method as described by Fossati and Prencipe (1982). Serum cholesterol was determined using enzymatic end point method (Roschlau et al., 1974). Serum total protein was analyzed using bromo cresol purple method of Varley et al. (1980). Serum albumin was determined by the bromo cresol green (BCG) method as described by Doumas et al. (1971). Serum globulin was calculated as the difference between total serum protein and serum albumin. Serum creatinine was analyzed using colorimetric method (Bowers and Wong, 1980).

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Table 2 - Ingredient composition of finisher experimental diet (4-8 weeks)

Ingredients (%) \ Diets	1	2	3	4	5
Maize	55.00	55.00	55.00	55.00	55.00
Wheat offal	6.00	6.00	6.00	6.00	6.00
Fish meal (72%)	2.00	2.00	2.00	2.00	2.00
Soybean meal	18.00	18.00	18.00	18.00	18.00
Groundnut cake	13.00	13.00	13.00	13.00	13.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Oyster shell	3.00	3.00	3.00	3.00	3.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30	0.30
*Premix	0.25	0.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25
[1]OXYT®	-	+	-	-	-
[2]GRO-UP®	-	-	+	-	-
[3]MOS®	-	-	-	+	++
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Crude Protein (%)	20.71	20.71	20.71	20.71	20.71
ME (Kcal/kg)	2911	2911	2911	2911	2911

*A kilogramme premix contains Vit. A: 10000000 IU, Vit. D_3: 2500000 IU, Vit. E: 20000 mg, Vit. K_3: 3000 mg, Vit. B: 30000 mg, Vit. B_3: 3000 mg, Vit. B_2: 7000 mg, Vit. B_6: 5000 mg, Vit. B_{12}: 25mg, Panthotenic acid: 10000mg, Folic acid: 800 mg, Biotin: 50mg, Manganese: 80000 mg, Iron: 40000 mg, Zinc: 60000 mg, Copper: 8000 mg, Cobalt: 250 mg, Iodine: 1000 mg, Selenium (1%), 150 mg, Choline: 200000 mg and Antioxidant: 100000 mg.
-: no additive; +: 500 ppm of MOS; ++: 1000 ppm of MOS
[1]OXYT® (antibiotics) at 600 mg/kg inclusion; [2]GRO-UP® (probiotics) at 500 mg/kg inclusion and [3]Mannan oligosaccharides® (prebiotics) at levels of 500 mg/kg and 1000 mg/kg respectively.

Statistical analysis

Data obtained were subjected to Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) using SAS (2003). Significant means among variables were separated using Duncan's Multiple Range Test.

RESULTS

The haematological and serum biochemical parameters of broiler chickens fed diets containing feed additives at Week 4 are presented in Tables 3 and 4 respectively. Variations in the parameters measured were not statistically significant (P>0.05).

The haematological parameters of broiler chickens fed diets containing feed additives at Week 8 are shown in Table 5. There were no significant (P>0.05) differences in all the parameters measured except in the heterophils and eosinophils where birds fed the control diets had the lowest value among all the treatments. The serum biochemical parameters of broiler chickens fed diets containing feed additives at Week 8 are shown in Table 6. There were no significant (P>0.05) differences in all the parameters measured except in serum globulin where birds fed diets containing OXYT (antibiotics) recorded the lowest value among all the treatments.

Table 3 - Haematological parameters of broiler chickens fed diets containing feed additives at Week 4

Parameters	Control	OXYT	GRO-UP	MOS (500ppm)	MOS (1000ppm)	SEM
PCV (%)	30.00	26.33	28.33	28.00	26.33	0.56
Hb (g/dl)	9.77	8.57	9.30	8.97	8.57	0.18
RBC ($\times 10^{12}$/l)	1.67	1.44	1.55	1.52	1.43	0.04
MCH (pg)	58.44	59.69	60.15	59.22	61.26	0.71
MCHC (g/dl)	32.54	32.54	31.67	32.06	33.26	0.28
MCV(fl)	17.95	18.34	18.30	18.46	18.44	0.17
WBC ($\times 10^3$/l)	8.03	6.27	7.13	6.90	7.30	0.35
Heterophils (%)	37.00	37.33	42.00	35.67	42.00	1.33
Lymphocytes (%)	56.67	56.00	52.33	55.00	53.33	1.43
Monocytes (%)	0.33	1.00	0.67	1.00	1.33	0.17
Eosinophils (%)	3.00	3.33	3.00	3.00	3.33	0.09
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00

Means on the same row having different superscripts are significantly different (P<0.05). Control (no additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan Oligosaccharide (prebiotics)

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Table 4 - Serum biochemical parameters of broiler chickens fed diets containing feed additives at Week 4

Parameters	Control	OXYT	GRO-UP	MOS (500ppm)	MOS (1000ppm)	SEM
Glucose (mg/dl)	146.67	149.33	151.33	147.67	152.33	1.14
Total protein (g/dl)	26.34	27.33	27.67	27.00	27.00	0.29
Serum albumin (g/dl)	12.67	13.33	13.00	12.67	13.00	0.18
Serum globulin (g/dl)	13.67	14.00	14.67	14.33	14.00	0.19
Uric acid (mg/dl)	5.07	5.10	5.47	5.03	5.49	0.10
Cholesterol (mg/dl)	97.67	90.00	114.00	99.00	104.33	4.40
Creatinine (mg/dl)	0.97	0.93	0.87	0.97	0.97	0.34

Means on the same row having different superscripts are significantly different (P<0.05). Control (no additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan Oligosaccharide (prebiotics)

Table 5 - Haematological parameters of broiler chickens fed diets containing feed additives at Week 8

Parameters	Control	OXYT	GRO-UP	MOS (500ppm)	MOS (1000ppm)	SEM
PCV (%)	29.67	29.33	30.00	29.33	30.33	0.32
Hb (g/dl)	9.70	9.87	9.70	9.77	10.00	0.10
RBC ($\times 10^{12}$/l)	2.48	2.45	2.51	2.46	2.54	0.32
MCH (pg)	29.19	40.28	38.73	39.76	39.44	2.24
MCHC (g/dl)	32.73	33.66	32.01	33.31	32.98	0.31
MCV(fl)	9.15	12.17	11.97	11.93	11.97	0.55
WBC ($\times 10^{3}$/l)	6.90	6.40	5.67	6.50	6.77	0.40
Heterophils (%)	46.33^{c}	50.67^{a}	50.00^{ab}	47.00^{bc}	50.00^{ab}	0.59
Lymphocytes (%)	50.33	49.33	47.00	48.00	46.00	0.72
Monocytes (%)	0.00	0.00	0.33	0.00	0.33	0.09
Eosinophils (%)	3.00^{b}	4.33^{ab}	5.33^{a}	4.33^{ab}	4.67^{ab}	0.30
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00

Means on the same row having different superscripts are significantly different (P<0.05). Control (no additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan Oligosaccharide (prebiotics)

Table 6 - Serum biochemical parameters of broiler chickens fed diets containing feed additives at Week 8

Parameters	Control	OXYT	GRO-UP	MOS (500ppm)	MOS (1000ppm)	SEM
Glucose (mg/dl)	147.00	149.00	148.33	154.67	147.33	1.49
Total protein (g/dl)	26.67	25.33	27.00	27.33	27.67	0.35
Serum albumin (g/dl)	12.00	12.33	13.00	13.00	13.00	0.19
Serum globulin (g/dl)	14.67^{a}	13.00^{b}	14.00^{ab}	14.33^{ab}	14.67^{a}	0.24
Uric acid (mg/dl)	5.00	5.07	5.17	5.27	5.17	0.63

Means on the same row having different superscripts are significantly different (P<0.05). Control (no additive); OXYT: Oxytetracycline (antibiotics); GRO-UP (probiotics); MOS: Mannan Oligosaccharide (prebiotics)

DISCUSSION

The PCV values obtained in the present study were within the normal range (Ridell, 2011) but lower than 35.9 % reported for chickens in Nigeria (Oyewale and Ajibade, 1990). Under normal conditions, blood composition is reasonably constant for any particular species with changes falling with fairly narrow limits (Banergee et al., 2002). The RBC counts and PCV are known to be mostly affected by dietary treatment (Banergee et al., 2002). The results obtained for Hb follow the same pattern with that of PCV with values for birds fed control diets reducing while others increased at the finishing phase. The Hb values obtained were close to the average values of 10.27 g/dl. This showed that probiotic based diets are nutritionally adequate to meet the protein needs of the birds since the haemoglobin concentrations decreased in animals on low protein intake and in parasitic infection of liver damage (Lindsay, 1997). The MCH reduced at the 8th week of the experiment for all groups. Birds fed diets supplemented with feed additives had MCH values within the normal range of 33.00-47.00 pg (Bounous and Stedman, 2000) while birds on control diet had MCH value below the normal range. This result suggests that the blood of the birds had an appreciable oxygen-carrying capacity which showed that nutrient transport by the blood was not impaired by feeding diets containing prebiotics. WBC plays a prominent role in disease resistance especially with respect to generation of antibodies and the process of phagocytosis. WBC values for birds fed OXYT supplemented diets increased while others reduced at finishing phase with birds fed on GRO-UP supplemented diets having the lowest value. An elevated value of WBC could be an indication of birds reacting to one or more

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factors in the feed (Oduguwa, 2006). At the finisher phase, values of 46.33-50.67% for heterophils and 3.00-5.33% for eosinophils aligns with the normal range (Ridell, 2011). This may be an indication of positive effect of additives exerted in birds to suppress any effect of antibodies at the finisher phase. Heterophils and eosinophils are granulocytes of the WBC. Heterophils plays critical role in immune response. Eosinophils destroy parasites and also help to modulate inflammatory responses (Britannica, 2013). Lymphocytes and monocytes did not follow any trend even though the values were not significant. Glucose values in this study were lower than the values obtained by Priya and Gomathy (2008) who observed a mean value of 160.92 mg/dl. Serum total protein consists of albumin and globulin; a change in nutritional status and malnutrition is often revealed in total protein values (Allison, 1995). The increased serum protein, albumin and glucose and decreased serum activity observed in broilers fed probiotics and prebiotics were due to improvement in protein synthesis, carbohydrate and lipid metabolism (Rosa et al., 2001). Serum globulin was lowest in birds fed OXYT supplemented diets at the finisher phase. This may suggest poor immune response and insufficient antibody production in the birds. The serum cholesterol in this study did not agree with the report of Mohan et al. (1996). Uric acid recorded high values at starter phase and lower values at finisher phase which is in line with the finding of Szabo et al. (2005) who reported a direct relationship between the amounts of ingested protein and serum uric acid. Uric acid gives an indication of the quality of protein fed and high levels in the serum utilization of protein.

CONCLUSION

The inclusion of prebiotics and probiotics in the diets of broiler chickens elicited no adverse effects on the haematological and serum biochemical parameters, thus, they can be used as replacement for antibiotics.

Competing interests

The authors have declared that no competing interest exists.

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