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The Dynamics of Dietary Supplementation of Direct Fed Microbial and Antibiotic on the Haemato-Biochemical Values of Broiler Chickens

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Abstract
With the restriction in the use of antibiotics in animal nutrition as growth promoters there has been increased interest in the discovery and use of natural alternatives. This experiment was conducted to investigate the influence of Direct fed microbial (RE3) and antibiotics (FYSAL®-DRY SP) on haematological and serum biochemistry of broiler chickens. Two hundred and forty one-day-old unsexed Marshal strain of broiler chicks were used for the experiment. The birds were randomly divided into 3 groups of 4 replicates of 20 birds each to make a total of 80 birds per treatment in a complete randomized design experiment. Three diets were formulated as follows Diet 1 was the control without probiotic or antibiotic, Diet 2- control diet plus Direct fed microbial (1.5ml/kg of feed) and Diet 3- control diet plus antibiotics (2g/kg of feed). The experiment lasted for 8 weeks. Blood samples were collected from the birds for haematological studies and serum biochemistry. Results showed that haematological parameters were not adversely ($p>0.05$) affected by the dietary treatments. The values obtained for serum total protein, globulin and albumin were not significantly ($p>0.05$) affected among the dietary treatments. However, blood cholesterol level was significantly lowered in diets containing direct fed microbial and antibiotics. It was concluded that addition of probiotic could be more beneficial in reducing cholesterol in broiler chickens.
Keywords: Antibiotic, Broilers, Cholesterol, Direct fed Microbial, Haematological parameters

INTRODUCTION

The needed improved performance and quick attainment of market weight of birds especially meat type chicken can be achieved by the use of growth promoters, they are available in form of feed additives which are added to animal feed in order to stimulate their growth and improve profitability of commercial poultry enterprise (Mandal *et al*., 2000). The well-known additives are the antibiotics used at sub-therapeutic levels. Antibiotic growth promoters (AGPs) cause improvements in feed efficiency, growth rate, egg production and subsequent economic benefits (Brorsen *et al*., 2002, Gaskin *et al.,* 2002). Besides, it decreases feed usage per production unit. However, there are concerns about the routine use of antibiotics and anti-microbial resistance development and transference gene from animal to human microbiota thus making make it unsafe for use (Castanon, 2007). The restriction in the use of antibiotics in animal nutrition as growth promoters has increased the interest in the discovery and use of alternative natural products. Among many alternatives, probiotics, prebiotics, symbiosis and enzymes are promising feed additives that have been hypothesized to effectively replace antibiotics as natural growth promoters in animal diets (Gaskin *et al.,* 2002). As an alternative to AGPs, prebiotics and probiotics have no withdrawal time and no residual effect (Ezeokeke, 2008). In addition, their use allows maintenance of high productivity, reduction of morbidity and mortality in intensive farms. A probiotic feed additive is a live microbial feed supplement that beneficially affects the host animal by improving its microbial intestinal balance (Fuller, 1989). According to Miles and Bootwalla, (1991), Direct fed microbial (DFM) or probiotic can be defined as a source of live (viable) naturally occurring micro-organisms and this includes bacteria, fungi and yeast.

Haematological parameters are index and reflection of the effects of dietary treatments on the animals in terms of type and quantity ingested and available for the animal to meet its physiological, biochemical and metabolical requirements (Ewuola and Egbunike, 2008). On the other hand, serum biochemical analysis according to Harper *et al*., (1999) is used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acid utilization in animals. This assertion corroborate the observation of Iyayi and Tewe, (1998) that the chemistry of serum is routinely used for detection of organ disease in domestic animal and the amount of available protein in the diets. Probiotic and prebiotic have been shown to reduce serum cholesterol and abdominal fat in broiler chickens (Gaggia *et al*., 2010),

The present study was designed to compare the effect of dietary supplementation of Direct fed Microbial and antibiotic on the health status of broiler chickens via haematological and serum biochemical indices in the derived savannah zone of Nigeria.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The agro-ecological description of the study area had earlier been done by Oguntoyinbo (1976).

Test ingredients and formulation of experimental diets: The test materials used for the experiment were probiotics **(**RE3) ™ obtained from Basic Environmental System and Technology Limited, Ghana. **(**FYSAL®-DRY SP) an Antibiotic obtained from a veterinary outlet in Ogbomoso, Oyo State, Nigeria.

Three experimental diets were formulated at both the starter and finisher phases. Diet 1 – Control diet, Diet 2 – Control diet + RE3 and Diet 3 – Control diet + Fysal-Dry sp (Table I).

Table I: Gross composition of experimental diet (Starter and Finisher phases)

Ingredients (%)	Starter			Finisher		
	T 1	**T 2**	**T 3**	**T1**	**T 2**	**T 3**
Maize	55.00	55.00	55.00	57.00	57.00	57.00
Soybean meal	35.20	35.20	35.20	25.50	25.50	25.50
Wheat offals	1.50	1.50	1.50	10.00	10.00	10.00
Palm kernel cake	2.00	2.00	2.00	2.00	2.00	2.00
Fishmeal(72%)	2.50	2.50	2.50	1.50	1.50	1.50
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Bone meal	1.5	1.5	1.5	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.12	0.12	0.12	0.25	0.25	0.25
Methionine	0.18	0.18	0.18	0.25	0.25	0.25
*Premix	0.25[1]	0.25[1]	0.25[1]	0.25[2]	0.25[2]	0.25[2]
RE3	-	+	-	-	+	-
Fysal®-Dry Sp	-	-	+	-	-	+
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
DETERMINED ANALYSIS						
Dry matter (%)	91.28	90.63	90.72	90.05	89.98	90.13
Crude protein (%)	23.43	23.31	23.62	19.74	19.08	19.43
Crude fibre (%)	3.62	3.86	3.93	5.77	5.87	6.10
Ether extract (%)	3.53	3.68	3.76	3.68	3.57	3.60
Ash (%)	5.63	5.49	5.36	9.09	8.94	9.07
**Nitrogen Free Extract (NFE%)	55.07	54.27	54.05	51.77	52.52	51.93
Metabolizable Energy (kcal/kg)	2982.15	2982.15	2982.15	2982.15	2919.28	2982.15

T1= Treatment 1(control diet), T2= Treatment 2(control + RE3), T3= Treatment 3(control diet + Fysal®-Dry sp), **NFE= Nitrogen Free Extract = 100 – (%crude protein + %crude fibre + %moisture + %ash + %Ether extract)

*[1]*Premix composition per kg of diet: vitamin A, 12,000IU; vitamin D3, 2500IU; vitamin E, 30IU; vitamin K, 2.0mg; vitamin B1, 2.25mg; vitamin B2, 6.0mg; vitamin B6, 4.5mg; vitamin B12, 0.015mg; Niacin 40mg,Panthetonic acid 15mg; Folic acid 1.5mg; Biotin 0.05mg; Chlorine chloride 300mg; Manganese 80mg; Zinc 50mg; Iron 20mg; Copper 5.0mg; Iodine 1.0mg; selenium 0.2mg; Cobalt0.5mg; Antioxidant125mg.*

*[2]*Premix composition per kg of diet: vitamin A, 12,000IU; vitamin D3, 2500IU; vitamin E, 30IU; vitamin K, 2.0mg; vitamin B1, 2.25mg; vitamin B2, 6.0mg; vitamin B6, 4.5mg; vitamin B12, 0.015mg; Niacin 40mg; Panthetonic acid 15mg; Folic acid 1.5mg; Biotin 0.05mg; Chlorine chloride 300mg; Manganese 80mg; Zinc 50mg; Iron 20mg; Copper 5.0mg; Iodine 1.0mg; selenium 0.2mg; Cobalt0.5mg; Antioxidant125mg.*

Experimental Animal and Management: Two hundred and forty (240) one day old Marshal strain of broiler chicks was used for the experiment. The birds were randomly divided into three (3) treatments of four (4) replicates each at the rate of twenty (20) birds per replicate to make a total of eighty (80) birds per treatment in a complete randomized design (CRD) experiment. The birds were intensively managed on deep litter with woodshavings as litter materials. The birds were offered feed *ad-libitum*, clean and fresh drinking water was also supplied on daily basis throughout the experiment which lasted for eight (8) weeks. All necessary vaccinations and medications as recommended were done.

Feed samples were analyzed using the procedure of AOAC (2005).

***Collection of Blood Sample for Haematological and serum analysis*:** At the end of the experiment,

two sets of blood samples were collected from two (2) birds per replicate by cutting of the jugular vein.

The first sets of blood samples were dispensed into labeled bottle (sterilized) containing EDTA (Ethylene Di amine Acetic Acid) which serves as anticoagulant. Packed cell volume (PCV) and haemoglobin (Hb) were determined using Micro-Haematocrit method and Cyan Meth-Haemoglobin methods respectively as described by Mitruka and Rawnsley (1977). Red Blood Cells and White Blood Cells will be determined using the method described by Jain (1986)

The second sets of blood samples were collected into bottles devoid of anti-coagulant to allow clotting of the blood for serum biochemical analysis. The biuret method was utilized in the determination of the total protein fraction while the serum was subjected to the direct colorimetric method for albumin with Bromocresol Green (BCG) as the dye as described by Peters *et al*. (1982). The globulin concentration was obtained by subtracting albumin from the total protein. Total serum cholesterol was determined according to the methods of Roschlan *et al.* (1974). The globulin concentration was obtained by subtracting albumin from the total protein.

RESULTS AND DISCUSSION

The proximate composition of the experimental diet presented in Table 1, showed that the diets had comparable composition. Crude protein (23.31-23.62% for starter and 19.08-19.74% for finisher) and Metabolizable energy values of the diets were within the range recommended by Oluyemi and Roberts (2000) for broilers. The crude protein value was greater than 17.3% used by Herberts (1998) but close to the values 21.46% for starter and 19.77% for finisher of Chawla *et al*. (2013) while the metabolizable energy of finisher is lower than the value used by Chawla *et al*. (2013) but higher than that of Egbunike *et al*. (2009).

Haematological values are used to appraise the health status of animals. The haematological parameters of birds (Table II) under this experiment revealed that none of the parameters showed significant ($p>0.05$) differences between the treatments.

Table II: Haematological Parameters of Broiler chicken fed control, RE3 and antibiotic containing diets

Parameters	T1	T2	T3	SEM	P-value
PCV (%)	27.25	26.86	27.25	1.31	0.97
Hb (g/dl)	8.58	8.67	8.74	0.29	0.93
RBC ($x10^6/\mu l$)	3.27	3.63	3.32	0.27	0.60
WBC ($x10^3/\mu l$)	16.92	18.04	18.39	1.10	0.62
MCV (μ^3)	84.32	73.88	87.83	4.98	0.14
MCH (%)	265.79	243.83	282.49	17.29	0.31
MCHC (%)	31.49	33.29	32.11	1.15	0.54
Lymphocytes (%)	64.25	64.86	68.63	2.47	0.41
Heterophils (%)	29.38	30.86	23.88	2.39	0.12
Monocytes (%)	3.75	2.14	4.25	0.52	0.02
Eosinophils (%)	2.50	1.86	3.13	0.54	0.28
Basophils (%)	0.13	0.14	0.13	0.12	0.99
Platelets	139,000	135,143	129,750	10,847.53	0.83

SEM: standard error of mean

T1= Treatment 1(control diet), T2= Treatment 2(control + RE3),T3= Treatment 3(control diet + Fysal®-Dry sp)

However, values obtained falls within the normal range and values for chickens (Mitruka and Rawnsley, 1977; Ariello and Mays, 1998). This result corroborate the findings of Health and Olusanya (1985) who reported that inclusion of antibiotic in the diet of broiler had little or no effect on the relative quantity of blood cells as compared with the total volume of blood. This result is an indication that animals' health status was not compromised. The non-significant values between treatments for haemoglobin, PCV and blood constant suggests good quality nature of the test protein. The WBC and RBC were not affected by the dietary treatments thereby indicating that no pathological effect was induced. The absence of any effect of treatment on RBC may also be an indication that the test ingredients had the ability to improve iron metabolism which had been observed to increase the number of red blood cell and blood concentration of haemoglobin (NRC, 1994).

Serum biochemical analysis is used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acid utilization in animals (Harper *et al*., 1999). Table III shows that there were no significant differences ($P>0.05$) in the blood total protein, albumin and globulin values. This agrees with the findings of Nwambe and Elechi (2009) and Omoikhoje *et al* (2004).

Table III: Serum Biochemical Parameters of broiler chicken fed control, RE3 and antibiotic containing diets

Parameters	T1	T2	T3	SEM	P-value
Total protein (g/dl)	3.69	3.91	3.56	0.16	0.31
Albumin (g/dl)	1.39	1.45	1.44	0.08	0.83
Globulin (g/dl)	2.30	2.46	2.12	0.16	0.30
Cholesterol (mg/dl)	125.08[a]	97.52[b]	96.41[b]	6.30	0.01

[abc] Means within the same row with different superscripts differ significantly ($p<0.05$)
SEM: standard error of mean
T1= Treatment 1(control diet), T2= Treatment 2(control + RE3), T3= Treatment 3(control diet + Fysal®-Dry sp)

The total protein and albumin values among the treatments were within the normal physiological range reported by C.C.A.C (1980) for birds implying adequate nutrient utilization by the experimental animals.

Cholesterol is widely distributed in the body and it plays an important role in the synthesis of steroid hormones, bile salts and vitamin D. The cholesterol values in the present study were significantly ($P>0.05$) influenced by dietary treatments which contradicts the findings of Stanley *et al*. (1997). The value of cholesterol declined in broilers supplemented with antibiotic and probiotic treatments compared with the control treatment. The lower cholesterol level in birds on probiotic supplementation could be attributed to probiotic effect and its ability to bind cholesterol in the small intestines. Usman (1999) reported that strains of *Lactobacillus gasseri* could remove cholesterol from laboratory media via binding onto cellular surfaces. This is in agreement with the reports of Panda *et al.*, 2001;Kalavathy *et al.*, 2003; Jin *et al.*, 1998) and Kannan *et al*. (2005) that probiotic supplementation significantly reduces the serum cholesterol level of the chickens. Earlier, Tizard *et al.* (1989) had reported that manna oligosaccharide obtained from yeast in the ration of broiler chickens significantly reduces the serum cholesterol level and prevent cholesterol absorption in gastro intestinal tract. Synthesis of bile acids from cholesterol in the liver according to Wilson *et al*., (1998) is the most important way of cholesterol excretion. The mechanism as explained by some authors is that the use of probiotics and prebiotic can disintegrate bile salts and de-conjugate production of enzymes by the activity of lactic acid bacteria. Reduction of the pH in the intestinal tract can also be effective in reducing the cholesterol concentration. Solubility 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 (Klaver and Van der Meer, 1993). Consequently, the liver for re-establishment of the hepatic cycle of bile acids, convert more cholesterol concentration into the tissues and therefore their concentrations in the blood is reduced (Ros, 2000). Also Klaver and Van der Meer (1993) suggested that co-precipitation with bile acids might be of importance for decreasing serum cholesterol concentrations. The reduction of the value of cholesterol could also be as a result of the binding effect of the test material on the bile acids excreting such and thus resulting in lowering of serum cholesterol. This is in agreement with the finding of Onilude (1999) who reported consistent significant reduction in cholesterol concentration of birds. He adduced that the low cholesterol could be as a result of slight reduction in lipogenesis. Possible conversion of cholesterol into coprostanol by bacteria has also been evaluated by Chiang *et al*. (2008).

CONCLUSION

In this study the use of probiotic and antibiotic had beneficial effect in reducing serum cholesterol which has been a great barrier for consumption of poultry products. The haematological and serum biochemical indices observed showed that addition of probiotic to broiler diets proved beneficial and may serve as worthy alternative growth promoters to antibiotics the usage of which has been a serious concern especially the indiscriminate use as feed supplement in poultry. The fear of residue deposition of antibiotics in animal products which invariably pose health hazard to humans will be allayed.

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