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Growth, haematology and serum biochemistry of broilers fed probiotics based diets

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An experiment was conducted with a total of one hundred and twenty one-day old Marshall broiler chicks to determine the effect of probiotics (Biovet-YC and Gro-Up) on the performance characteristics, haematology and serum biochemistry of broilers. A completely randomized design was used and the experiment lasted for eight weeks. The birds were grouped into three treatments (T_1, T_2 and T_3 respectively). T_1 served as the control, T2 contained 0.1% Biovet$^{(R)}$ and T3 contained 0.1% Gro-up$^{(TM)}$ both at starter and finisher phase respectively. Data were collected on feed intake and weight gain, while blood samples were collected for haematological indices and serum biochemical indices examination. Bird on T3 had the highest live weight (2321.10 g), while those on control had the lowest (2117.00 g); the same trend was elicited for the feed conversion ratio. The results of haematology and serum biochemistry revealed significant *(P<0.05)* difference in the values obtained for lymphocytes, neutrophil, and cholesterol. The highest lymphocytes value was obtained in T1 (59.00%) while the lowest value was obtained in T2 (44.75%), but for neutrophil, T2 has the highest value of (54.75%) while T1 has the lowest (40.00%). The same trend was noticed for cholesterol. It can be concluded that the inclusion of probiotics in the diets of broilers will bring about improved live weight and feed conversion ratio, but can elevate the serum cholesterol value.

Key words: Probiotics, broilers, performance, haematology, serum.

INTRODUCTION

In the gut there are pathogenic bacteria, but the balance between pathogenic and beneficial bacteria determines whether or not disease will occur. The bacteria considered beneficial to the gut, include lactic acid forming bacteria like Lactobacillus spp, which prevent proliferation of pathogens, such as Salmonella spp., through competitive exclusion for nutrients and for receptor sites on the gut wall (Patterson and Burkholder, 2003). It can also produce an adverse environment for pathogenic bacteria to colonise and grow, by the production of short-chain fatty acids which lower the pH and prevent growth of pH sensitive pathogenic bacteria (Patterson, and Burkholder, 2003). The intestine is the biggest immune organ in the body, but to achieve appropriate protection from pathogens a complex gut microflora is essential. The microflora also have functions in the development of the digestive and immune tissue in the host animal, it can produce nutrients that can be used by the host as a nutrient source and also can neutralize some feed toxins and promote an environment in the gut where anti-nutritional factors and toxins are minimized (Dawson, 2001).

However, with the severe restriction of antibiotic feed additive use in the EU and increasing consumer concern

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Table 1. Gross composition of experimental diet at starter phase.

Ingredients (%)	T1	T2	T3
Maize	52.00	52.00	52.00
Wheat bran	9.32	9.32	9.32
Fish meal (72%)	3.00	3.00	3.00
Soya bean meal	17.00	17.00	17.00
Ground nut cake	15.00	15.00	15.00
Salt	0.24	0.24	0.24
Bone meal	2.00	2.00	2.00
Lysine	0.12	0.12	0.12
Methionine	0.12	0.12	0.12
Limestone	1.00	1.00	1.00
Premix (Starter)*	0.20	0.20	0.20
Total	100.00	100.00	100.00
Metabolisable energy (kcal/kg)	2864.60	2864.60	2864.60
Crude protein	22.23	22.23	22.23

*Vitamin-mineral premix (2.5 kg/1000 kg); vitamin A (10,000,000 IU), vitamin D3 (3,000,000IU), vitamin E (30,000 IU), vitamin K (2.3 g), vitamin B1 (2.0 g), Riboflavin (5.0 gr), Pyridoxine (3.0 g), vitamin B12 (160 mg), Biotin (60 mg), Niacin (31 g), panthotenic acid (8 g), folic acid (1 g),manganese (85 g), zinc (50 g), iron (25 g), copper (6 g), iodine (1 g), selenium (120 g), cobalt (220 mg), antioxidant (125 g), choline chloride (200 g).

in markets such as Japan, alternatives to antibiotic feed additives have been investigated and found to significantly influence this balance.

Probiotics are microorganisms that have a positive effect on the host by improving the balance of pathogenic to beneficial bacteria in the gut (Simon et al., 2001). The benefits of probiotics are based on two main functions, stimulating the growth of beneficial microflora and suppressing the growth of pathogenic bacteria. The potential health benefits associated with using a probiotics include improved digestion, stimulation of gastrointestinal immunity and increased natural resistance to enteric disease (Tellez et al., 2001). Modes of action that have been suggested include increasing the number of beneficial bacteria in the intestine and therefore improving the ratio of beneficial bacteria to pathogens (Simon et al., 2001). When a higher number of beneficial bacteria are present they are more likely to out-compete the pathogens for both nutrients and adhesion sites on the gut wall, a process known as competitive exclusion. Beneficial bacteria, such as Lactobacillus, are also known to release short chain fatty acids, bacteriocins and hydrogen peroxide, which have antagonistic effects on pathogenic bacteria (Patterson and Burkholder, 2003). This study was conducted to assess the effects of probiotics on the performance, haematology and serum biochemical indices of broilers fed at 1 g kg^{-1} of feed.

MATERIALS AND METHODS

Experimental plan

One hundred and twenty (120) unsexed day old chicks of Marshal strains were divided into three experimental group of 40 birds each. Each treatment group were replicated four times with each replicate comprising of 10 chicks. The birds were randomly assigned to each of the following dietary treatment: Diet 1 (0% probiotics inclusion), Diet 2 (0.1% Bio vet- YC®) and Diet 3 (0.1% Gro up ™) at starter and finisher phase respectively (Tables 1 and 2). The birds were acclimatized for 7 days to allow for physiological adjustment, after which data collection started. The parameters recorded were feed intake, body weights and mortality. Feed conversion and body weight gain were calculated using feed intake and body weight records. Body weight was recorded on weekly basis, while the feed intake was recorded on daily basis. All vaccinations and medication procedures were strictly adhered to. The starter ration was fed for 3 weeks, while finisher ration was fed for 4 weeks.

Haematological and biochemical indices examination

At week 8, two birds per replicate were bled using hypodermic needle and syringe. Blood was drained into two different carefully labelled bottles for haematological and serum biochemistry investigation. The blood samples for haematological parameters were collected into the bottle pre-treated with EDTA, an anticoagulant. Blood samples for biochemical indices were collected into another sample bottle containing no anti coagulant. These samples were spurned in the centrifuge at 3,000 rpm and the clearer portion decanted (after centrifugation) into small sample tubes stored in a freezer. The haematological indices examined include Red Blood count (RBC), white blood cell (WBC), Packed cell volume (PCV), Leucocytes differential count (monocyte, lymphocyte, eosinophil e.t.c) and haemoglobin concentration (Hb). Serum biochemical indices investigated were total protein, globulin, albumin, albumin:globulin ratio, creatinine, glucose, uric acid, cholesterol, alanine amino transferase (ALT) and aspartate amino transferase (AST).

The packed cell volume (PCV) was determined by spinning about 75 µl of each blood samples in heparinised capillary tube in a haematocrit centrifuge for about 5 min and read on haematocrit reader (Benson et al., 1989). Erythrocyte and Leucocytes counts

Table 2. Gross composition of experimental diet (Finisher phase).

Ingredients (%)	T1	T2	T3
Maize	59.00	59.00	59.00
Wheat bran	7.20	7.20	7.20
Fish meal (72%)	1.50	1.50	1.50
Soya bean meal	18.00	18.00	18.00
Ground nut cake	10.00	10.00	10.00
Salt	0.25	0.25	0.25
Bone meal	2.00	2.00	2.00
Lysine	0.10	0.10	0.10
Methionine	0.10	0.10	0.10
Limestone	1.60	1.60	1.60
Premix (finisher)*	0.25	0.25	0.25
Total	100.00	100.00	100.00
Metabolisable Energy (Kcal/Kg)	2913.76	2913.75	2913.76
Crude protein	19.63	19.63	19.63

*Vitamin-mineral premix (2.5 kg/1000 kg); vitamin A (11,000,000 IU), vitamin D3 (3,000,000 IU),vitamin E (30,000 IU), vitamin K (2.3 g), vitamin B1 (1.7 g), Riboflavin (5.2 g), Pyridoxin (3.7 g),vitamin B12 (18 mg), Biotin (76 mg), Niacin (37 g), panthotenic acid (9.2 g), folic acid (0.9 g),manganese (85 g), zinc (50 g), iron (25 g), copper (6 g), iodine (1 g), selenium (120 g), cobalt (220 mg), antioxidant (126 g), choline chloride (180 g).

were determined using Neubaur chamber method as described by Lamb (1981). The blood sample collected in each treatment was diluted at a ratio of 1: 200 for RBC counts using red cell diluting fluid while a dilution ratio of 1: 20 (blood: white cell diluting fluid) was used for WBC counts. Samples of RBC and WBC counts were obtained using the relationship:

RBC/µl = Numbers of red blood cells counted × 5 × 10 × 200

WBC/µ = Numbers of white blood cells counted × 0.25 × 10 × 20.

Haemoglobin was estimated using cyanomethaemoglobin method. 0.02 ml of blood was expelled into 4 ml drakkins solution. The mixture was allowed to stand for 5 min for full colour development. Sample haemoglobin concentration was obtained using this relationship:

$$\text{Sample haemoglobin} = \frac{\text{Reading of test x standard haemoglobin concentration (g/100 ml)}}{\text{Reading of Standard}}$$

The haemoglobin (Hb) concentration and the blood constants: mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean cell volume were determined using cyaethaemoglobin method and appropriate formula, respectively (Jain, 1986):

$$\text{MCH} = \frac{\text{Haemoglobin (g per 100 ml)}}{\text{RBC count million per cu.mm}} \times 10$$

or

$$\text{MCH} = \frac{\text{Haemoglobin (g/L)}}{\text{RBC } (10^{12}/\text{L})}$$

$$\text{MCHC} = \frac{\text{Haemoglobin (g/dl)}}{\text{PCV \%}} \times 100$$

The smear of each blood samples in the bottles containing EDTA was made on a clean slide, air dried and fixed in methanol for three minutes. The smear was stained in Giemsa stain for 30 min, rinsed in water and air- dried. The cell count for each sample was carried out (Lamb, 1981). During the process, the slide was viewed under the microscope and cells were counted. From the cell counted, the percentage of different cells (neutrophils, eosinophils, lymphocytes and monocytes) was determined.

The serum total protein was determined by the Biuret method (Reinhold, 1953) using a commercial kit (Randox Laboratories Ltd, U.K), while albumin value was obtained by bromocresol green method (Doumas and Biggs, 1971). The globulin and albumin-globulin ratio were determined according to the method of Coles (1986). The serum creatinine and urea nitrogen were estimated by deproteinisation and Urease-Berhelot colorimetric methods, using a commercial kit (Randox Laboratories Ltd, U.K). Also the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods, respectively using commercial kit (Quimica Clinica Applicada, S.A), while the serum enzymes; Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits.

Data analysis

All data generated on performance, haematology and serum biochemistry of the experimental birds were subjected to statistical analysis of variance procedures of SAS Institute Inc (SAS, 2006). The treatment means were compared using the Duncan's procedures of the same software.

RESULTS AND DISCUSSION

Performance characteristics of broilers fed the experimental diets

The mean average final weight (AFW) was 2117.00,

Table 3. Proximate composition of the experimental diet.

Parameter (%)	T1	T2	T3
Dry matter	88.80	88.20	88.20
Crude protein	19.30	19.00	18.60
Ether extract	5.40	5.20	5.00
Crude fibre	4.70	4.40	4.70
Ash content	11.60	11.90	12.00
Nitrogen free extract	47.80	46.80	47.90

Table 4. Performance characteristics of broilers fed probiotics based diets.

Parameter	T1	T2	T3	±SEM
Initial weight (g/bird)	143.50	147.75	152.50	1.63
Final weight (g/bird)	2117.00^{b}	2250.00^{ab}	2321.10^{a}	67.03
Weight gain (g/bird)	1973.50	2103.20	2168.60	66.45
Total feed intake	5759.80	5867.10	5738.00	79.03
Feed conversion ratio	2.92^{a}	2.79^{ab}	2.65^{b}	0.09
Mortality	1.75	1.25	1.00	0.31

abc, Mean with difference superscript are significantly different (P<0.05).

2250.00 and 2321.10 g for treatments T1, T2 and T3, respectively. Significant P<0.05) difference was observed among treatment for AFW. Birds on probiotic based diets had significant higher final weight compared to those on the control (Table 4). Although the birds consumed similar feed and had similar weight gain, significant difference were observed in the mean values obtained for feed conversion ratio. The FCR ranged from 2.65 to 2.92 with birds on 0.1% Group ™ having the best FCR, while those on control diet had the lowest. It was hypothesised that the increasing the level of probiotics would consistently increase the live weight and weight gain of experimental birds (Altaf et al., 2009). This could be because the microbes present in probiotics would secrete amylase, protease, and lipase, which would enhance the catalytic activities of the endogenous enzymes to liberate more energy from hydrolysing the energy in the feed ingredients. Such higher quantity of liberated energy would help to improve the weight gain and live weight of chicks fed probiotics rations compared the chicks fed rations containing no probiotics. The present study was in agreement with the above hypothesis as the birds on probiotics based diet had higher live weight compared to the control.

The inclusion of probiotics in the intestinal tract of the birds may be secreting amylolytic, cellulolytic, proteolytic, and lypolytic enzymes (Biswas et al., 1999; Was et al., 1999; Bedford, 2001; Lazaro et al., 2003; Józefiak et al., 2004), which provide maximum help to enhance the digestibility of starch, protein and fat component and liberated maximum energy. Such energy would not only improve the overall vital activities in the birds, but also improve live weight and weight gain. The results of this study are in agreement with those reported by Chiang and Hsieh (1995), who obtained maximum growth response in broilers, even using a probiotic at very low level such as 0.25 or 0.5 g/kg feed starter ration. Omprakash et al. (1996) also found the highest weight gain (500 to 550 g/chicks) by incorporating probiotic at 15 or 20 ml/L drinking water in broiler at started phase. The present findings are further supported by the results of many researchers (Shoeib and Madian, 2002; Sklan, 2002; Cross, 2002; Muneer et al., 2002; Lazaro et al., 2003; Josefiak et al., 2004; Kabir et al., 2004); they all predicted that the all beneficial species of *Lactobacillus* if added in the form of probiotic had the efficiency to produce energy nutrient digesting enzyme, which could be able to accelerate the catalytic activities of the endogenous enzymes, which could be highly effective in improving weight gain and live weight in broiler chicks. The non significant effect of the probiotics on the weight gain in this study agrees with the findings of Eden (2003), who reported that the addition of a probiotic, did not affect weight gain of broilers at 42 days of age; however, it improves feed conversion.

Birds on control ration showed poor feed conversion ratio compared to those on probiotic ration (Table 3). These findings are further supported by the work of many researchers (Jin et al., 1997; Pedron et al., 1997; Nezami et al., 2000; Gonzalez et al., 2001), they also reported best FCR for chicken raised on ration containing high levels (5 to 10 g/kg feed) of probiotics. The improved FCR might be due to maintaining normal intestinal micro flora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activities and by promoting digestion rate of energy nutrient.

Table 5. Cost analysis of broilers fed probiotic based diets.

Parameter	T1	T2	T3	±SEM
Feed intake (g)	5759.80	5867.10	5738.00	79.03
Cost of feed/kg (#)	88.41	89.71	89.71	0.00
Cost of feed consumed/bird (#)	510.01	519.72	518.24	7.46
Weight gain (g)	1973.50	2103.30	2168.60	66.46
Cost of feed/kg gain	259.34	247.57	245.63	9.26

abc, Mean with difference superscript are significantly different (P<0.05).

Table 6. Haematological indices of broilers fed probiotics based diets.

Parameter	T1	T2	T3	±SEM
Packed cell volume (%)	27.75	25.00	25.50	1.00
Haemoglobin (g/100ml)	9.87	8.35	8.35	0.36
Red blood cell (x 10^3/mm^3)	3.20	3.19	3.08	0.20
White blood cell (x10^3 /mm^3)	5.80	8.45	6.90	1.03
Mean cell volume (μ^3)	95.00	80.75	84.25	3.99
Mean corpuscular haemoglobin (Fl)	31.00	26.50	27.00	1.28
Mean cell haemoglobin concentration (%)	33.00	33.00	32.25	0.25
Lymphocytes (%)	59.00^a	44.75^b	49.00^b	2.18
Neutrophil (%)	40.00^b	54.75^a	50.50^a	2.25

Abc, mean with difference superscript are significantly different (p<0.05).

Table 7. Serum biochemical indices of broilers fed probiotics based diets.

Parameter	T1	T2	T3	±SEM
Total protein (g/dl)	2.88	2.09	2.04	0.18
Albumin (g/dl)	1.06	1.31	1.39	0.16
Urea (mg/dl)	0.72	0.68	0.72	0.03
Creatinine (mg/dl)	0.92	0.92	0.80	0.05
Aspartate aminotransferase (IU/L)	114.69	119.75	112.55	2.15
Alanine aminotransferase (IU/L)	19.80	13.96	13.84	1.71
Globulin (mg/dl)	1.24	0.79	0.65	0.14
Cholesterol (mg/dl)	100.96^b	105.64^{ab}	122.02^a	4.17
Triglyceride (mg/dl)	123.95	121.57	127.88	3.16

abc, mean with difference superscript are significantly different (P<0.05).

The results of the cost benefit ratio are as presented in Table 5. There was no significant (P>0.05) difference in the mean values obtained for all parameters measured. This results agrees with the findings of Tarum (2008), who reported a non significant in cost of feeding broilers with difference strains of probiotics.

Haematological and serum biochemical indices

The result of haematological and serum biochemical parameter are as presented in Tables 6 and 7. Apart from leucocyte differential counts that was significantly (P<0.05) influenced by the dietary treaments, all other haematological parameters measured were not significant (P>0.05). There was no significant (P>0.05) in all the serum biochemical indices examined except for the cholesterol which was significantly influenced by the dietary treatment. It was observed that the lymphocyte and neutrophil counts were similar for the probiotic based diets which were significantly (P>0.05) lower than the values obtained for the control. However, the control had the lowest cholesterol level. The reduction in lymphocytes counts in the present study agrees with the findings of Kamruzzaman et al. (2005), who reported a significant reduction in the lymphocytes counts of broilers fed

200 mg/kg of probiotics compared with the control. However, the increased in the values of cholesterol of broilers fed probiotic based diets did not conformed with report of Islam et al. (2004) and Jouybari et al. (2010), who reported a reduced cholesterol and triglycerides in broilers fed feed containing probiotic in starter, grower and finisher phase.

Conclusion

The results of this study revealed that broilers can be fed with 0.1% probiotics in broilers diets without any detrimental effects on performance parameters, haematology and serum biochemical indices, but bring about improvement in live weight and feed conversion ratio.

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