



International Journal of
**Agricultural
Research**

ISSN 1816-4897



Academic
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Effects of some Growth Promoters on Blood Hematology and Serum Composition of Broiler Chickens

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ABSTRACT

This study was designed to compare the effects of antibiotic, probiotic, prebiotic and organic acid supplements on the hematological and biochemical constituents of blood of broiler chickens. The 450 broiler chickens of the commercial Hubbard Flex strain were used in a randomized completely design with 5 groups, 3 replicates in each group and 30 birds replicates. Birds were raised on floor pens for 42 days. Group (1) Basal diet with no additives (control), group (2) Basal diet+100 g ton⁻¹ antibiotic (lincomycin), group (3) Basal diet+1000 g ton⁻¹ probiotic (*Bacillus subtilis* spp), group (4) Basal diet+1000 g ton⁻¹ prebiotic (Mannan oligosaccharide MOS) and group (5) Basal diet+1000 g ton⁻¹ organic acid (sorbic acid, propionic acid, benzoic acid, phosphoric acid). On day 42 of the experiment, two birds from each replicate were selected and the blood samples were taken from the wing artery to determine some blood indicators. The results showed that there was no significant differences ($p>0.05$) in the Red Blood Cells (RBC) number and blood hemoglobin of birds in all groups. Significant increase in number of White Blood Cells (WBC) in blood samples of probiotic and organic acids groups compared to antibiotic group, significant increase was obvious in the HCT % of the group fed on probiotic compared to the other groups ($p<0.05$). There was no significant difference in blood glucose levels between all groups, a decrease in the blood level of total cholesterol and triglyceride was observed in groups fed on probiotic and organic acids compared to other groups. A significant increase was also observed ($p<0.05$) in HDL cholesterol level in groups fed on probiotic and prebiotic and a significant decrease ($p<0.05$) in LDL cholesterol level in the same groups as compared with the other groups.

Key words: Broiler, growth promoters, antibiotic, hematology, serum

INTRODUCTION

Antibiotics have been used to protect the animal health and to improve growth for many years. Aim of using antibiotics in feed sector was to get advantage from feeding and increasing protection against some diseases, toxins and making better the absorption of nutrients in intestines. Confidence has diminished about antibiotics used for improving performance and reducing stress factors because of the risk development of bioresistance against bacteria in human. The recent European Union ban on the prophylactic use of in-feed antibiotics and has escalated the search for alternatives for use within the poultry industry (Janardhana *et al.*, 2009). Currently, natural alternatives like probiotic, prebiotic, plant extracts and the organic acids seem the most interesting alternative supplement in regard of minimizing economic loses (Yang *et al.*, 2008; Pirgozliev *et al.*, 2008).

Probiotics are defined as live bacteria-yeast cultures or biological products that are added to drinking water or feed to regulate the ecological balance of microflora in the digestive tract of animals. These substances prevent the harmful effects of potentially pathogenic microorganisms and allow animals to derive increased benefits from the feed (Dibner and Richards, 2005; Al-Mansour *et al.*, 2011). A prebiotic defined as, a non-digestible food ingredient that can be utilized by intestinal microflora which beneficially affects the host (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003). Oligosaccharides are carbohydrates composed of short chains of monosaccharide. Some are thought to enhance the growth of beneficial organisms in the gut and others are thought to function by competing with pathogenic bacteria for attachment sites in the lumen. In this way, prebiotic oligosaccharides may improve host health. Organic acids (for example, lactic acid, fumaric acid, propionic acid, citric acid, formic acid and acetic acid) create an acidic environment by decreasing the pH in the digestive tract which prevents the development of pathogenic microorganisms and increases enzyme activity. Moreover, this increases the digestibility and utility of minerals such as iron, calcium, phosphorous, magnesium and zinc as well as proteins and amino acids (Gunal *et al.*, 2006). Therefore, the objectives of the present study were to compare the effects of antibiotic, probiotic, prebiotic and organic acids on hematological parameters and serum biochemical of broiler chickens.

MATERIALS AND METHODS

Experimental design: In this study, 450 broiler chickens of the commercial “Hubbard Flex” strain were used in a randomized completely design with 5 groups; 4 treatment groups and a control group, 3 replicates in each group and 30 birds replicates. Birds were raised on floor pens (300×300×100 cm) for 42 days and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness.

Feeding: The chicks were fed as standard starter (1-14 days), grower (15-35 days) and finisher (36-42 days) diets according to feed mixture followed in the Department of Animal Production at the University of Damascus (Table 1). Groups were randomly assigned to following treatment groups:

Group 1: Basal diet with no additives (control)

Group 2: Basal diet+100 g ton⁻¹ antibiotic (lincomycin)

Group 3: Basal diet+1000 g ton⁻¹ probiotic (*Bacillus subtilis* spp)

Group 4: Basal diet+1000 g ton⁻¹ prebiotic (Mannan oligosaccharide MOS)

Group 5: Basal diet+1000 g ton⁻¹ organic acid (sorbic acid, propionic acid, benzoic acid, phosphoric acid)

Table 2 shows the analysis of nutrient material in testing portion.

Hematological parameters: At 42 days of age, two birds were chosen randomly from each replicate for bleeding. The 3 mL of blood was collected from wing vein from two birds of each replicate into anticoagulant EDTA treated tubes for determination of total Red Blood Cell (RBC), total White Blood Cell (WBC), hemoglobin (HB) and hematocrit (HCT). The following hematological parameters were assessed by using Automatic Fully Digital Hematology Analyzer, BC- 3000 Plus, Shenzhen Mindray, Bio-Medical Electronics Co., LTD.

Table 1: Composition of basal diet in different periods of the experiment

Ingredient (%)	Period (days)		
	Starter (0-14)	Grower (15-35)	Finisher (36-42)
Corn	60.2	69.0	74.0
Soybean meal (44%)	35.8	27.0	22.0
Dicalcium phosphate	2.2	2.2	2.2
Calcium carbonate	1.0	1.0	1.0
Salt	0.4	0.4	0.4
DL-Methionine	0.1	0.1	0.1
Colin chloride	0.1	0.1	0.1
Vitamin premix	0.1	0.1	0.1
Mineral premix	0.1	0.1	0.1
Total	100.0	100.0	100.0

Table 2: Nutrient content of the basal diets

Nutrient content	Period (days)		
	Starter (0-14)	Grower (15-35)	Finisher (36-42)
ME (kcal kg ⁻¹)	2866.80	2971.00	3031.00
Crude protein (%)	21.17	18.10	16.34
ME/cP	135.40	164.30	185.50
Crude fat (%)	2.77	3.03	3.18
Calcium (%)	0.96	0.94	0.93
P available (%)	0.77	0.74	0.73
Methionine (%)	0.47	0.42	0.40
Lysine (%)	1.27	1.00	0.85

Serum biochemical parameters: On 42 day of experimental period, 3 mL of blood was collected from wing vein from two birds of each replicate (from six birds of each treatment). Serum was isolated by centrifugation at 2,000×rpm for 10 min. The serum concentrations of glucose, total triglyceride, cholesterol, High Density Lipoprotein (HDL), cholesterol and Low Density Lipoprotein (LDL) ratio in serum samples were analyzed by an automatic biochemical analyzer (Clima, Ral. Co, Spain).

Statistical analysis: Data was subjected to a one-way analysis of variance (ANOVA) and the comparison of means was carried out through Least Significant Differences (LSD) test. All statistical analyses were done using SPSS program (SPSS, 2002).

RESULTS AND DISCUSSION

Table 3 shows the effect of different diets on hematological blood parameters, no significant differences were observed in the RBC number of birds of different treatment except for a non significant ($p>0.05$) increase in the red blood cells number in antibiotic and probiotic treatments. Also, results showed that a significant increase in the number of White Blood Cells (WBC) ($p<0.05$) was observed in all treatments except for antibiotic treatment which showed a significant decrease compared to organic acid and probiotic treatment, respectively. It seems that the microbial interactions and effects on local immune stimulation results increase of white blood cells and immunity. These results are accordance with findings of Zarehshahneh *et al.* (2007) and EFSA (2010) but are not accordance with findings of Shareef and Al-Dabbagh (2009).

Table 3: Values of blood hematological parameters of experimental groups

Hematological parameters	Dietary treatments					LSD (5%)
	Control	Antibiotic	Probiotic	Prebiotic	Organic acid	
RBC ($\times 10^6 \mu\text{L}^{-1}$)	2.92 ^a	2.96 ^a	2.94 ^a	2.81 ^a	2.83 ^a	-
WBC ($\times 10^3 \mu\text{L}^{-1}$)	25.78 ^{ab}	24.82 ^a	26.43 ^b	25.85 ^{ab}	26.85 ^b	1.24
Hemoglobin HB (mg/100 mL)	11.36 ^a	12.15 ^a	11.72 ^a	11.87 ^a	11.05 ^a	-
Hematocrit (HCT) (%)	31.17 ^a	31.67 ^{ab}	33.00 ^b	31.33 ^{ab}	30.17 ^a	1.69

Means in each row with different superscripts are significantly different ($p < 0.05$)

Table 4: Values of serum biochemical parameters of experimental groups

Serum biochemical parameters	Dietary treatments					LSD (5%)
	Control	Antibiotic	Probiotic	Prebiotic	Organic acid	
Glucose (mg dL ⁻¹)	233.87 ^a	237.28 ^a	234.84 ^a	233.30 ^a	229.39 ^a	-
Triglycerides (mg dL ⁻¹)	56.40 ^a	63.43 ^a	45.33 ^{bc}	57.84 ^a	53.67 ^{bc}	10.28
Total cholesterol (mg dL ⁻¹)	143.53 ^a	152.84 ^b	130.02 ^a	140.87 ^{ad}	132.77 ^{cd}	9.02
HDL (mg dL ⁻¹)	65.53 ^a	67.08 ^{bc}	73.28 ^{bc}	76.77 ^b	66.53 ^{bc}	7.32
LDL (mg dL ⁻¹)	42.38 ^a	43.74 ^a	31.67 ^b	35.77 ^{bc}	40.52 ^{bc}	5.41

Means in each row with different superscripts are significantly different ($p < 0.05$)

No significant differences were found in the blood hemoglobin levels in all treatments ($p > 0.05$) in spite of an increase in the antibiotic treatment. This result did not resemble the results of the Al-Mayah and Al-Ahmed (2005). It may be due to the type of antibiotic and its mechanism of action or place of impact.

Significant increase was also noted in haematocrit values (HCT) in probiotic treatment compared with the another treatments and this is in agreement with the study done by Cetin *et al.* (2005), who observed that the probiotic supplementation caused statistically significant increase in the erythrocyte count, haemoglobin concentration and haematocrit values of Turkeys. In contrast, the findings disagree with Djouvinov *et al.* (2005), who found that the probiotic supplementation did not affect the blood constituents comprising haemoglobin concentrations. The differences may be attributed to type and number of species of bacteria present in probiotics.

Levels of serum glucose, triglyceride, total cholesterol, HDL and LDL are shown in Table 4. There was no significant effect of treatments on glucose levels, the non significant decrease in blood glucose level of organic acids treatment is in agreement with Adil *et al.* (2010). A significant decrease was observed in triglycerides and cholesterol values in the probiotic, organic acid and prebiotic treatments, respectively. A decrease of serum cholesterol via adding acidifier to diet was also reported by Abdel-Fattah *et al.* (2008). The role of organic acids in decreasing blood fat may be explained by their effect on decreasing intracellular microbes by preventing microbial enzymes activity and forcing cellular bacteria to use energy in order to release protons which cause forming of mass intracellular anions. In agreement to present results, Taherpour *et al.* (2009) and Ashayerzadeh *et al.* (2010) noted the decline of plasma cholesterol level by adding probiotic and prebiotic to broiler diets the significant reduction in serum cholesterol level could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic or prebiotic supplementation (Mohan *et al.*, 1995, 1996) also Patterson and Burkholder (2003) noted that decreasing in triglycerides and attributed the reason that probiotic and prebiotic secrete inulin which have an inhibitory effect on the process of building triglycerides.

Addition of antibiotic showed a higher blood triglyceride and cholesterol which is in agreement with Li *et al.* (2007) and Mansoub (2011), who attributed it to the low effect of these antibiotics on the absorption of fat in the GI. The serum HDL concentration was significantly increased in probiotic and prebiotic treatments compared to control ($p < 0.05$). This is in agreement with Kalavathy *et al.* (2003), Jouybari *et al.* (2009) and Hashemzadeh *et al.* (2013). Results also showed significant decrease ($p < 0.05$) in the serum LDL concentration in probiotic and prebiotic treatment compared to other groups which agrees with Panda *et al.* (2006) who attributed this decrease to the relationship between LDL and triglycerides.

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