

ISSN 1682-8356  
ansinet.org/ijps



INTERNATIONAL JOURNAL OF  
**POULTRY SCIENCE**

**ANSI***net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Effects of Dietary Supplementation of Probiotic and Synbiotic on Broiler Chickens Hematology and Intestinal Integrity

S.S.M. Beski<sup>1</sup> and S.Y.T. Al-Sardary<sup>2</sup>

<sup>1</sup>School of Animal Production, University of Duhok, Kurdistan Region, Iraq

<sup>2</sup>College of Agriculture, University of Salahaddin, Kurdistan Region, Iraq

**Abstract:** A total of 525 d-old male Ross 308 chicks, were obtained from a local hatchery (Jin hatchery, Duhok, Kurdistan region, Iraq) and randomly assigned to five dietary treatments, each with three replicates, 35 chickens per replicate. The birds were reared in floor pens. The five diet treatments were the control (no supplement), diets supplemented with probiotic at 2.5 or 5 g/kg and diets supplemented with synbiotic at 2.5 or 5 g/kg diet. The five treatments were arranged in a completely randomized design. Probiotic and synbiotic were supplemented throughout the 42 d experimental period. Feed and water were provided *ad libitum*. This study was conducted to investigate the effects of dietary supplementation of probiotic and synbiotic on some haematological and serum biochemical parameters of broiler chicken at 42 d of age. Supplementation the broiler diet with probiotics and synbiotics resulted in a significant ( $p < 0.05$ ) increase in the concentration of Hb. The H/L ratio also reduced due to the addition of supplements to the broiler diets. Supplementation of broiler diet with synbiotic significantly ( $p < 0.05$ ) reduced the concentration of serum cholesterol and LDL of broiler chickens. Histological results showed that broiler chickens that fed on diets supplemented with probiotics and synbiotics have higher villi than control group. Supplementation of probiotic and synbiotics could have positive effects on the productive performance of broiler thereby improving their haematological and intestinal histological aspects.

**Key words:** Productive performance, broiler chickens, broiler diet

### INTRODUCTION

Animals including poultry are vulnerable to potentially pathogenic microorganisms in the small intestine which competes with the host for nutrients (Engberg *et al.*, 2000). This leads to decrease growth performance and to increase the incidence of disease. Antimicrobial compounds have been used in the poultry industry to improve the health status and performance of birds by reducing the population of the bacteria present in the gastrointestinal tract (Fairchild *et al.*, 2001). Growth stimulating antibiotic, by the spread of antibiotic resistant bacteria, are a threat to human health (Turnidge, 2004). Therefore, restrictions on the use of antibiotics as a growth-stimulating feed supplements imposed in many countries resulted in intensification of scientific researching for various alternative feed supplements which could replace those used in the past. Alternative feed supplements include enzymes; probiotic, organic acids, as well as various prebiotic and phytogetic preparations are available to poultry (Giedrius *et al.*, 2008). The probiotic is a product that contains a dynastic vital microorganisms 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). Prebiotics have been defined as non digestible feed

ingredients which are growth substrates specifically directed towards potentially beneficial bacteria. Several studies have shown that the addition of prebiotics to the diet of broiler, layer and pig leads to improved performance through improving gut microflora (Xu *et al.*, 2003; Pelicano *et al.*, 2004). The combination of a prebiotic and probiotic as a single administration is called synbiotic which is characterized by antimicrobial, anticarcinogenic, antiallergic and immune stimulating actions. It also improves the absorption of minerals, protects from diarrhea and optimizes nutrient digestion processes (Gruzauskas *et al.*, 2004). This study was aimed to evaluate the effect of organic acids mixture, probiotic and synbiotic on some hematological and serum biochemical contents of 42 days old broiler chicks of both sexes.

### MATERIALS AND METHODS

A total of 525 d-old male Ross 308 chicks, were obtained from a local hatchery (Jin hatchery, Duhok, Kurdistan region, Iraq) and randomly assigned to five dietary treatments, each with three replicates, 35 chickens per replicate. The birds were reared in floor pens. The five diet treatments were the control (no supplement), diets supplemented with probiotic at 2.5 or 5 g/kg and diets supplemented with synbiotic at 2.5 or 5

g/kg diet. The five treatments were arranged in a completely randomized design. Probiotic and synbiotic were supplemented throughout the 42 d experimental period. Feed and water were provided *ad libitum*. The room temperature was gradually decreased from 33°C on d 1 to 24±1°C at 35 d. Eighteen hours of lighting were provided/day throughout the duration of the experiment, apart from days 1 to 7 when 23 h of lighting were provided. On d 42 blood samples were collected to measure the whole blood hematological parameters and serum was harvested for serum biochemistry.

**Synbiotic preparation:** The synbiotic used in this study was prepared by mixing the probiotic, prebiotic and sugar according to (Mirza, 2009). The probiotic used in this trial was a locally product which was prepared by Dr. Saad Naji (College of Agriculture, Bagdad University). Types and numbers of microorganisms contained are shown in (Table 1).

Jerusalem artichoke was used as the source of prebiotic. It contains inulin in the form of fructooligosaccharide (FOS). The Jerusalem artichoke was cut into small pieces and then dried in the oven at temperature of 50°C for 48 h and then the dried pieces were grinded to powder and mixed with the probiotic. About 250 g of table sugar was heated until it changed to light brown color and dissolved in 0.5 L of water and added to the mixture of probiotic and prebiotic and well mixed to form the locally prepared synbiotic which was a mixture of 1:0.75:0.25 probiotic, prebiotic and table sugar, respectively.

**Measurements:** Blood samples (approximately 3 mL) from four birds (2 males and 2 females) were collected from the jugular vein into heparinized tubes for estimation of whole blood red blood cells, packed cell volume (PCV), Hb and Erythrocyte sedimentation rate. PCV% was determined by using micro-hematocrite method according to the method of (Archer, 1965). Hemoglobin (Hb) concentration was determined by Drabkin's reagent. Erythrocyte Sedimentation Rate (ESR) was determined by using Standard Wistergreen tubes according to (AL-Darajy *et al.*, 2008). The determination of H/L ratio was performed by preparing a blood film stained by Wright Giemsa stain. At minimum 100 cells from the film were examined. The H/L ratio was estimated by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel, 1983). Subsequently, serum was harvested by collecting the blood into non-heparinized tubes and centrifuged at 3000 rpm for 15 min and stored in the freezer for analyses. Serum biochemical contents including blood glucose (mg/dL), lipid profile test including Total cholesterol, Triglycerides, HDL-C, LDL-C (mg/dL) and Total protein (g/dL) were determined Spectrophotometrically. The serum biochemical

Table 1: Types and numbers of microorganisms in the probiotic

No. of microorganisms in each g of product	Kind of microorganisms
10 <sup>6</sup>	<i>Acidophilus Lactobacillus</i>
10 <sup>6</sup>	<i>Bacillus subtilis</i>
10 <sup>6</sup>	<i>Bifidobacterium</i>
10 <sup>6</sup>	<i>Saccharomyces cerevisiae</i>

contents were measured by using commercial kits. LDL was calculated by the Friedewald formula (Friedewald *et al.*, 1972):

$$\text{LDL-cholesterol} = \frac{\text{Total cholesterol-HDL-cholesterol-Triglycerides}}{5}$$

Tissue samples from ileum of experimental chicken (1 male and 1 female) of each replicate were removed and fixed in (10%) neutral buffer formalin.

The tissue samples were by auto-processing apparatus, thereafter the slide sections were prepared by rotary microtome type (manubeni, Erma-Tokyo). The sections were examined to determine the ileum villus length and crypt depth by using microscope (TRIUP international corp.) and the villus length and crypt depth were measured by using micrometer stage for calibration objective lens (10X) with ocular micrometer.

**Statistical analysis:** All data collected were subjected to analysis using one way ANOVA procedure of SAS (SAS/STAT vs 9.1, SAS Institute Inc., Cary, NC) followed by comparisons using Duncan's multiple range tests.

## RESULTS AND DISCUSSION

**Hematology:** For the selected hematological parameters-RBC, Hb concentration, PCV percentage, ESR and H/L ratio-the significantly ( $p < 0.05$ ) higher Hb concentration and lower H/L ratio were observed in chickens fed on diets with probiotic and symbiotic than the control group (Table 2). The results were in line with the findings of AL-Kassie *et al.* (2008) who found that the supplementation of probiotic to the broiler diet at a rate of 10 g/kg significantly increased Hb concentration and significantly decreased the H/L ratio at 42 days old of chicks compared to the control. Silvia *et al.* (2008) found that the addition of probiotic and synbiotic to broiler diet had no significant effects on RBC count at 42 days. In contrast Agawane and Lonkar (2004) found no differences in Hb concentration due the addition of probiotic to the diet of broiler for 42 d. also Sarinee *et al.* (2008) did not find any effect of probiotic on the H/L ratio of broiler at 42 days when added to their drinking water in comparison with the control. The higher Hb concentration in the chicks received probiotics and synbiotic may be due to the acidic media of the alimentary tract caused by prebiotic fermentation which resulted in better iron salt absorption from the small intestine. This may also caused better vitamins B

Table 2: Effect of probiotics and symbiotic on some hematological parameters of 42 d old broilers (means±standard error)

Treatments	RBC*10 <sup>9</sup>	Hb g/dL	PCV (%)	ESR (mm/h)	H/L ratio
Control	2.7±0.16 <sup>b</sup>	8.6±0.38 <sup>b</sup>	29.8±0.91 <sup>ab</sup>	2.0±0.08 <sup>ab</sup>	0.71±0.049 <sup>a</sup>
2.5 g/kg probiotic	2.9±0.15 <sup>ab</sup>	8.8±0.37 <sup>bc</sup>	28.5±0.49 <sup>ab</sup>	2.3±0.17 <sup>a</sup>	0.43±0.054 <sup>b</sup>
5 g/kg probiotic	2.9±0.16 <sup>ab</sup>	9.7±0.43 <sup>ab</sup>	28.2±0.83 <sup>b</sup>	2.1±0.10 <sup>ab</sup>	0.47±0.036 <sup>b</sup>
2.5 g/kg synbiotic	3.1±0.10 <sup>ab</sup>	10.6±0.34 <sup>a</sup>	30.3±0.68 <sup>a</sup>	1.8±0.10 <sup>b</sup>	0.52±0.039 <sup>b</sup>
5 g/kg synbiotic	3.0±0.08 <sup>ab</sup>	10.0±0.40 <sup>ab</sup>	29.1±0.44 <sup>ab</sup>	2.3±0.10 <sup>a</sup>	0.46±0.042 <sup>b</sup>
Overall mean	2.9±0.06	9.3±0.17	29.3±0.28	2.1±0.05	0.52±0.023

<sup>a,b,c</sup>: Mean values on the same column not sharing a superscript are significantly different (p<0.05)

Table 3: Effect of probiotics and symbiotic on serum biochemical content of 42 d old broilers (means±standard error)

Treatments	Glucose <sup>a</sup>	TP <sup>b</sup>	Chol <sup>c</sup>	Trigl <sup>d</sup>	HDL <sup>e</sup>	LDL <sup>f</sup>
Control	227.7±11.0 <sup>a</sup>	3.1±0.1 <sup>a</sup>	183.8±7.0 <sup>ab</sup>	120.5±6.0 <sup>a</sup>	87.3±3.2 <sup>ab</sup>	72.4±7.6 <sup>a</sup>
2.5 g/kg probiotic	239.7±8.2 <sup>a</sup>	3.1±0.1 <sup>a</sup>	179.0±4.1 <sup>ab</sup>	124.2±8.9 <sup>a</sup>	94.3±4.8 <sup>a</sup>	59.8±5.8 <sup>ab</sup>
5 g/kg probiotic	230.3±8.19 <sup>a</sup>	2.9±0.07 <sup>a</sup>	168.3±6.8 <sup>bc</sup>	122.5±6.4 <sup>a</sup>	80.0±3.7 <sup>b</sup>	63.8±3.9 <sup>ab</sup>
2.5 g/kg synbiotic	232.0±13.8 <sup>a</sup>	3.0±0.2 <sup>a</sup>	160.0±1.6 <sup>c</sup>	104.0±5.3 <sup>a</sup>	89.7±3.5 <sup>ab</sup>	49.5±1.6 <sup>bc</sup>
5 g/kg synbiotic	228.7±10.2 <sup>a</sup>	2.8±0.3 <sup>a</sup>	156.7±7.7 <sup>c</sup>	104.5±5.4 <sup>a</sup>	90.0±1.7 <sup>ab</sup>	45.8±6.1 <sup>c</sup>
Overall mean	228.1±4.0	3.0±0.1	173.2±3.1	114.8±2.9	89.2±1.5	61.0±2.7

<sup>a,b,c</sup>: Mean values on the same column not sharing a superscript are significantly different (p<0.05)

<sup>1</sup>: Total protein; <sup>2</sup>: Cholesterol; <sup>3</sup>: Triglycerides; <sup>4</sup>: mg/dL; <sup>5</sup>: g/dL

Table 4: Effect of probiotics and synbiotic on ileum villus length and crypt depth of 42 d old broilers (means±standard error)

Treatments	Villus length (µm)	Crypt depth (µm)
Control	458.3±37.45 <sup>c</sup>	225.0±25.00 <sup>a</sup>
2.5 g/kg probiotic	675.0±25.00 <sup>b</sup>	204.2±13.56 <sup>a</sup>
5 g/kg probiotic	637.5±42.69 <sup>b</sup>	209.2±16.35 <sup>a</sup>
2.5 g/kg synbiotic	883.3±33.33 <sup>a</sup>	225.0±17.07 <sup>a</sup>
5 g/kg synbiotic	666.7±94.57 <sup>b</sup>	216.7±16.66 <sup>a</sup>
Overall mean	668.1±30.59	219.72±7.87

<sup>a,b,c</sup>: Mean values on the same column not sharing a superscript are significantly different (p<0.05)

complex production by useful bacteria which may results in positively affecting blood-forming processes (Kander, 2004). Meanwhile, normal iron supply leading to intensification of erythropoiesis was probably as a consequence of hyper synthesis of erythropoietin (Sjaasted *et al.*, 1996). Factors that affect erythropoiesis and red blood cell numbers also affect hemoglobin level (Sturkie, 1986). Synbiotic could increase the digestibility percentage and availability of many nutrient elements such as proteins, mineral elements and vitamins (Naji, 2009). Absorption of these nutrients may elevate the concentration of hemoglobin.

The lower H/L ratio in the experimental diets may be due to that the probiotic effect in inhibiting the nutritional stress or any stress which causes an increase in H/L ratio (Karoglu and Drudage, 2005). Stress could stimulate adrenal gland to secrete hormones such as estrone which has a direct effect to analyze a lymphatic cell which causes an increase in H/L ratio (Gross and Siegel, 1983).

**Serum biochemical content:** Among the selected serum biochemical contents, the concentration of cholesterol and LDL were significantly (p<0.05) lower in chickens received synbiotic in their diets than those fed on control diet (Table 3). The significant differences among different treatments may be due to the different

doses. The results were in accordance with the findings of Panda *et al.* (2006) who stated that there was a significant reduction in serum cholesterol and LDL concentration at 42 d of broiler age due to the dietary supplementation of different levels of probiotic to the broiler diet. Paryad and Mahmoudi (2008) found a significant decrease in serum cholesterol as a result of different levels 0, 0.5, 1.5 and 2% of probiotic addition to the broiler diet at 42 days of age. In contrast Ashayerizadeh *et al.* (2009) found that probiotic, prebiotic and synbiotic addition to the broiler diet had no significant effect in serum LDL. Safalaoh (2006) did not find differences in serum cholesterol when the probiotic was added to the drinking water of broiler chicks.

The lower concentration of cholesterol in the groups fed synbiotic and probiotic may be due to that some microorganisms present in the probiotic had the ability of cholesterol utilization for their metabolism and depressed the cholesterol absorption from gastrointestinal tract (Nelson and Gilliland, 1984; Mohan *et al.*, 1995) in addition probiotic microorganism had the ability to inhibits the activity of hydroxymethyl-glutaryl-coenzymeA which involved in the cholesterol synthesis (Fukushima and Nakano, 1995). Prebiotic that present in the synbiotic mixture has hypocholesterolemic effects there by reducing the absorption of lipids in the intestine through binding bile acids, increasing cholesterol elimination and hepatic synthesis of new bile acid (Zhang *et al.*, 2003). Cholesterol is a precursor of bile acids; more molecules are spend for recovery of bile acids and thus reduced the cholesterol level in the serum (De Smet, 1994).

The lower serum LDL in the chicks fed on synbiotic may be due to that the large portion of LDL is cholesteryl esters (CE) and free cholesterol with little triglycerides (TG) (McEneay *et al.*, 2002). Synbiotic has the ability to decrease the concentration of CE in LDL (Min-Tze Liong *et al.*, 2007). Loss of CE from the core of LDL forms

smaller and denser LDL particles. Although smaller LDL appeared more atherogenic than larger LDL particles (Haffner, 2002), smaller LDL formed could be removed from plasma easier than larger particles (Fernandez *et al.*, 1993).

**Ileum morphology:** Dietary supplementation of probiotic and synbiotic significantly ( $p < 0.05$ ) increased the villus length and had no significant effect on crypt depth compared with the control (Table 4). Significantly longer villi were recorded for chickens that received (2.5 g/kg) synbiotic than the other experimental groups. The results were in line with the findings of Mirza (2009) who found a significant increase in ileum villus height at 42 days as a result of synbiotic supplementation to the broiler diet. Similar results have been obtained by Songsak *et al.* (2008) due to the addition of cassava yeast probiotic to the broiler diet and fed for 42 days. However, the results were in contrast with the finding of Ahmad (2004) who found an increase in crypt cell proliferation of the small intestine in broiler with the use of probiotic compared to the control. Also, Awad *et al.* (2009) reported that synbiotic supplementation to the broiler diet reduced crypt depth of the ileum when compared with the control.

The longer ileal villi observed in broiler chickens that fed on diets supplemented with probiotic and synbiotic may be due to the enhanced short chain organic acids formation induced by probiotics. Thus increase the acidity in the gut which reduces the growth of many pathogenic or non-pathogenic intestinal bacteria, therefore, reduce intestinal colonization and reduce infectious processes, ultimately decrease inflammatory processes at the intestinal mucosa, which increase villus height (Loddi *et al.*, 2004). The short chain organic acids which are by products of bacterial fermentation stimulate the proliferation of epithelial cells of the bowel (Ichikawa *et al.*, 1999). According to (Iji and Tivey, 1998) some bacteria may recognize binding sites on the prebiotic (present in the synbiotic mixture) as if they were from the intestinal mucosa and the colonization of the intestine by pathogenic bacteria is thus reduced, therefore besides a lower infection incidence, there is an increase in the absorption of available nutrients, a mechanism that directly affects the recovery of intestinal mucosa, increasing villi height.

**Conclusions:** It could be concluded that dietary supplementation of probiotics and synbiotics to the broiler chickens could have positive effects on the hematological and serum biochemical parameters and subsequent histomorphological aspects. This could have positive effects on broiler productive performance thereby improving the physiological and metabolic activities of the broiler body and increasing the availability of the nutrients required.

## REFERENCES

- Agawane, S.B. and P.S. Lonkar, 2004. Effect of probiotic containing *Saccharomyces boulardii* on experimental ochratoxicosis in broilers: hematobiochemical studies. *J. Vet. Sci.*, 5: 359-367.
- Ahmad, I., 2004. Effect of probiotic (Protectin) on growth of broilers with special reference to the small intestinal crypt cells proliferation. M. Phil Thesis. Centre of Biotechnology, Univ. Peshawar.
- AL-Darajy, H.J., W.K. AL-Hayany and A.S. AL-Hasany, 2008. Avian Blood Physiology. Ministry of higher education and scientific research, University of Baghdad, College of Agriculture.
- Al-Kassie, G.A.M., Y.M.F. Al-Jumaa and Y.J. Jameel, 2008. Effect of probiotic (*Aspergillus niger*) and prebiotic (*Taraxacum officinale*) on blood picture and biochemical properties of broiler chicks. *Int. J. Poult. Sci.*, 7: 1182-1184.
- Archer, R.K., 1965. Hematological techniques for use on animals. Blackwell Scientific Publications Oxford.
- Ashayerizadeh, A., N. Dabiri, O. Ashayerizadeh, K.H. Mirzadeh, H. Roshanfekr and M. Mamooee, 2009. Effect of dietary antibiotic, probiotic and prebiotic as growth promoters, on growth performance, carcass characteristics and hematological indices of broiler chickens. *Pak. J. Biol. Sci.*, 12: 52-57.
- Awad, W.A., K. Ghareeb, S. Abdel-Raheem and J. Bohm, 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. *Poult. Sci.*, 88: 49-56.
- De Smet, I., Van L. Hoorde, De Saeyer, Van M. de Woestyne and W. Verstraelle, 1994. *In vitro* study of bile salt hydrolase (BSH) activity of BSH isogenic *Lactobacillus plantarum* 80 strains and estimation of cholesterol lowering through enhanced BSH activity. *Microbial Ecol. Health Dis.*, 7: 315-329.
- Engberg, R.M., M.S. Hedemann, T.D. Leser and B.B. Jensen, 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poult. Sci.*, 79: 1311-1319.
- Fairchild, A.S., J.L. Grimes, F.T. Jones, M.J. Wineland, F.W. Edens and A.E. Sefton, 2001. Effects of hen age, bio-mos and flavomycin on poult susceptibility to oral *Escherichia coli* challenge. *Poult. Sci.*, 80: 562-571.
- Fernandez, M.L., G. Abdel-Fattah and D.J. McNamara, 1993. Dietary fat saturation modifies the metabolism of LDL sub-fractions in guinea pigs. *Arterioscler Thromb Vasc Biol.*, 13: 1418-1428.
- Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, 18: 499-502.
- Fukushima, M. and M. Nakano, 1995. The effect of probiotic on faecal and liver lipid classes in rats. *Br. J. Nutr.*, 73: 701-710.

- Giedrius, B., P. Matusевичius, A. Januskevicius, J. Jankowski, D. Mikulski, J. Blok and K. Kozlowski, 2008. Use of synbiotic preparations in turkey diets and their effect on growth performance. *Veterinarija IR Zootechnika T.*, 43.
- Gross, W.B. and H.S. Siegel, 1983. Evaluation of heterophile/lymphocyte ratio as a measure of stress in chickens. *Avian Dis.*, 27: 972-979.
- Gruzauskas, R., R. Lekavicius, A. Raceviciute-Stupeliene, V. Sasyte and F. Guarner, 2004. Inulin and oligofructose: Impact on intestinal diseases and disorders. *Br. J. Nutr.*, 93: 61-65.
- Haffner, S.M., 2002. Lipoprotein disorders associated with type-2 diabetes and insulin resistance. *Am. J. Cardiol.*, 90: 55-61.
- Ichikawa, H., T. Kuroiwa, A. Inagaki, R. Shineha, Nishihira, S. Satomi and T. Sakata, 1999. Probiotic bacteria stimulate gut epithelial cell proliferation in rat. *Digestive Dis. and Sci.*, 44: 2119-2123.
- Iji, P.A. and D.R. Tivey, 1998. Natural and synthetic oligosaccharides in broiler chicken diets. *Word Poult. Sci. J.*, 54: 129-143.
- Kander, M., 2004. Effect of *Bifidobacterium* sp. on the health state of piglets, determined on the basis of hematological and biochemical indices, *EJPAU.*, 7: 07.
- Karoglu, M. and H. Durdag, 2005. The influence of probiotic (*Saccharomyces cerviciae*) supplementation and different slaughter age on the performance, slaughter and carcass properties of broiler. *Int. J. Poult. Sci.*, 4: 309-316.
- Loddi, M.M., V.M.B. Moraes, L.S.O. Nakaghi, F.M. Tucci, M.I. Hannas and J.A. Ariki, 2004. Mannan oligosaccharide and organic acids on performance and intestinal morphometric characteristics of broiler chickens. In: *Proceedings of the 20 th Annual Symposium*, p: 45.
- McEneny, J., C. McMaster, E.R. Trimble and I.S. Young, 2002. Rapid isolation of VLDL subfractions: assessment of composition and susceptibility to copper-mediated oxidation. *J. Lipid Res.*, 43: 824-831.
- Min-Tze, L., F.R. Dunshea and N.P. Shah, 2007. Effect of synbiotic containing *Lactobacillus acidophilus* ATCC 4962 on plasma lipid profiles and morphology of erythrocytes in Hypercholestromaemic pigs on high- and low-fat diets. *Br. J. Nutr.*, 98: 736-744.
- Mirza, R.A., 2009. Using prepared synbiotic in early feeding of broiler chicken and it's effect on some productive, physiological and histological performance and carcass characteristics. M. Sc. thesis, University of Salahddin-Erbil-Agriculture College. (Arabic).
- Mohan, B., R. Kadirvel, M. Bhaskaran and A. Natarajan, 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *Br. Poult. Sci.*, 36: 799-803.
- Naji, S.A.H., G.A. Al-kassie, M.F. Al-Hadithi, A.H. Al-Hillali and Y.J. Jameel, 2009. Poultry health management. Brochure No (27). Iraqi Poultry Producer association. (Arabic).
- Nelson, C.R. and S.E. Gilliland, 1984. Cholesterol uptake by *Lactobacillus acidophilus*. *J. Dairy Sci.*, p: 67.
- Panda, A.K., S.V.R. Rao, M.V.L.N. Raju and S.R. Sharma, 2006. Dietary supplementation of *Lactobacillus sporogenes* on performance and serum biochemico-lipid profile of broiler chickens. *J. Poult. Sci.*, 43: 235-240.
- Paryad, A. and M. Mahmoudi, 2008. Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broilerchicks. *Afr. J. Agric. Res.*, 3: 835-842.
- Pelicano, E.R.L., P.A. De Souza, H.B.A. De Souza, F.R. Leonel, N.M.B.L. Zeola and M.M. Boiago, 2004. Productive traits of broiler chickens fed diets containing different growth promoters. In: *Asian Tec. Conf. Brazil.*, pp: 18.
- Safalaoh, A.C.L., 2006. Body weight gain, dressing percentage, abdominal fat and serum cholesterol of broilers supplemented with a microbial preparation. *Afr. J. Food. Agric. Nutr. Devel.*, 6: 1.
- Sarinee, K., Thongsong, T. Boonrit and C. Vivat, 2008. Blood haematological-cholesterol profile and antibody titer response of broilers with added probiotic containing both bacteria and yeast or an antibiotic in drinking water. *Thai. J. Vet. Med.*, 38: 45-56.
- Schrezenmier, J. and M.D. Vrese, 2001. Probiotics and synbiotics approaching a definition. *Am. J. Ch. Nutr.*, 73: 305-3645.
- Silvia, M., A. Jan, T. Marta, K.E. Jana, N. Radomira, G. Monika and B. Darina, 2008. Effect of probiotics, prebiotics and herb oil on performance and metabolic parameters of broiler chickens. *Medycyna Wet.*, 64.
- Sjaasted, Q.V., T. Franstad and A.K. Blom, 1996. Effect of iron on erythropoietin production in anemic piglets. *Acta. Vet. Scand.*, 37: 133-138.
- Songsak, C., O. Chinrasri, T. Somchan, S. Ngamluan and S. Soychuta, 2008. Effect of dietary inclusion of cassava yeast as probiotic source on growth performance, small intestine (ileum) morphology and carcass characteristic in broilers. *Int. J. Poult. Sci.*, 7: 246-250.
- Sturkie, P.D., 1986. *Avian Physiology* 4th ed. Springer-Verlag-NewYork-Berlin-Heidelberg. Tokyo.

- Turnidge, J., 2004. Antibiotic use in animals-prejudices, perceptions and realities. *J. Antimicrob. Chemother.*, 53: 26-27.
- Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang, 2003. Effects of dietary fructo oligosaccharide on digestive enzyme activities. Intestinal Microflora and morphology of male broilers. *J. Anim. Sci.*, 82: 1030-1036.
- Zhang, W.F., D.F. Li, W.Q. Lu and G.F. Yi, 2003. Effect of isomaltooligosaccharides on broiler performance and intestinal micro flora. *Poult. Sci.*, 82: 657-663.