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Effectiveness of Prebiotic as an Alternative to the Antimicrobial Growth Promoter on Growth Performance, Blood Constituents, Intestinal Healthiness and Immunity of Broilers.

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Key words

prebiotic; chlortetracycline; growth; hematology; biochemistry; intestine; immunity

ABSTRACT:

This study was designed to compare the effect of the prebiotic and antimicrobial growth promoter (AGP) on the growth performance, blood constituents, intestinal bacteriology and histomorphometric parameters as well as humeral immunity of broiler chicks. A total of 90 unsexed commercial Cobb chicks were randomly assigned to 3 dietary treatments (control, AGP and prebiotic groups), each group contains 30 chicks. Each group subdivided into 3 replicates, 10 chicks each, and was reared for 42 days. The prebiotic supplemented group showed a significant improvement in growth performance parameters in comparison to the control and AGP-supplemented groups. Total leukcocytic count, lymphocyte percent, total protein, total globulin and gamma globulin were significantly increased in the broilers fed on prebiotics. Moreover, prebiotics supplementation significantly reduced heterophil percent, heterophil/ lymphocyte ratio (H/L ratio), albumin/globulin ratio, aspartate and alanine aminotransferase (AST and ALT), uric acid and creatinine compared to the AGP-supplemented and control groups. The AGP-supplemented group exhibited a significant reduction in the total aerobic count when compared to the control and prebiotic-supplemented groups. However, the prebiotic supplemented group showed a significant reduction in the coliform count when compared to the control and antibiotic supplemented groups. The prebiotic supplemented group induced a significant increase in the villus height (VH) all over the small intestine. In addition, it induced a significant increase in villus height: crypt depth ratio in the duodenum and jejunum in comparison to the control and antibiotic supplemented groups. However, there were no significant differences among the different groups regard to the crypt depth (CD) in the duodenum and jejunum. Prebiotics could be considered as safe and effective antimicrobial alternatives for broiler chicks' growth performance, immunity and intestinal bacteriology and morphology.

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1. INTRODUCTION

Recently, the poultry industry was developed rapidly in order to achieve the high demand for a higher and safer protein source. To overcome this high inquiry, the intensive rearing system was more necessary, but this led to challenges of various diseases, and increased uses of antibiotics for prophylactic, therapeutic, and growth promoting purposes (Caprioli *et al.*, 2000). Antibiotic growth promoters (AGPs) were used in the poultry and other livestock production for many years. They were reported to enhance the growth performance of poultry and other food producing animals (Toghyani *et al.*, 2010). However, continuous and sub-therapeutic uses of AGPs led to the development of undesirable antibiotic resistance in poultry, which had hazardous effects on the consumer health (Toghyani *et al.*, 2010). Thus, many countries banned the use of antibiotics in animal and poultry feed. The ban of antibiotic growth promoters (AGPs), increases the demand to find AGPs replacers. Although natural products have been used for their medicinal benefits, there is still huge demand for these products to solve many emerging economic and health issues (Abdel-Daim *et al*, 2013 and Abdel-Daim, 2014). There are many natural alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immunostimulant and other management practices, which can be used safely in the poultry and animal industry (Banerjee, 1998). Chlortetracycline is a member of the tetracycline family and has a broad-spectrum activity that is commonly given to poultry and livestock due to

its great effect on controlling the enteric pathogens (Sande and Mandell, 1990).

Prebiotics are AGPs alternatives having three criteria; non-digestible by host enzymes, fermentable in the gastrointestinal tract and selectivity in stimulation of intestinal flora and metabolic activity (Gibson *et al.*, 2004 and Van Loo, 2004). Therefore, they improve the intestinal ecosystem, intestinal tissue, immunity and general host status (Gibson *et al.*, 2004). The prebiotic, Mannan-oligosaccharides (MOS), is a carbohydrate, derived from yeast cell walls, and can inhibit the growth of harmful bacteria and stimulate the non-specific immune system; thus activates the healthiness and growth performance of birds (Ferket, 2004). Prebiotics based on Mannan-oligosaccharides reduced AST, ALT and serum cholesterol. While had no effect on the triglycerides (Yalçinkaya *et al.*, 2008). Prebiotics could alter the intestinal ecosystem via promoting competitive exclusion of pathogenic microbes and selective colonization by beneficial microbes, leading to improvement poultry performance (Biggs *et al.*, 2007).

This study was aimed to compare the effect of prebiotic and antibiotic growth promoters on the growth performance, blood constituent, intestinal bacteriology, intestinal histomorphometery and humoral immunity of broiler chicks.

2. Materials and methods:

2.1. Feed additives:

In this study, there were two feed additives used; AGP and prebiotic. The AGP was chlortetracycline (Chlorfeed® manufactured by ATCO Pharma for pharmaceutical drugs, Egypt). Prebiotic used in this experiment was (Organoferm dry®) a product of Organic Chemical Solutions, L. L. C (OSCLLC, USA). It was derived from the cell wall of *Saccharomyces cerevisiae* by special fermentation technology, and its components were mannanoligosaccharides (M.O.S.) 11.7%, beta glucans (9.2%) and balanced mixture of vitamin B complex, essential amino acids, minerals and vitamin E.

2.2. Birds, management and diets:

Ninety unsexed one day old Cobb broiler chicks were obtained from Elshrooq Poultry Company, El-Sharkia, Egypt. The birds were housed on deep litter floor system (with wheat straw from day of hatch) at the experimental house of Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. All chamber partitions, feeders, drinkers, and heaters were cleaned and disinfected before the study. Environmental temperature was adjusted according to the age. It was set at 32˚C for the first week of age and then, decreased by 2˚C per week till reach 22˚C at 6th week of age. Relative humidity was set at 50-60% throughout the study. Ventilation was controlled to maintain birds' comfort during the rearing period. Birds were provided 24 hours of lighting and checked three times daily (at 6 am, 2 pm and 10 pm) for feed, water and mortality. Feed and water were provided *ad libitum*. Diets were formulated as starter and grower finisher diets. The chicks were fed on the formulated broiler starter basal ration from one day old to 3 weeks of age, and then the formulated grower finisher ration was used until the end of the experiment at 6 weeks of age. The diet was formulated to meet the nutritional requirements as recommended by the NRC (1994) as shown in table (1).

2.3. Vaccines:

The birds were routinely vaccinated against Newcastle (ND), Gumboro (IBD), Infectious Bronchitis (IB) and Avian Influenza (AI) as following: All birds were vaccinated with (IB and ND); Izovac H120 & Hitchner B1 (Izo S.p.A, Italy) at 7th day-old by eye drops, (ND and AI); Inactivated ND&H5N1 vaccine (Schering-Plough Animal Health, Holland) at 8th day-old by subcutaneous injection, IBD; Nobilis® Gumboro -D.78 (Intervet, Holland) at 14th day-old by eye drops and at 24th day-old in drinking water, (IB and ND); Izovac H120 & Lasota (Izo S.p.A, Italy) at 18th day-old by eye drops and ND; Nobilis® ND-Vac Clone 30 (Intervet, Holland) at 28th day-old in drinking water.

2.4. Experimental design:

The chicks were classified randomly into three groups (each of 30). Each group was subdivided into three replicates, each of 10. The experimental groups were; control group received basal diet as NRC (1994), AGP- treated group received basal diet plus antibiotic (Chlorfeed®) at concentration of 1 g/kg feed, and prebiotic-treated group received basal diet plus prebiotic (Organoferm Dry®) at a concentration of 0.5g/kg feed. This experiment was conducted for 6 weeks.

2.5. Growth performance parameters evaluation:

Final body weight, total weight gain, total feed intake, total food conversion ratio (FCR) and

total feed efficiency (FE) were determined at the end of the experiment.

2.6. Blood samples:

At 21st and 42nd days of age, 6 birds in each group (two birds per replicate) were randomly chosen for blood sampling. Two blood samples were collected from the wing vein of each bird into two different centrifuge tubes (heparinized and non-heparinized). The blood samples with heparin were immediately used for determination of hematological parameter values. Sera were separated by centrifugation at 3000 rpm for 10 minutes, and then collected in eppendorf tubes and stored at -20˚C to be used in evaluation of different biochemical parameters.

2.7. Hematological parameters:

Total erythrocyte and total leukocyte counts were performed by using the improved Neuober hemocytometer with Natt and Herrick solution as diluting fluid according to the method described by Natt and Herrick (1952), hemoglobin (Hb) was estimated using the methodology of Lamberg and Rothstein (1977), and the packed cell volume (PCV) was determined according to Lamberg and Rothstein (1977). Differential leukocyte count was carried out in blood films prepared and stained with Giemsa and counting up to 100 cells then take the percent of eosinophils, lymphocytes, basophils, heterophils and monocytes (Jain, 2000). The heterophils / lymphocytes (H/L) ratio was calculated by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel, 1983).

2.8. Serum biochemical parameters:

At 21 and 42 days of the experiment, the serum samples were biochemically examined as the following;

2.8.1. Liver and kidney functions:

Aspartate and alanine aminotransferase (AST and ALT) were quantitatively estimated according to the method described by Retiman and Frankel (1957), Creatinine and uric acids were determined according to the methods of Young *et al.*, (1975) and Caraway (1963). These parameters were spectrophotometrically assayed by using semi-automated spectrophotometer (Erba-Chem7, Germany) and using commercial kits purchased from (Spectrum, Cairo, Egypt).

2.8.2 Lipid profile:

Triglycerides, cholesterols, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) at both 21 and 42 days old were spectrophotometrically assayed using semi-automated spectrophotometer (Erba-Chem7, Germany) and by using diagnostic reagent kits (Spectrum, Cairo, Egypt) as described by Kannan *et al.* (2005).

2.7.1

2.8.3. Serum protein electrophoresis:

Serum protein electrophoresis was performed according to the methodology of Laemmli (1970).

2.8.4. Hemagglutination inhibition (HI) test:

At 28 and 42 days, six serum samples were collected from each group (Shahir *et al.*, 2014), and humoral immune response was investigated by detecting serum antibody titers against ND and AI viruses by hemagglutination inhibition test as described by Alexander and Chettle (1977).

2.9. Bacteriological Evaluation:

At 21 and 42 days of age, two birds per each replicate (6 birds per group) were randomly selected and slaughtered (Abdel-Raheem *et al.*, 2012). All viscera of each carcass were removed carefully by hand and one gram of the intestinal content from the ileocecal junction portion of the intestinal tract was collected and weighted in clean previously sterilized Petridishs. Then, was transferred to a series of sterile test tubes containing 9 ml of 0.1% sterile buffered peptone water and well mixed to prepare decimal serial dilutions of sample homogenate up to 10^{-8}. Then, the total aerobic and total coliform counts were carried out by pour plate method (FDA, 2002).

2.10. Histomorphometric parameters of intestinal tract:

At the end of the experiment (42 days) two birds per each replicate (6 birds per group) were randomly selected and slaughtered (Sayrafi *et al.*, 2011). The abdomen of each carcass was opened, and the small intestine was excised. Two-cm long segment were taken from the middle parts of the duodenum, jejunum and ileum then washed with normal saline and immersed in 10% buffered formalin for fixation. Routine histological laboratory methods, including dehydration, clearing and paraffin embedding was applied and paraffin blocks were made. Sections were cut at 6-µm thickness using a Sliding Microtome (MIC509, Euromax, Tokyo, Japan). Then, the sections were stained with the standard hematoxylin and eosin method (Gridley, 1960). Then slides were examined under light microscope (Nikon, Japan) for measuring villus height, crypt depth and villus height to crypt depth ratio. Morphometric analyses of the intestinal

epithelium were carried out by TSview software, ImageJ software using standard calibrated stage micrometer, Calibrated standard digital microscope camera (Tucsen digital camera) using Olympus CX21 microscope, with resolution of 5 MP (2592 x 1944 pixel each image) by Rasband (1997) was used for estimating villus height (VH, µm), which was measured from the top of the villus to the top of the lamina propria. Crypt depth (CD, µm) was defined as the depth of the invagination between the adjacent villi Sakamoto *et al.* (2000).

2.11. Statistical analysis:

The obtained data were statistically analyzed by variance method (ANOVA) considering $P < 0.05$ using SPSS 18.0 SPSS Inc. (2009) software. The significant differences were taken to Duncan multiple range tests to compare the means.

3. RESULTS and DISCUSSION

In the modern intensive poultry rearing system, newly hatched chicks reared away from their mother; therefore, normal micro flora are slow to be established in the intestine (Fuller, 1989). This situation makes chicks easily to be affected by any pathogenic microbes due to the sterile condition of the intestine, subsequently causing food-borne disease in human beings (Pivnick and Nurmi, 1982). Prebiotics showed significant effects on performance, blood constituent, intestinal environment, small intestinal morphology and immunity of broiler chickens (Houshmand *et al*., 2012).

Table (1): The composition of the basal ration:

Ingredients kg / 100 kg	Starter (0-3 weeks)	Grower finisher (3-6 weeks)
Grounded yellow corn (8.5%)	57	63.1
Soya bean meal (45%)	34.4	26.2
Corn gluten (62%)	5	5.0
Soya oil	-	2.5
Supplements kg/100 kg	`	
Dical.Phosphate (22%Ca&19%P)	1.6	1.1
Limestone ground (38%)	1.4	1.5
Common salt	0.1	0.1
DL-Methionine (98%)	0.1	0.1
Lysine (98%)	0.1	0.1
Mineral& Vitamin premix**	0.3	0.3
Calculated Composition		
Crude protein (CP%)	23	20
(Kcal/Kg) ME	2900	3100

** Each 3 kg contains the following vitamins and minerals: Vit. A 12 mIU, vit. D_3 2 mIU, vit. E 1000mg, vit. k_3 1000mg, vit. B_1 1000mg, vit. B_2 5000mg, vit. B_6 1500mg, vit. B_{12} 10mg, biotin 50mg, pantothinic acid 10g, nicotinic acid 30g, folic acid 1000mg, manganese 60g, zinc 50g, iron 30g, copper 4g, iodine 300mg, selenium 100mg, cobalt 100mg, carrier($CaCO_3$) to 3kg. (Golden premix- Selim Pharm Elasher, Egypt. patch No. 8181, production 3-2013).

Table (2): Effects of different feed additives on the total body performance:

	Control	AGP	Prebiotic
Intial body weight	40.63±0.3	40.33±0.46	40.58±0.46
Final body weight (g/bird)	2177.67 ± 7.77^{c}	2326.73 ± 9.52^{b}	2607.61 ± 12.98^{a}
Total weight gain	2137.03 ± 7.76^{c}	2287.32 ± 6.52^{b}	2567.29 ± 13.12^{a}
Total feed intake (g/bird)	4881.33 ± 8.52^{a}	4798.46 ± 3.94^{b}	4628.68 ± 2.63^{c}
Total FCR	2.24 ± 0.04^{a}	2.06 ± 0.01^{b}	1.78 ± 0.01^{c}
Total FE	0.45 ± 0.001^{c}	0.48 ± 0.001^{b}	0.56 ± 0.002^{a}

Values are expressed as means ± standard error (SE); n=6

Means within the same row and experimental period with different superscripts are significantly different ($P<0.05$).

AGP= Antimicrobial Growth Promoter; FCR= feed conversion ratio; FE= Feed efficiency.

Table (3): Effect of different feed additives on the hematological parameters of the experimental chicks at 21 and 42 days:

	Control	AGP	Prebiotic	Control	AGP	Prebiotic
	21 days			42 days		
RBCs (10^6/ul)	2.83±0.15 [a]	2.67±0.13[a]	2.94±0.13[a]	2.98±0.12[a]	3.02±0.12[a]	3.02±0.12[a]
Hb (gm/dl)	10.37±0.36[a]	10.48±0.34[a]	10.68±0.24[a]	10.74±0.27[a]	10.62±0.25[a]	10.74±0.24[a]
PCV (%)	29.83±0.74[a]	29.83±1.74[a]	29.52±1.24[a]	30.54±1.28[a]	31.65±1.29[a]	31.04±1.46[a]
WBCs (1000/ul)	22.35±1.18[b]	21.54±0.89[b]	28.17±1.31[a]	25.45±1.66[b]	24.73±0.99[b]	31.87±1.69[a]
Heterophil (%)	37.33±0.72[a]	36.65±0.65[a]	32.94±0.67[b]	38.25±0.93[a]	37.82±0.71[a]	32.61±0.62[b]
Lymphocyte(%)	52.77±0.64[b]	52.84±0.81[b]	56.42±0.62[a]	50.42±0.84[b]	51.28±0.64[b]	56.58±0.92[a]
Monocyte (%)	4.87±0.48[a]	5.36±0.61[a]	5.24±0.58[a]	6.04±0.58[a]	5.75±0.65 [a]	5.79±0.62[a]
Eosinophil (%)	2.86±0.31[a]	2.81±0.31[a]	2.94±0.48[a]	2.84±0.43[a]	2.79±0.55[a]	2.75±0.43[a]
Basophil (%)	2.17±0.31[a]	2.34±0.31[a]	2.46±0.35[a]	2.45±0.33[a]	2.36±0.33[a]	2.27±0.43[a]
H/L ratio	0.71±0.02[a]	0.69±0.01[a]	0.58±0.01[b]	0.76±0.09[a]	0.74±0.02[a]	0.58±0.01[b]

Values are expressed as means ± standard error (SE); n=6

Means within the same row and experimental period with different superscripts are significantly different (P<0.05).

AGP= Antimicrobial Growth Promoter; RBCs= Red Blood Cells; Hb= Hemoglobin; PCV= Packed Cell Volume; WBCs= White Blood Cells; H/L ratio= Heterophil/Lymphocyte ratio.

The effects of dietary supplementation of prebiotic and AGP on broiler growth performance have been shown in Table (2). The results indicated that the broiler chicks supplemented with dietary prebiotic elicited a significant increase (P≤ 0.05) in the final body weight, total weight gain and total feed efficiency. Meanwhile, a significant reduction (P≤ 0.05) in the total feed intake and feed conversion rate compared to the control and antibiotic-supplemented groups. These results revealed that the prebiotic was an effective replacer to the AGP in growth promotion and economic purposes. The beneficial effects of prebiotic on broiler performance in the present study came in concord with Baurhoo *et al.* (2007), Markovic *et al.* (2009), Kim *et al.* (2010) and Ghahri *et al.*, (2013) who reported that the prebiotic could be considered as an effective AGPs alternative; it showed a significant improvement in body performance parameters than the control and antibiotic supplemented broilers. On the other hand, this result was in disagreement with Oliveira *et al.* (2008) who recorded that the supplementation of prebiotic had no significant effect on body performance of broiler chicks and also, Kamaran *et al* (2013) who concluded that the effect of mannan-oligosaccharides (MOS) on the broilers growth performance was inferior to AGP.

The prebiotic based on MOS improving broiler performance through inhibition of pathogenic bacteria which possess type-1 fimbriae (mannose-sensitive lectin), modulation of intestinal morphology and expression of mucin and brush border enzymes (Ferket, 2004). Prebiotic improves digestion in broiler by increase digestive enzyme (intestinal amylase and protease) activity (Xu *et al.,* 2003). Prebiotic improves the intestinal morphology; increases the absorption area and improves energy and protein utilization by the bird (Santin *et al.,* 2001). Prebiotic reduces the pathogenic bacteria and maintain the beneficial bacteria in the intestine. Therefore, improve the digestibility of amino acids (Biggs *et al.,* 2007). Prebiotic could alter the intestinal ecosystem via promoting competitive exclusion of pathogenic microbes and selective colonization by beneficial microbes, thus it improved the poultry performance parameters (Xu *et al.,* 2003, Hooge, 2004 and Biggs *et al.,* 2007). Therefore the improved performance parameters in poultry fed prebiotic supplemented diets in this study could be related to the above mentioned effects.

The effects of dietary supplementation of prebiotic and AGP on the hematological parameters at 21 and 42 days were tabulated in table (3). The prebiotic-supplemented group showed a significant (p<0.05) increase in the total leukocytic count and lymphocyte percent as well as significant (p<0.05) decrease in the heterophil percent and H/L ratio than the antibiotic-supplemented group and control. These results indicated the immune-stimulant effect of prebiotics. These results were in line with the results of Sadeghi *et al.* (2013) who reported that the

prebiotic based on β-glucan and Mannan-oligosaccharides caused a significant increase in the white blood cells and decrease in the heterophils / lymphocyte ratio in the chicken infected with *Salmonella enteritidis.* On the other hand, the results were inconsistent with Ghasemi *et al.* (2014) and Shahir *et al.* (2014) who reported that prebiotic had no effect on the leukocyte count, heterophil / lymphocyte ratio, lymphocyte and heterophil percent when compared with the probiotic-fed group and control.

The effects of prebiotic and AGP supplementation in broilers diet on the liver and kidney functions were illustrated in table (4). There were a significant reduction in the ALT, AST, uric acid and creatinine levels in the chicks received prebiotic in their diet as compared with chicks fed on antibiotic and basal ration at both 21 and 42 days. These results revealed that the prebiotic had renoprotective and hepatoprotective effects. Our results were compatible with Yalçinkaya *et al*. (2008) who reported that the addition of the MOS (prebiotic) in the broiler diet caused a significant reduction in AST and ALT levels. In the same context, Salim *et al.* (2011) reported that the prebiotic caused a significant reduction in the AST, ALT, uric acid and creatinine either in the infected and non-infected broiler chicks.

The effects of dietary supplementation of prebiotic and AGP on broilers lipid profiles at 21 and 42 days were shown in table (4). There were no significant differences among the three groups in the levels of serum cholesterol, triglycerides, HDL, LDL and VLDL. These results were in accordance with the findings of Yalcinkaya *et al.* (2008) who reported that the use of MOS in broiler diet could not significantly reduce the serum cholesterol and triglyceride levels as compared with the control group. Furthermore, Ashayerizadeh *et al.* (2009) concluded that the prebiotic (Biolex-MB) had no significant effect on the levels of cholesterol, triglycerides, HDL, LDL and VLDL in broiler chicks. While, these results disagreed with Kannan *et al.* (2005) who reported that the use of 0.5 g kg-1MOS obtained from yeast in the ration of broiler chickens, significantly reduced the serum cholesterol level on day 35 as compared with the control.

The effects of dietary addition of prebiotic and AGP on the plasma protein electrophoresis at 21 and 42 days were presented in tables (5). There were a significant increase ($p<0.05$) in the levels of total protein, total globulin, total gamma-globulin, total beta-globulin and total alpha-globulin. Meanwhile, a significant decrease ($p<0.05$) in albumin / globulin ratio in broilers fed on prebiotic as compared with the antibiotic-supplemented group and control. On the other hand, there were no significant differences among the different groups in albumen levels. The high globulin level and low (A/G) ratio signify better disease resistance and immune response (Griminger, 1986).

Table (4): Effects of different feed additives on the liver & kidney functions and lipid profiles of broiler chicks at 21 and 42 days:

	Control	AGP	Prebiotic	Control	AGP	Prebiotic
	21 days			42 days		
AST (u/l)	127.83±1.45 ^{a}	131.08 ±1.24 ^{a}	76.58 ±1.79^{b}	161.67±2.73^{a}	166.33±2.87^{a}	97.67±3.53^{b}
ALT (u/l)	27.83±1.62^{a}	27.78±1.76^{a}	17.11±0.97^{b}	30.83±0.92^{a}	31.33±0.58^{a}	21.83±1.12^{b}
Creatinine (mg/dl)	0.62±0.05^{a}	0.63±0.07^{a}	0.38±0.04^{b}	0.65±0.05^{a}	0.68±0.05^{a}	0.37±0.06^{b}
Uric acid (mg/dl)	7.53±0.32^{a}	7.71±0.19^{a}	5.18±0.25^{b}	8.32±0.21^{a}	8.65±0.19^{a}	4.75±0.17^{b}
TG (mg/dl)	56.92±2.31^{a}	59.67±1.82^{a}	55.83±1.72^{a}	54.83±1.88^{a}	56.33±2.06^{a}	56.16±2.13^{a}
Cholesterol (mg/dl)	93.55±3.15^{a}	92.48±2.29^{a}	93.21±2.07^{a}	99.71±1.92 ^{a}	99.91±1.54 ^{a}	98.84 ±1.42 ^{a}
HDL (mg/dl)	63.51±1.67^{a}	64.67±1.58^{a}	63.83±1.51^{a}	67.82±1.05 ^{a}	67.16 ±1.53 ^{a}	67.32 ±1.25 ^{a}
LDL (mg/dl)	20.42±0.16^{a}	20.38±0.42^{a}	20.32±0.40^{a}	20.84 ±0.50 ^{a}	20.56±0.33 ^{a}	20.25 ±0.32 ^{a}
VLDL (mg/dl)	12.17±0.70^{a}	11.83±0.48^{a}	11.66±0.88^{a}	12.09 ±0.58 ^{a}	12.56 ±0.81^{a}	12.54 ±0.76 ^{a}

Values are expressed as means ± standard error (SE); n=6

Means within the same row and experimental period with different superscripts are significantly different ($P<0.05$).

AGP= Antimicrobial Growth Promoter; AST= Aspartate aminotransferase; ALT= Alanine aminotransferase; TG= Triglycerides; HDL= High density lipoprotein; LDL=Low density lipoprotein; VLDL= Very low density lipoprotein.

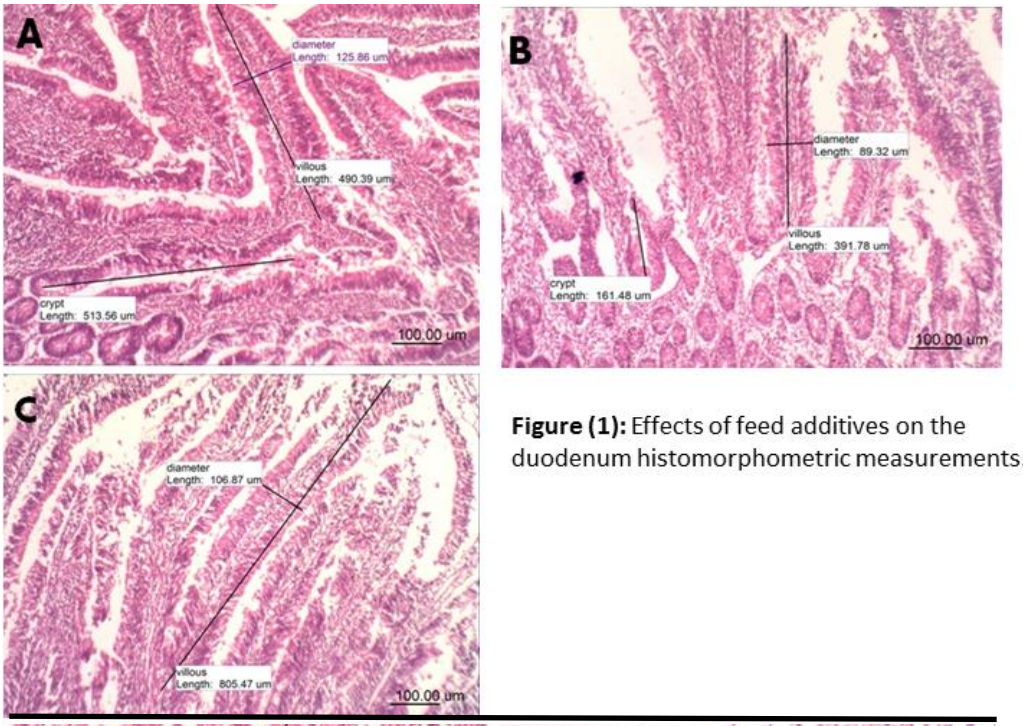

Figure (1): Effects of feed additives on the duodenum histomorphometric measurements.

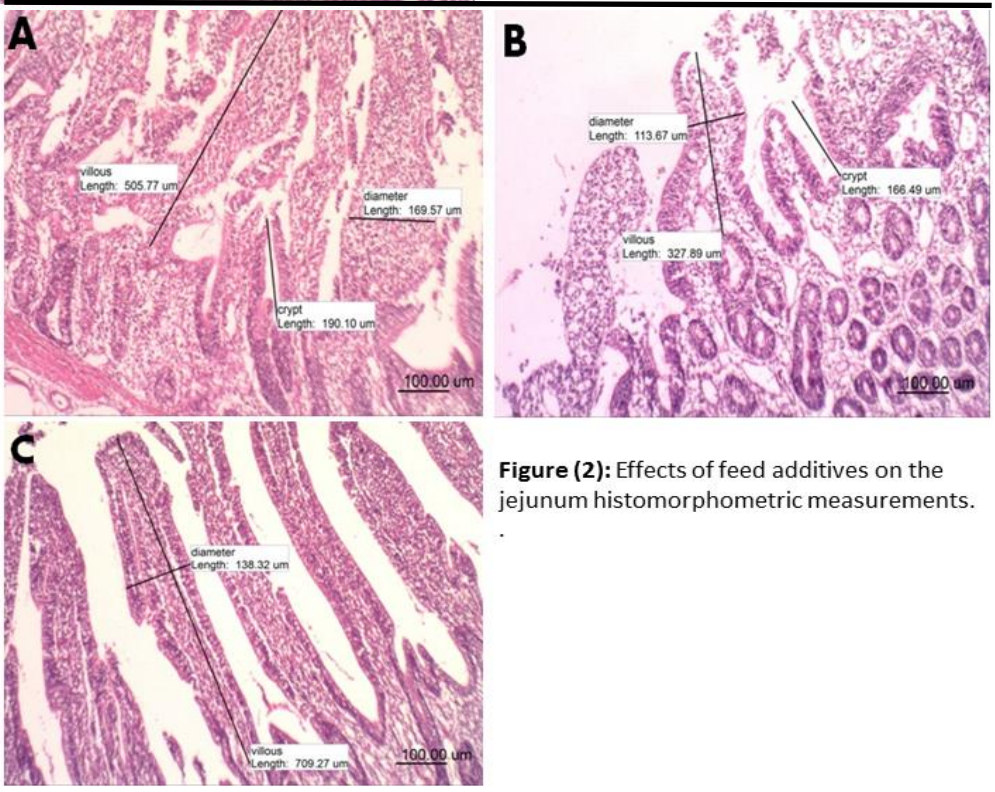

Figure (2): Effects of feed additives on the jejunum histomorphometric measurements.

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Table (5): Effects of different feed additives on the plasma protein fractionation of experimental chickens at 21 and 42 days:

	Control	AGP	Prebiotic	Control	AGP	Prebiotic
	21 days			42 days		
Total protein (g/dl)	2.25±0.11[a]	2.22±0.09[a]	2.23±0.09[a]	2.02±0.02[b]	2.03±0.07[b]	2.89±0.11[a]
Total globulin (g/dl)	1.53±0.03[a]	1.51±0.07[a]	1.54±0.12[a]	1.33±0.04[b]	1.36±0.06[b]	2.23±0.14[C]
Total gamma-globulin (g/dl)	0.68±0.03[a]	0.71±0.03[a]	0.69±0.05[a]	0.54±0.02[b]	0.61±0.01[b]	0.91±0.03[a]
Gamma-globulin 1 (g/dl)	0.49±0.03[a]	0.51±0.02[a]	0.47±0.02[a]	0.37±0.02[b]	0.45±0.03[b]	0.73±0.03[C]
Gamma-globulin 2 (g/dl)	0.19±0.01[a]	0.20±0.02[a]	0.22±0.01[a]	0.17±0.02[a]	0.16±0.01[a]	0.18±0.01[a]
Total beta-globulin (g/dl)	0.44±0.03[a]	0.41±0.01[a]	0.42±0.04[a]	0.41±0.03[b]	0.44±0.01[b]	0.52±0.02[C]
Beta-globulin 1 (g/dl)	0.22±0.03[a]	0.22±0.03[a]	0.19±0.02[a]	0.22±0.02[b]	0.28±0.02[b]	0.36±0.01[a]
Beta-globulin 2 (g/dl)	0.22±0.03[a]	0.19±0.02[a]	0.23±0.02[a]	0.19±0.03[a]	0.16±0.03[a]	0.16±0.01[a]
Total alpha-globulin (g/dl)	0.41±0.03[a]	0.39±0.02[a]	0.43±0.03[a]	0.38±0.02[b]	0.31±0.03[b]	0.80±0.12[a]
Alpha-globulin 1 (g/dl)	0.24±0.03[a]	0.18±0.03[a]	0.18±0.06[a]	0.17±0.02[b]	0.08±0.01[b]	0.37±0.07[a]
Alpha-globulin 2 (g/dl)	0.17±0.02[a]	0.21±0.04[a]	0.25±0.03[a]	0.21±0.01[b]	0.23±0.03[b]	0.43±0.02[a]
Total albumin (g/dl)	0.72±0.07[a]	0.71±0.05[a]	0.69±0.07[a]	0.69±0.04[a]	0.67±0.03[a]	0.66±0.04[a]
Pre-albumin (g/dl)	0.08±0.04[a]	0.07±0.02[a]	0.08±0.01[a]	0.11±0.02[a]	0.08±0.02[a]	0.08±0.12[a]
Albumin (g/dl)	0.64±0.05[a]	0.64±0.03[a]	0.61±0.05[a]	0.58±0.05[a]	0.59±0.03[a]	0.58±0.04[a]
Albumin/Globulin ratio	0.47±0.07[a]	0.47±0.06[a]	0.45±0.04[a]	0.54±0.03[a]	0.51±0.03[a]	0.31±0.04[b]

Values are means ± standard error (SE); n=6
Means within the same row and experimental period with different superscripts are significantly different (P<0.05).
AGP= Antimicrobial Growth Promoter.

Therefore, Prebiotic had positive effect on broiler immunity (Ferket, 2004). These results were confirmed by Vytautas *et al.* (2006) who reported that feeding broiler chickens on a prebiotic supplemented diets increased serum total protein and globulin levels. In addition, Abd-El-Samee *et al.* (2013) revealed that the prebiotic inclusion in the quail's diet caused a significant increase in the concentrations of total plasma protein and total globulin. On the other hand, these results disagreed with Shahir *et al.* (2014) who reported that supplementation of broiler diet with prebiotic had no effect on total protein, albumin, globulin and albumin to globulin ratio.

Table (6), shows the effects of dietary inclusion of prebiotic and AGP on humoral immune response, which were investigated by detecting serum antibody titers against ND and IBD viruses by hemagglutination inhibition test (HIT) in this experiment at both 28 and 42 days, there were no significant differences among the three groups in the antibody titters against ND and AI at both 28 and 42 days. These results agreed with Silva *et al.* (2009) exhibited that the prebiotic had no significant effect on antibody titer against ND in broiler chicks. And also, Shahir *et al.* (2014) revealed that the prebiotic had no significant effect on the antibody titers against both Influenza and Newcastle Disease. Where, these results disagreed with Huang *et al.* (2007) reported that the prebiotic (MOS) caused a significant improvement in the broiler immunity and better immunity response as measured by elevated levels of serum antibody titer against ND.

The effects of prebiotic and AGP feeding on the intestinal bacteriology were shown in table (7). The antibiotic-supplemented group showed a significant ($p<0.05$) reduction in the total aerobic count than prebiotic-supplemented group and control at both 21and 42 days. These results were hand in hand with Kim *et al.* (2010) who reported that the addition of avilomycin antibiotic in the broiler diet

caused a significant reduction in the total bacterial count than the control, probiotic, prebiotic and synbiotic fed groups. Also, Banerjee *et al.* (2013) recorded that the dietary supplementation of Bacitracin Methylene Disalicylate (BMD) caused a significant reduction in the *Lactobacillus* count in broiler chicks. These results explain the bad effect of antibiotic on the intestinal tissue healthiness and morphology and this seem consistent with Baurhoo *et al.* (2007) who reported that the antibiotic was less effective in maintaining of the intestinal tissue healthiness and morphology than prebiotic due to its bad effect on the beneficial intestinal bacteria.

By increasing the growth of beneficial microbes or by reduction and removal of potential pathogens, the alternatives to AGP possibly can improve the health and performance of birds (Yang *et al.*, 2009). Regarding the coliform count in this experiment, the prebiotic-supplemented group showed a significant ($p<0.05$) reduction in the coliform count than the control and antibiotic-supplemented groups at 42 days. These results were consistent with Ferket, (2004) who mentioned that prebiotic (MOS) possessed inhibitory effect on intestinal pathogens which could be related to their effects on pathogenic or potential pathogenic bacteria which possess type-1 fimbriae, resulting in better performance.

Table (6): Effects of different feed additives on humoral immune response investigated by detecting serum antibody titers against ND and AI viruses by hemagglutination inhibition test (HIT) at both 28 and 42 days:

	Control	AGP	Prebiotic	Control	AGP	Prebiotic
	28 days			42 days		
ND	5±0.41[a]	4.83±0.38[a]	5.17±0.28[a]	6.67±0.24[a]	6.83±0.31[a]	7±0.37[a]
AI	1.83±0.32[a]	1.83±0.47[a]	2.17±0.33[a]	2.5±0.22[a]	2.33±0.47[a]	2.83±0.23[a]

Values are expressed as means ± standard error (SE); n=6.
Means within the same row and experimental period with different superscripts are significantly different ($P<0.05$). AGP= Antimicrobial Growth Promoter; ND= Newcastle; AI= Avian Influenza.

Table (7): Effects of different feed additives on the intestinal bacterial count at 21 and 42 days:

	Control	AGP	Prebiotic	Control	AGP	Prebiotic
	21 days			42 days		
Total aerobic count (log CFU/g)	8.05±0.05[a]	6.91±0.04[b]	8.15±0.12[a]	8.52±0.04[a]	8.35±0.01[b]	8.59±0.04[a]
Total coliform Count (log CFU/g)	5.67±0.06[a]	5.81±0.01[a]	5.65±0.08[a]	7.75±0.2[a]	7.8±0.05[a]	5.08±0.14[b]

Values are expressed as means ± standard error (SE); n=6
Means within the same row and experimental period with different superscripts are significantly different ($P<0.05$). AGP=Antimicrobial Growth Promoter; CFU = cell forming unit.

Table (8): Effects of different feed additives on the histomorphometric measurements of the intestinal tract of broilers at 42 days (µm):

	Control	AGP	Prebiotic	Control	AGP	Prebiotic	Control	AGP	Prebiotic
	Duodenum			Jejunum			Ileum		
VH (µm)	531.51± 2.69[b]	535.93± 1.42[b]	745.85± 2.77[a]	496.94± 2.05[b]	382.12± 2.97[c]	606.41± 2.25[a]	436.02± 3.18[b]	432.52± 2.51[b]	681.73± 2.14[a]
CD (µm)	333.21± 2.92[a]	337.93± 3.26[a]	339.02± 2.94[a]	303.59± 1.98[a]	305.42± 1.79[a]	301.73± 1.96[a]	207.37± 1.56[b]	201.42± 1.58[b]	314.92± 1.96[a]
VH:CD R	1.59± 0.02[b]	1.58± 0.02[b]	2.22± 0.03[a]	1.64± 0.01[b]	1.25± 0.01[c]	2.01± 0.02[a]	2.1± 0.02[a]	2.15± 0.02[a]	2.16± 0.02[a]

Values are expressed as means ± standard error (SE); n=6.
Means within the same row and experimental period with different superscripts are significantly different ($P<0.05$).
AGP= Antimicrobial growth promoters; VH= Villus height; CD= Crypt depth; VH: CD R= Villus height: Crypt depth ratio

Baurhoo *et al*. (2007) revealed that the prebiotic-fed groups based on MOS showed a significant reduction in the Litter *E. coli* load than control and virginamycin-fed group. Similarly, Yang *et al.* (2008) demonstrated considerable decrease in the ileal and cecal populations of coliforms in broilers fed diets containing MOS. Furthermore, Kim *et al.* (2010) concluded that the addition of prebiotic (MOS and fructo-oligosacchride) in the broiler diet caused a significant reduction in the total coliform count than the control and antibiotic received groups.

The effects of prebiotic and AGP supplementation on the histomorphometric measurements of the intestinal tract at the end of the experiment were shown in table (8). Broilers received dietary prebiotic revealed a significant ($p<0.05$) increase in the villus height (VH) and VH: CD ratio and no significant differences in the crypts depth (CD) in duodenum and jejunum as compared with antibiotic and control groups. While, the antibiotic had a bad effect on the jejunal VH and VH: CD ratio than the control and prebiotic-supplemented groups as shown in figures (1 and 2). The prebiotic-supplemented group showed a significant ($p<0.05$) increase in the VH and CD and no significant differences in the VH: CD ratio when compared with control and antibiotic-supplemented groups in the ileum as in figure (3). Intestinal villi play a great role in nutrient digestion and absorption, as the villi greatly increase small intestine's surface area and are the first tissues in the intestine to make contact with nutrients (Gartner and Hiatt, 2001). Villus height, crypt depth and their ratio are important indications of gut health in broiler chickens (Pluske *et al.,* 1996). In general, the longer villus height and higher villus height to crypt ratio resulted in epithelial turnover therefore, increased the absorptive surface of intestine leading to proper absorption and improved performance (Xu *et al.,* 2003). This may explain the better performance in broilers fed on prebiotic supplemented ration.

The positive effect of prebiotic on the intestinal morphology mainly arose from its ability to create a favorable intestinal environment (through reducing of pathogenic bacteria and maintaining of beneficial bacteria in the intestine) which had a better effect on intestinal morphology (Xu *et al.,* 2003). Prebiotics increase production of fatty acids and reduce intestinal pH therefore; intestinal tissue health and morphology are achieved (Oliveira *et al.,* 2008). These results were compatible with Baurhoo *et al*. (2007), Markovic *et al.* (2009) and sayrafi *et al.* (2011) who reported that the prebiotic could be effective alternatives to the AGP as the prebiotic caused significant increase in VH and VA: CD ratio than the antibiotic and control groups that due to the ability of prebiotics to modulate the intestinal microbial communities. More recently, these results were confirmed by Houshmand *et al*. (2012), Abdel-Raheem *et al.* (2012), Ghasemi and Taherpour (2013) and Gharhi *et al.* (2013) concluded the positive effect of prebiotics on the intestinal morphology. However, the bad effect of antibiotic on the intestinal morphology mainly due to its negative effect on the beneficial intestinal bacteria (Baurhoo *et al*., 2007). Moreover, Gunal *et al.* (2006) reported that the antibiotic had no significant effects on jejunum and ileum height and width of the villi, depth of crypts and VH: CD ratio.

4. CONCLUSION

Dietary prebiotic supplementation could be an effective alternative to AGP that caused significant improvement in productive performance, hematological and serum biochemical constituent, intestinal bacteriology & morphometrical parameters and humeral immunity of broiler chicken.

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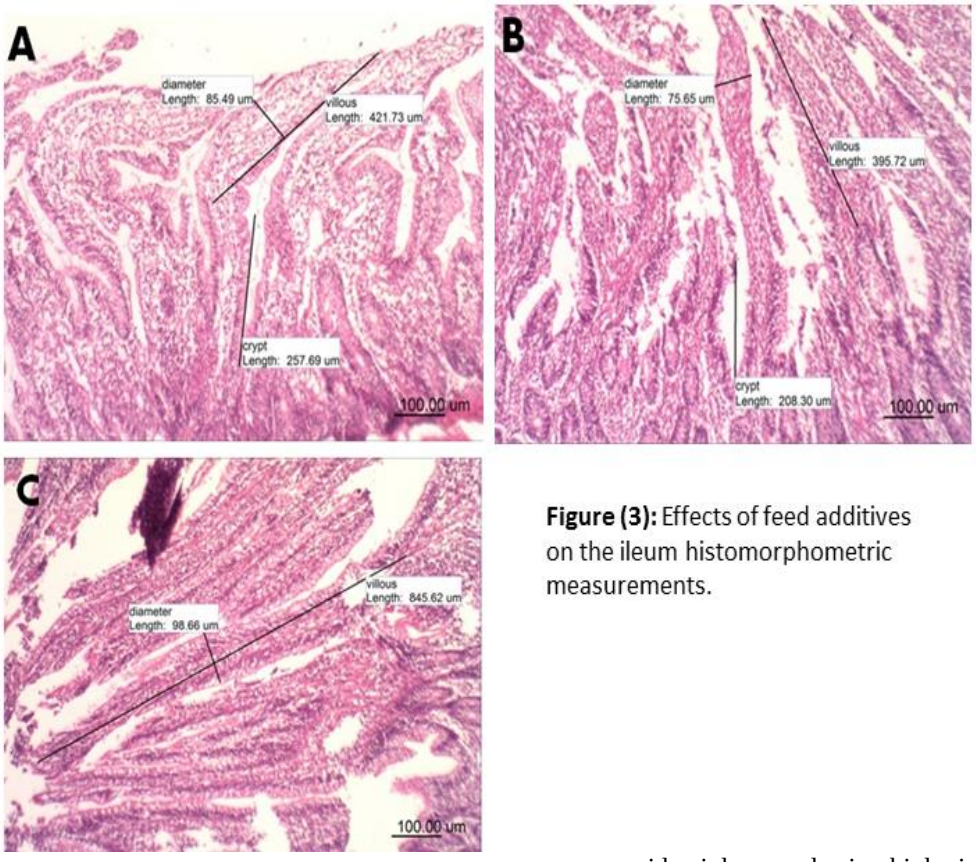

Figure (3): Effects of feed additives on the ileum histomorphometric measurements.

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