Evaluation of Anherbal Compound, a Commercial Probiotic, and an Antibiotic Growth Promoter on the Performance, Intestinal Bacterial Population, Antibody Titers, and Morphology of the Jejunum and Ileum of broilers

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ABSTRACT

The current study was conducted to examine the effects of an herbal compound, a probiotic and an antibiotic growth promoter (AGP) on the performance, intestinal bacterial population, antibody titers, and morphology of the jejunum and ileum of broilers. A number of 240 male Ross 308 broilers were distributed into four treatments, with five replicates of 12 bird each. The experimental period was 42 days. Treatments includes: 1) basal diet; 2) basal diet supplemented with an AGP (phospho-flavomycin at 450 mg/kg of diet); 3) basal diet supplemented with a *Lactobacillus*-containing probiotic (250 mg/kg of diet); and 4) basal diet supplemented with an herbal compound (containing thyme, oregano, chamomile, and peppermint essential oils at 1 g/kg of diet). Body weight (BW) and cumulative feed intake (CFI) were measured weekly. Blood parameters, intestinal morphology, vaccine immunity titers, and intestinal microbial population were measured on day 42. The results showed that probiotic and herbal compound supplementation significantly increased body weight (BW) and decreased feed conversion ratio (FCR) ($p<0.05$), in comparison with the basal diet. A significant increase in vaccine titers against Avian Influenza, Newcastle disease, and Infectious Bursal Disease were achieved with the treatment with herbal compound supplementation. Herbal compound significantly reduced triglycerides, cholesterol, LDL concentration and ALP, AST and ALT activities and increased the HDL levels in blood serum of chicks ($p<0.05$). The bacterial load of *E. coli*, Salmonella and coliforms of the AGP-fed group was significantly lower than those fed the control diet. Significant increases in villus height and decrease in crypt depth and goblet cells were seen in the ileum and jejunum of probiotic-fed broilers ($p<0.05$). In conclusion, better overall immune status and blood biochemical parameters were obtained with the herbal compound than with the probiotic; however, the performance of broilers fed the probiotic was better than that of the broilers fed the herbal compound and the AGP.

INTRODUCTION

The addition of antibiotic growth promoters (AGP) in poultry diets can reduce the negative impact of gastrointestinal tract infections, enhancing nutrient absorption in the intestinal mucosal layer (Ferket, 2002; Ayasan *et al.,* 2006). The use of AGP is banned in most countries, and, therefore, several alternatives to AGPs, including probiotics, prebiotics, acidifiers, and essential oils have been researched (Fuller, 2003).

Probiotics are living organisms that promote intestinal microflora balance, resulting in beneficial support of gastrointestinal tract functions, and, consequently, overall animal performance (Ayasan *et al.,* 2013;

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Seidavi *et al.,* 2017). The probiotics typically used in broiler nutrition contain microorganisms belonging to different species of *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifido bacterium*, Enterococcus, *Aspergillus*, and *Saccharomyces*. These have proven favorable impact on animal performance and intestinal microflora balance by preventing the presence of enteric pathogens, changes in intestinal histology, as well as by promoting immune sustainability and improving blood serum biochemical profile (Irshad, 2006; Ayasan *et al.,* 2016). By enhancing the activity of beneficial microorganisms, FCR and the growth rate of livestock and poultry are improved as they prevent the damaging effects of harmful microorganisms on animal health and growth performance (Murry *et al.,* 2006; Seidavi *et al.,* 2017).

The presence of mannan, chitin, and glucan in the cell wall of the yeast *Saccharomyces* spp. enhances the immunity and reduces the load of harmful intestinal bacteria in broilers (Nosrati *et al.,* 2017). In the study of Zaker *et al.* (2011), the dietary addition of a chemical growth promoter was compared with natural growth promoters, and the results showed that natural growth promoters, such as acidifiers, improved FCR, growth factors, and performance of broilers, as well as enhanced immunity and feed digestion and absorption. Moreover, those authors also reported significant reduction of feed costs with the use of natural growth promoters. Previous research has shown that various medicinal plants components have beneficial effects on the intestinal environment and its bacterial population (Omar *et al.,* 2016). Medicinal herbs and their extracts have antibacterial, antiparasitic, antiviral and antioxidant properties and stimulate the immune and hormonal systems of different bird species (Sharifi *et al.,* 2013). Herbal essential oils stimulate digestive enzymes and may have a positive impact on fat metabolism and digestion (Hashemipour *et al.,* 2013). Sharifi *et al.* (2013) showed that thymol and carvacrol present in thyme were effective in enhancing the immune system and increasing the livability of broilers. Previous studies showed that the addition of artemisia, thyme, oregano and rosemary extract to broiler diets increased the growth rate, improved intestinal digestion, and increased starch digestibility and dry matter utilization (Nosrati *et al.,* 2017). Higher antibody titers against Newcastle disease (Houshmand *et al.,* 2012), higher weight gain, better FCR, and lower cholesterol and triglyceride levels were recently reported by Ghaderi-Jouybari *et al.* (2012) in broilers fed probiotic. Therefore, the objective of this experiment was to evaluate the effects of the dietary inclusion of an herbal compound consisting of thyme, oregano and peppermint essential oils, in replacement of an antibiotic growth promoter on the performance, intestinal bacterial population, antibody titers, and morphology of the jejunum and ileum of broilers.

MATERIAL AND METHODS

Experimental design, Housing, Management and Experimental Diets

The experiment was carried out under the supervision of the Ethics committee of the Malayer University.

A number of 240 day-old male Ross 308 broilers, with 40 ginitial weight, were evaluated for 42 days. The chicks were housed in an environmentally-controlled room at the Malayer University Poultry Research farm. Initial room temperature set at 33°C and was gradually decreased by 3°C per week after the first week until 20°C at the end of trial. A photoperiod of 23L:1D was adopted, with the lights turned off at 23:00 h. Birds had free access to feed and water during the entire experimental period of 42 days.

Birds were distributed according to completely randomized design, consisting of four treatments with five replicates of 12 birds each. The basal diet was based on corn and soybean formulated according to the genetic company recommendations (Ross company Ross 308, 2014). A three-phase feeding program was adopted: starter diet (1-10 days), grower diet (11-24days), finisher diet (25-42 days). The treatments included: T1) basal diet; T2) basal diet supplemented with addition of the antibiotic phospho-flavomycinat the rate of 450 mg/kg of diet (each g of phospho-flavomycin contains 75 mg of flavophospholipol; HUVEPHARMA, Sofia, Bulgaria); 3) basal diet supplemented with a Lactobacillus-containing probiotic (250 mg/kg of diet); and 4) basal diet with the herbal compound (1 g/kg of diet). The probiotic product contained 10^8 *Lactobacillus* spores/g and was obtained from Lallemand Health Solutions, Paris, France. The herbal compound consisted of a mixture of thyme, oregano, and peppermint essential oils (Natusol® A property product of Zeus Biotech Private Limited, Mysore, India).

Chicks were vaccinated against Newcastle and Avian Influenza diseases as per the guidelines of Iranian Veterinary Organization, considering the maternal

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antibody levels as well as antibody titer levels obtained at day 4.

EVALUATED PARAMETERS

Performance Parameters

Body weight gain (BWG), feed intake (FI) were measured on a weekly basis used to calculate feed conversion ratio (FCR). Livability was calculated as the percentage of birds alive at the end of the experiment relative to the initial number of birds.

Biochemical Parameters and Antibody Titers

At the end of the experimental period (day 42), two birds per experimental unit (replicate) were randomly selected for collection of 5 mL of blood from the wing vein for blood analysis and antibody titering.

Vaccine titers against Avian Influenza (AI) and Newcastle disease (ND) were determined by hemoagglutination inhibition test (HI), and Infectious Bursal Disease (IBD)titers was measured by Enzyme-linked Immunosorbent Assay (ELISA kit, IDEXX, USA).

Blood samples were centrifuged at 3000xg for 10 minutes to separate the serum, and the serum levels of triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and the activities of the liver enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate transaminase (AST) were measured using an auto analyzer system (Technicon RA-1000, USA), (Technicon RA-1000, USA and Pars Azmoon test kits (Tehran, Iran) (Hedayati *et al.,* 2015).

Bacterial Count

Two birds per experimental unit were randomly selected and sacrificed. After *post-mortem* exami-nation, the cecal content was collected and submitted in sterile containers with ice to the laboratory for coliform, *Salmonella* spp., and *E. coli*counts. In the lab, individual cecal contents were pooled per replicate to prepare serial dilutions (10^{-4} to 10^{-6}) of cecal samples in anaerobic diluents. The serial dilutions were seeded on Petri dishes with sterile agar, according to Gunal *et al.* (2006). Eosin methylene blue agar (EMB) was used for *E. coli* growth, Salmonella-Shigella agar (SS Agar) (Merck, Germany) for *Salmonella* spp. growth, and MacConkey agar (Merck, Germany) for coliform growth. *E. coli* growth medium was aerobically incubated at 37°C. Colonies were counted between 24 and 48 h after incubation. Colony forming units (cfu) were defined as distinct colonies measuring 1 mm in diameter. Then, 9 sterile test tubes with lids containing 9mL of phosphate buffer solution (PBS, Ph 7.4) as diluent were prepared. Approximately 1g of the cecal contents taken by sterile swab and homogenized for 3 min before transferring to the microbiology lab in cold conditions (Gunal *et al.*, 2006), aseptically mixed, added to the tubes, and diluted up to 10^9. Later, 1mL of the contents of each test tube was transferred to one of three selective agar media on petri plates, respectively, and each petri plate incubated in 37°C for 24h. Finally, the intestinal bacterial colony populations formed in each plate was counted, manually adjusted to X 10^6, and reported.

Intestinal Morphology

A 2X2 cm^2 section of the ileum and the jejunum were collected from the middle of the ileum and jejunum and flushed with PBS (pH 7.4) twice. Tissue sections were immediately fixed in formalin at 10%, which was changed thrice to complete the fixation process. A single 0.5-cm sample was cut from each tissue section, dehydrated with increasing concentrations (70, 80, 95 and 100%) of ethanol, cleared with xylene, and embedded in polyfin wax. Tissue sections (2 μm) were cut using a microtome (Leitz-1512 Microtome, Leitz, Wetzlar, Germany), floated onto slides, and stained with hematoxylin (Gill no. 2, Sigma, St. Louis, MO) and eosin (Sigma) (H&E staining). To measure villus height and crypt depth, images of the samples were made using a digital camera under light microscopy. Twelve images of four tissue sections of ileum and of the jejunum were taken, and 12 villus heights and crypt depths were measured by imaging software (Toupe view software 2.3, 2010). Villus length was measured from the tip of the villus to the valley, and crypt depth were taken from the valley to the basolateral membrane (Xu *et al.,* 2003).Villus height and crypt depth were measured per each segment in order to determine villus height, crypt depth, and villus height:crypt depth ratio (villus:crypt ratio).

Statistical Analysis

Data were submitted to analysis of variance using the GLM procedure of SAS 9.3 software (SAS Institute, 2007). Differences between treatment means were tested using Duncan's multiple comparison test. Statistical significance was declared at $p<0.05$.

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Table 1 – Ingredients and analyzed nutritional composition of the basal diets (as-fed basis)

Feed Ingredient (%)	Starter (1–10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn (8% CP)	53.20	55.88	57.25
Soybean meal (43% CP)	38.41	34.90	33.31
Wheat bran	2.02	2.02	2.02
Vegetable oil	2.08	3.60	4.10
Limestone	1.30	1.10	1.04
Dicalcium phosphate	1.65	1.40	1.31
Salt	0.42	0.42	0.40
DL-Met	0.15	0.10	0.07
HCl-Lys	0.21	0.08	0.00
Thr	0.06	0.00	0.00
Vitamin premix [a]	0.25	0.25	0.25
Mineral premix [b]	0.25	0.25	0.25
Analyzed composition (%, unless otherwise noted)			
Metabolizable energy (kcal/kg)	2850	2970	3020
Crude protein	22.1	20.7	19.8
Calcium	1.00	0.85	0.80
Available phosphorous	0.47	0.42	0.39
Sodium	0.18	0.18	0.17
Lys	1.35	1.17	1.03
Met	0.48	0.42	0.39
Met + Cys	1.01	0.90	0.81
Thr	0.89	0.78	0.70

Dicalcium phosphate contained: 16% phosphorous and 23% calcium.

[a] Vitamin premix provided the following per kilogram of diet: vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,500 IU; vitamin E (DL-α-tocopheryl acetate), 60 mg; vitamin K (menadione), 3 mg; thiamine, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B12 (cyanocobalamin), 0.01 mg; niacin, 45 mg; pantothenic acid (D-calcium panto-thenate), 11 mg; folic acid, 1 mg; biotin, 0.15 mg; choline chloride, 500 mg; ethoxyquin (antioxidant), 150 mg.

[b] Mineral premix provided the following per kilogram of diet: Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co, 0.2 mg; Se, 0.15 mg.

RESULTS

Live performance

Body weight

There was a significant effect ($p<0.05$) the treatments on BW. Probiotic-fed broilers presented higher BW compared with the other treatments in all evaluated periods, except on day 21, when their BW was statistically similar to the AGP-fed birds. The BW of the control group was lower compared with that obtained in the other treatment groups, except for day 7 and 14, when it was not different from the AGP and HC groups, and from the AGP group, respectively (Table 2).

Table 2 – Effects of the dietary addition of a herbal compound, a Lactobacillus-based probiotic and of an AGP on broiler performance.

Parameter	Treatments					
	Control	AGP	Probiotic	HC	P-Value	SEM
Body weight (g)						
1 - 7 d	200[b]	201[b]	205[a]	201[b]	0.0431	19.03
1 – 14 d	430[b]	245[ab]	455[a]	452[a]	0.0310	39.77
1 - 21 d	800[c]	820[a]	825[a]	812[b]	0.0218	82.19
1 - 28 d	1300[c]	1340[b]	1350[a]	1344[ab]	0.0186	145.76
1 - 35 d	1750[c]	1810[b]	1850[a]	1780[c]	0.0318	181.06
1 - 42 d	2305[d]	2355[c]	2430[a]	2390[b]	0.0298	291.25
Feed Intake						
1 - 42 d	4300[a]	4260[ab]	4250[ab]	4230[b]	0.0311	501.56
FCR						
1 - 42 d	1.865[a]	1.809[ab]	1.748[b]	1.769[b]	0.0217	0.249
Livability						
1 - 42 d	98	99	99	99	0.0677	10.24

[a-d]Means in a same row with different superscripts are significantly different ($p\leq0.05$). Control: basal diet with no addition of AGP, probiotic, or HC; AGP: control plus 0.045% phospho-flavomycin; Probiotic: control plus 0.025% of a commercial probiotic; HC: control plus 0.1% of an herbal compound.

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Feed intake

The highest cumulative FI was observed in the control group and the lowest in the HC group (*p*<0.05), while the AGP and probiotic groups presented statistically intermediate values (Table 2).

FCR and livability

The highest FCR was obtained in the control group, and the lowest in the probiotic group (*p*<0.05). Livability was not statistically influenced by the treatments

(*p*>0.05), although it was numerically lower in the control group (Table 2).

Vaccinal antibody titers

The birds fed HC presented were significantly higher (*p*<0.05) antibody titers against ND and IBD compared with the other treatment groups. Relative to AI, the HC and the AGP groups presented higher (*p*<0.05) antibody titers than the probiotic group, whereas the control group showed intermediate values (Table 3).

Table 3 – Effects of the dietary addition of an herbal compound, a Lactobacillus-based probiotic and of an AGP on the antibody titers of 42-d-old broilers.

Antibody titers	Treatments					
	Control	AGP	Probiotic	HC	P-Value	SEM
ND[1]	5[b]	5.10[b]	5.15[b]	6[a]	0.0368	0.65
AI[2]	4.55[ab]	4.80[a]	4.00[b]	5[a]	0.0419	0.61
IBD[3]	444[b]	455[b]	448[b]	484[a]	0.0371	58.36

[a-b]Mean values (n=10/treatment) in a same row with different superscripts are significantly different (*p≤0.05*)

Control: basal diet with no addition of AGP, probiotic, or HC; AGP: control plus 0.045% phospho-flavomycin; Probiotic: control plus 0.025% of a commercial probiotic; HC: control plus 0.1% of an herbal compound. [1] ND: Newcastle disease. [2] AI: Avian Influenza. [3] IBD: Infectious Bursal Disease.

Serumbiochemical parameters

The highest and lowest blood serum triglyceride levels were determined in the control and herbal compound groups, respectively. The lowest and the highest cholesterol levels (*p*<0.05) were observed in the HC and control groups, respectively. The HC and Probiotic groups presented lower LDL levels (*p*<0.05) compared with the control group, whereas the AGP group showed intermediate values. The highest HDL levels were obtained in the HC and Probiotic groups, and the lowest in the AGP group (*p*<0.05). The

activity of the liver enzyme ALT was not influenced by the treatments (*p*>0.05). The highest AST activity was observed in the Probiotic group, followed by the AGP group (*p*<0.05), and the lowest and statistically similar values were obtained in the control and HC groups. The highest ALP activity was observed in the Probiotic group, followed by the control group, and the lowest activity in the AGP group (*p*<0.05). The ALP activity value obtained in the HC group was statistically similar to those of the control and AGP groups (Table 4).

Table 4 – Effects of the dietary addition of an herbal compound, a Lactobacillus-based probiotic and of an AGP on blood serum biochemical parameters and liver enzyme activities of 42-d-old broilers

Items	Treatments					
	Control	AGP	Probiotic	HC	P-Value	SEM
Triglyceride (mg/dL)	122[a]	120[a]	115[b]	112[c]	0.0338	14.42
Cholesterol (mg/dL)	195[a]	180[b]	175[b]	115[c]	0.0219	12.29
LDL (mg/dL)[1]	74[a]	70[ab]	65[b]	62[b]	0.0321	7.24
HDL (mg/dL)[2]	50[b]	45[c]	52[a]	55[a]	0.0421	6.81
ALT (IU/L)[3]	5	4.8	5	4.9	0.0618	0.53
AST (IU/L)[4]	175[b]	170[c]	180[a]	168[c]	0.0191	18.26
ALP (IU/L)[5]	15[b]	140[c]	160[a]	155[bc]	0.0319	16.83

[a-b]Mean values (n=10/treatment) in a same row with different superscripts are significantly different (*p≤0.05*)

Control: basal diet with no addition of AGP, probiotic, or HC; AGP: control plus 0.045% phospho-flavomycin; Probiotic: control plus 0.025% of a commercial probiotic; HC: control plus 0.1% of an herbal compound. [1] LDL: Low-density lipoprotein. [2] HDL: High-density lipoprotein. [3]ALT: Alanine Aminotransferase. [4]AST: Aspartate transaminase. [5]ALP: Alkaline phosphatase.

Cecal bacteria count

The cecal bacterial count results showed that the AGP-fed broilers presented significantly (*p*<0.05)

lower populations of *E. coli* and coliforms compared with the other groups (Table 5). Lower *Salmonella* spp. counts were detected in the AGP and Probiotic groups compared with the HC group (*p*<0.05).

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Table 5 – Effects of the dietary addition of an herbal compound, a Lactobacillus-based probiotic and of an AGP on intestinal bacterial counts of 42-d-old broilers[1]

Bacteria	Treatments					
	Control	AGP	Probiotic	HC	P-Value	SEM
Escherichia coli	3.52[a]	1.75[c]	2.15[bc]	2.33[b]	0.0384	0.31
Salmonella spp.	2.10[a]	1.50[b]	1.65[b]	2.00[a]	0.0218	0.19
Coliforms	3.50[a]	1.15[c]	2.00[b]	2.30[b]	0.0443	0.28

[a-b]Mean values (n=10/treatment) in a same row with different superscripts are significantly different ($P \leq 0.05$)

Control: basal diet with no addition of AGP, probiotic, or HC; AGP: control plus 0.045% phospho-flavomycin; Probiotic: control plus 0.025% of a commercial probiotic; HC: control plus 0.1% of an herbal compound.

[1]Calculated as per $\log_{10}$cfu/g (10^6).

Intestinal morphology

In the jejunum (Table 6), probiotic-fed broilers presented higher villi compared with the AGP-fed and control birds ($p<0.05$), while the HC-fed group presented intermediate values. Lower crypt depth was obtained in the probiotic and HC groups compared

with the control and AGP groups ($p<0.05$). Treatments significantly influenced the number of goblet cells ($p<0.05$), with the highest and lowest numbers observed in the control and the probiotic groups, respectively. Treatments did not influence VH/DC ratio ($p>0.05$).

Table 6 – Effects of the dietary addition of an herbal compound, a Lactobacillus-based probiotic and of an AGP on the morphology of the jejunum of 42-d-old broilers.

Items	Treatments					
	Control	AGP	Probiotic	HC	P-Value	SEM
Villus height (µm)	650[b]	600[b]	800[a]	750[ab]	0.028	37.19
Crypt depth (µm)	600[a]	580[a]	530[b]	540[b]	0.032	21.66
Number of goblet cell (per mm	9[a]	8[b]	6[d]	7[c]	0.001	0.01
VH/CD	1.08	1.03	1.50	1.38	0.067	0.01

[a-b]Mean values (n=10/treatment) in a same row with different superscripts are significantly different ($p \leq 0.05$)

Control: basal diet with no addition of AGP, probiotic, or HC; AGP: control plus 0.045% phospho-flavomycin; Probiotic: control plus 0.025% of a commercial probiotic; HC: control plus 0.1% of an herbal compound.

In the ileum (Table 7), the highest villi were measured in the Probiotic group, followed by the HC group, which presented statistically similar values to those obtained in the probiotic and AGP groups, and the AGP group, with the lowest villi observed in the control group ($p<0.05$). The probiotic and HC groups presented the deepest crypts, followed by the AGP and the control groups ($p<0.05$). The lowest number of goblet cells was observed in the Probiotic and HC groups, followed by the AGP group, while the control group presented the highest number ($p<0.05$). The Probiotic and HC

groups presented higher VH/CD ratios compared with the control and AGP groups ($p<0.05$).

DISCUSSION

Except for the first week, the different treatments resulted in significant increase in body weight. No significant differences in Flamong treatments was observed, but the highest and lowest FCR values were obtained in the control and probiotic-fed groups, respectively. Feeding probiotics considerably

Table 7 – Effects of the dietary addition of an herbal compound, a Lactobacillus-based probiotic and of an AGP on the morphology of the ileum of 42-d-old broilers.

Items	Treatments					
	Control	AGP	Probiotic	HC	P-Value	SEM
Villus height (µm)	500[c]	550[b]	600[a]	580[ab]	0.001	45.33
Crypt depth(µm)	450a	400b	300c	320c	0.023	29.61
Number of goblet cell (per mm)	8[a]	6[b]	5[c]	5[c]	0.001	0.05
VH/CD	1.11[b]	1.375[b]	2[a]	1.81[a]	0.038	0.009

[a-b]Mean values (n=10/treatment) in a same row with different superscripts are significantly different ($p \leq 0.05$)

Control: without AGP, Probiotic and HC supplementation; AGP: control plus 0.045%phospho-flavomycin; Probiotic: control plus 0.025% commercial probiotic; HC: control plus 0.1% Herbal Compound.

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improves the FCR of broilers reared in poor nutritional and management conditions. Baurhoo et al. (2009) reported that feeding broilers with *Saccharomyces cerevisiae* increased villus height and intestinal absorption, leading to optimal growth and FCR. Kabir (2004) observed that adding 2 g of a probiotic/L of drinking water promoted metabolic and morphological changes in the intestinal epithelial lining that were associated with better FCR, body weight gain, as well as carcass and cuts (breast and thigh) yields than the control diet. However, previous reports showed that broilers fed an AGP presented higher final body weight and better FCR compared with those fed a probiotic containing lactobacilli and Bifido bacteria (Mountzouris *et al.*, 2007, 2010). Safamehr & Nobakht (2008) reported that feeding broilers with 400 g/ton of a probiotic containing *Bacilussubtilis* significant increased body weight and decreased FCR. Gunal *et al.* (2006), reported that using combination of 0.1% probiotic and flavomycin antibiotic growth promoter in broiler diets had no impact on feed intake or weight gain. Sarica *et al.* (2005) reported no significant differences in weight gain of broilers fed with thyme and garlic extract compared with the control group. Galib *et al.* (2010) reported that using 1% of different herbal mixtures had no effect on broiler performance. Thyme affects the gastrointestinal tract of chickens, particularly the secretion of the digestive enzymes trypsin, amylase and chymotrypsin and increases nutrient absorption by the intestinal villi, resulting in better performance (Hernandez *et al.,* 2004). The antioxidant properties of medicinal plants may induce the secretion of protein digestion enzymes, and consequently improve FCR (Jafari *et al.*, 2011). It may also increase lactic acid bacteria populations in the jejunum of poultry, increasing feed intake and efficiency increases (Koc, 2010). The addition of thyme essential oil to the feed and water of broilers significantly improved FCR (Alcicek *et al.,* 2003). Medicinal plants and their extracts, as well as organic acids, may reduce feed intake due to reduced feed palatability. Since organic acids are components of nettle, it can reduce the feed intake of diets supplemented with nettle and organic acids (Bampidis *et al.,* 2005). Thymol and carvacrol in medicinal herbs added to broiler feeds stimulate digestion and increase daily feed intake and overall performance (Cabuk *et al.,* 2006). Manafi *et al.*(2016) reported that thyme affected the gastrointestinal tract, particularly the intestine, increasing the secretion of digestive enzymes like amylase and chymotrypsin.

The antioxidant properties of herbal compounds stimulate the secretion of proteases, which result in better FCR (Jafari *et al.,* 2011). It may also increase lactic-acid bacterial populations in the jejunum and improve feed efficiency (Koc, 2010). The addition of thyme essential oil to a feed and water improved broiler FCR (Alcicek *et al.,* 2003). Thymol and carvacrol present in medicinal herbs like nettle, oregano, thyme, and mint have antimicrobial properties, reducing the number of pathogenic bacteria in the digestive tract, consequently promoting better performance in broilers (Burt & Reinders, 2003). These positive effects are apparent when broilers are reared in unfavorable conditions. For instance, when broilers are fed a low digestibility diet or are reared in unhealthy conditions, medicinal plants may improve their FCR (Demir *et al.,* 2003).

Improved humoral immune responses against ND in broilers fed probiotics and a commercial prebiotic containing MOS were earlier reported by Zakeri *et al.* (2011). In the study of Rowghani *et al.* (2007), enhanced immune response against ND was observed when broilers were fed a probiotic. An increase in immune titers against ND was observed when broilers were fed thyme and oregano mixed with cinnamon (Sadeghi *et al.,* 2012). No differences in antibody titers against ND and AI were detected when a probiotic was compared with yarrow powder (Toghyani *et al.,* 2011). Oregano increases the antibody titers against Newcastle disease in broilers due to its antioxidant and antimicrobial properties (Mahboubi & Haghi, 2008). The phenolic compounds thymol and carvacrol present in savory enhances the immune response of broilers (Manafi, 2015).

The production of free radicals weakens the immune system, as the cell wall of many cells, such as lymphocytes and macrophages, are extremely sensitive to oxidative damage. Immune-cell damage caused by free radicals can be prevented by vitamins A and E, ascorbic acid, superoxide dismutase, glutathione reductase, glutathione peroxidase, and catalase. The imbalance between these compounds and free-radical producing systems cause oxidative stress. Damage caused by free radicals is an important mechanism of cell damage (Kumar *et al.,* 2006). Nettle phenolic compounds, such as carvacrol and thymol, have antimicrobial activities, reduce oxidative stress, and enhance overall immunity (Gulcin *et al.,* 2004). The beneficial effects of the thyme, nettle and oregano on bacterial activity and its direct impact on the immune system due to their powerful antioxidant properties,

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are due to the active ingredients thymol and carvacrol present in thyme essential oil (Hashemipour *et al.,* 2011). The addition of thyme extract (200 mg/kg) in broiler diets effectively enhanced their immune function and livability (Lee et al., 2004). In study of Saleh *et al.* (2014), the use thyme essence (200 mg/ton of feed) increased ND immune titers. There are many reports on the stimulation of the immune system by natural products (Manafi *et al.,* 2014; Hedayati *et al.,* 2015).

Broiler immune responses are enhanced by the dietary addition of probiotics and plant extracts (Houshmand *et al.,* 2012;Hedayati*et al.,* 2015). It was demonstrated that herbal extracts with high vitamin C content increased the phagocytic activity of immune cells (Kong *et al.,* 2004). Plant extracts increase the production of antibodies, in particular IgG, and therefore, through their anti-bacterial and anti-viral effects, they may indirectly improve the immune system (Kong *et al.,* 2004). Gunal *et al.* (2006) reported feeding garlic to broilers significantly increased their immune response against ND and IBD. Hernandez *et al.* (2004) reported that some herbal extracts increased the antibody titers against Newcastle and Avian Influenza viruses of broilers and turkeys, and increased the growth of immune organs in turkeys. A combination of savory and thyme extracts in broiler diets increased the percentage of lymphocytes and heterophils (Toghyani *et al.,* 2011). In another study, different levels of savory were fed to broilers, and higher ND titers were obtained compared with the control group (Zamani Moghaddam *et al.,* 2007). In another study, broilers fed wild mint presented higher antibody titers against ND (Al-Ankari *et al.,* 2004). It was reported that the addition of cinnamon and thyme in broiler diets decreased the lymphocyte heterophil ratio (Al-Kassie, 2010). In the study of Radwan (2003), broiler diets containing 0.5, 1 or 2% thyme leaves increased antibody titers. Broilers fed thyme supplements presented reduced lymphocyte to heterophil ratio (Galib *et al.,* 2010).

Demir *et al.* (2003) evaluated the addition of powder mixture of herbs (garlic, thyme, cinnamon and oregano) in broiler diets and did not report any significant effects on serum triglyceride concentrations. In another study (Dahiya *et al.*, 2006), a significant decrease in cholesterol and blood lipids of Japanese quails fed chamomile flowers of and thyme plants was observed. Lee *et al.* (2004) reported that broilers fed thymol and carvacrolin at 100 and 200 mg/kg, respectively, did not present any significant differences

in plasma cholesterol concentrations. On the other hand, Ghaderi-Jouybari *et al.* (2012) reported that a probiotic dietary supplement increased HDL and decreased LDL values in broilers. Ghalmkari *et al.* (2011) reported that thyme powder was effective in reducing cholesterol and triglyceride serum levels in broilers. Lee *et al.* (2004) fed broilers with thymol extract at 100 and 200 mg/kg and carvacrol extracts at 200 mg/kg and did not find any differences in plasma cholesterol concentrations. In the study of Yakhkeshi *et al.* (2011), a probiotic was more effective than an AGP in reducing serum triglyceride and cholesterol levels compared to the control group. Karimi *et al.* (2005), adding 1.0% probiotic to broiler diets, observed a significant reduction in serum cholesterol levels compared with the control group, and stated that the use of this probiotic could reduce serum LDL, HDL and triglycerides, although triglyceride reduction was not significant. It was reported that a probiotic did not significantly influence ALT and AST enzyme activity (Al-Khalaf *et al.,* 2010). Ali (2014) found that thyme powder at a dose of 500 g/ton of broiler feed increased albumin and decreased triglyceride and cholesterol levels. In another study, thyme essential oil at 2% in broiler diets decreased serum ALT and AST activities (Tawfeek *et al.,* 2012). Sarica *et al.* (2005) reported that consumption of garlic and thyme by broiler chickens numerically reduced serum cholesterol levels. This reduction is due to the inhibitory effects of these extracts on the activity of key enzymes, such as peroxidase and dehydrogenase, involved in lipid and cholesterol production. Blood cholesterol and triglyceride level reduction in broilers fed thyme and nettle is attributed to the actions of carvacrol and thymol, which reduce the levels of harmful metabolites in the blood of broilers. In addition, carvacrol stimulates the growth of lactobacilli, which play an important role in improving blood and serum lipids (Zargari, 2001).

In the present experiment, the HC-fed and control broilers presented the highest cecal bacterial counts, whereas the lowest counts were determined in the AGP-fed group. Antibiotics influence the gut microflora and effectively kill pathogens, reducing the production of bacterial toxins and the competition for nutrients by bacteria, increase the synthesis of vitamins and other growth factors, improve nutrient absorption by reducing the thickness of the internal layer of the intestine, and reduce bowel movements. On the hand, the lipopolysaccharides present in the membrane of some Gram-negative bacteria act as barrier against the passage of the active ingredients of essential oils and

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plant extracts through their cytoplasmic membrane, and as a result, some of these oils have no effect on the population of Gram-negative bacteria (Surono, 2003). The antibacterial properties of medicinal plants can be attributed mainly to their phenolic compounds. Phenols generally disrupt cell membrane activities, breaking the hold of active protons, electron flow stream and active transfer and clotting of the cell contents (Surono, 2003). It was reported that the ethanol extracts of yarrow and thyme prevented the growth of pathogenic bacteria (*Staphylococcus aureus*, *Bacillus cereus* and *E. coli*), but was not effective against *Pseudomonas aeruginosa* (Moosavi *et al.,* 2008). The effects of thyme and oregano essential oils against *Salmonella typhimurium* and *Staphylococcus aureus* were studied in a food system model, as well as their impact on cell wall morphology and structure using electronic microscopy. Cell wall destruction and leakage of cytoplasmic contents were observed (Moosavi *et al.,* 2008). Studies show that mannans of the cell wall of the yeast *Saccharomyces cerevisiae* are responsible for reducing the colonization and the presence of harmful intestinal bacteria (Oyouf *et al.,* 1989). Spring *et al.* (2000) showed that the fimbriae of pathogenic intestinal *Salmonella* spp. and *E. coli* bind to mannose in the intestine, and that *Saccharomyces cerevisiae* yeast cell wall binds to mannose, preventing the attachment of these bacteria to the intestinal epithelial cells of the small intestine, consequently reducing the population of harmful bacteria. Studies have shown that feeding *Saccharomyces cerevisiae* increased presence of lactobacilli and decrease the presence of *Salmonella* spp. and *E. coli* in the intestinal lumen (Murry *et al.,* 2006). Koc *et al.* (2010) reported that the use of *Saccharomyces cerevisiae* can be effective in reducing the presence of *E. coli* in the small intestine. It is also mentioned that both mannan oligosaccharides (MOS) and *Saccharomyces cerevisiae* significantly reduce the number of *E. coli* in the ceca of broilers (Spring *et al.,* 2000). Reduction of intestinal diameter and thickness and increased absorption level, along with reduction of harmful bacteria, can be extremely effective in improving the weight gain of broilers. Mountzouris *et al.* (2010) stated that probiotics may enhance the health and performance of broilers by preventing the growth of intestinal infectious bacteria, especially *Salmonella* spp. and *E. coli*. However, in the current study, the most significant reduction in cecal harmful bacteria was observed in the group receiving antibiotic, herbal compound and probiotics groups, respectively.

In the study of Sherief *et al.* (2012), the highest villi were measured in broilers fed a probiotic containing *Saccharomyces* and MOS, and the deepest crypts in probiotic-fed broilers. Concurrent addition of MOS and an acidifier in broiler diets by Pelicano *et al.* (2007) resulted in increased ileal villus height, while the lowest and highest crypt depth values were measured in broilers fed MOS and a *Bacillus subtilis* probiotic, respectively. In another study, the combination of probiotics and organic acids in broilers diets increased villus height (Gunal *et al.,* 2007). However, Pelicano *et al.* (2007) and Santin *et al.* (2010) did not find any difference in intestinal villus height when broilers were fed diets containing MOS and probiotic. The dietary addition of 2% *Saccharomyces cerevisiae* significantly increased villus height, reduced crypt depth, and increased the villus height to crypt depth ratio (Bradly *et al.,* 1994). According to the authors, the compounds in the cell wall of the yeast *Saccharomyces cerevisiae* increase villus height and reduce crypt depth and the number of goblet cells. Increasing intestinal villus height and reducing crypt depth enhances the absorptive capacity of the small intestine; higher villi reduce digesta passage rate and have larger absorption capacity and, therefore, optimize broiler performance.

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Erratum

In the article Evaluation of Anherbal Compound, a Commercial Probiotic, and an Antibiotic Growth Promoter on the Performance, Intestinal Bacterial Population, Antibody Titers, and Morphology of the Jejunum and Ileum of broilers published in the Revista Brasileira de Ciência Avícolas/Brazilian Journal of Poultry Science, v20 (2):377-386, in page 305, 306, 308, 309 and 310 where it was written:

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the correct form is

a herbal