



Comparison of Antibiotic, Probiotic and Great Plantain (*Plantago major* L.) on Growth Performance, Serum Metabolites, Immune Response and Ileal Microbial Population of Broilers

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Abstract

The objective of the study was to compare the effects of antibiotic virginiamycin, probiotic Protexin® and *Plantago major* L. (plantain) on performance, serum metabolites, immune response, and the ileal microbial population of broilers. The experiment was carried out with a total of 200 day-old male Ross 308 broiler chickens in a completely randomized design. Chickens were allocated to five groups consisting of T₁: control diet (Con), T₂: Con+0.02% virginiamycin, T₃: Con+0.01% Protexin, T₄: Con+0.5% plantain and T₅: Con+1% plantain. Each group was divided into four replicates consisting of ten chicks each. In comparison with the control group, body weight gain increased in chickens fed Protexin and 0.5% plantain groups in the starter period, as well as by antibiotic in grower and finisher periods and by 1% plantain in all periods ($P < 0.01$). Supplementation of plantain and virginiamycin increased ($P < 0.01$) feed intake in the starter and finisher periods, respectively. Feed conversion ratio improved ($P < 0.05$) in finisher period only by virginiamycin. All treated birds showed an elevated relative weight of carcass and bursa, and plantain increased relative weight of the spleen ($P < 0.01$). All treatments demonstrated a hypocholesterolemic effect ($P < 0.01$) and higher level of plantain (1%) decreased ($P < 0.05$) serum glucose, triglyceride and low-density lipoprotein-cholesterol as well. The inclusion of Protexin and plantain enhanced immune system with increased white and red blood cells as well as second anti-SRBC immune response and reduced heterophil/lymphocyte ratio in SRBC injected birds ($P < 0.05$). Virginiamycin decreased ileal microbial population of *Lactobacillus* while Protexin and plantain increased it ($P < 0.01$). Meanwhile, 1% plantain suppressed ileal *E. coli* counts. In conclusion, 1% *Plantago major* L. performed the best in this study because it led to increased body and carcass weight, lowered serum cholesterol and triglyceride, reduced heterophil/lymphocyte ratio, improved immune response, and ileal microflora.

Introduction

In modern poultry production, newly hatched chicks have no contact with maternal feces; thereby, normal microflora slowly colonizes in the intestine. Chicks also do not receive maternal

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antigens to develop the immune system. Accordingly, growth promoters are used in poultry feed to enhance the microflora of intestines and to develop the immune system to ultimately improve performance (Fuller, 1989). Antibiotics are a group of chemical materials which are used to reduce the spread of diseases and enhance the growth rate of broilers (Waldroup *et al.*, 2003). Antibiotics inhibit the activity of toxin-producing bacteria in the intestine which lead to nutrient availability and improved body weight gain and feed conversion ratio (FCR). Virginiamycin (Vm) is a common antibiotic used in the poultry industry to improve performance, and its effects may be due to the inhibition of intestinal pathogens (LaVorgna *et al.*, 2013). However, many countries deny antibiotic consumption as a growth promoter because of its persistence in poultry product which can lead to antibiotic resistance in consumers.

Probiotic is a culture of a single bacteria strain or the combination of different strains, and can be fed to poultry to manipulate intestinal microflora and to increase growth rate. Probiotic can affect intestinal mucosa by inhibiting the growth of harmful bacteria and enhancing nonpathogenic bacteria population (Hashemipour *et al.*, 2011). According to the enhanced intestinal microflora and improved performance in broilers fed diets supplemented with probiotic Protexin® (Pro), this additive has been affirmed as an antibiotic alternative (Khosravi *et al.*, 2008).

Using medicinal plants comes back to many thousands of years ago, but recently they have been considered specifically for human, animal, and poultry industry as health- and growth-promoting feed additives. Phytochemicals could be used as growth promoters due to their mode of action on stimulating the secretion of digestive enzymes, gut development, and antimicrobial activity (Khaksar *et al.*, 2012). There is good potency of cultivating medicinal plants in different parts of Iran. People show a high affinity to natural feed additives compared to antibiotics and other chemical products. Great plantain (*Plantago major* L.) contains five classes of biologically active compounds, namely flavonoids (baicalein, baicalin and luteolin), phenolic compounds (caffeic acid, chlorogenic acid, ferulic acid and p-coumaric acid), benzoic compound (vanillic acid), iridoid glycoside (aucubin) and triterpenes (oleanolic acid and

ursolic acid) (Oto *et al.*, 2011) which show a range of biological activities including wound healing, anti-inflammatory, analgesic, antioxidant, weak antibiotic, immunomodulating and antimicrobial activity (Kolak *et al.*, 2011). Therefore, the aim of this study was to compare the effects of antibiotic virginiamycin, probiotic Protexin® and *Plantago major* L. on performance, carcass characteristics, serum parameters, immune response, and the ileal microbial population of broilers.

Materials and Methods

Birds, diets and management

The experimental protocols were approved by the Animal Care Committee of University of Jiroft, Iran. A total number of 200 day-old male Ross 308 broiler chicks (average weight of 45±3 g) were obtained from Mahan commercial hatchery (Mahan, Kerman, Iran). The birds were fed to match the requirements recommended by the Ross 308 during the starter (1–10 d), grower (11–24 d) and finisher (25–42 d) periods (Table 1). Chicks were allocated to five experimental groups consisting of: T₁: control diet (Con), T₂: Con+0.02% antibiotic virginiamycin, T₃: Con+0.01% probiotic Protexin®, T₄: Con+0.5% *Plantago major* L. seed and T₅: Con+1% *Plantago major* L. seed in a completely randomized design.

The chemical composition of *Plantago major* L. seed was not measured in this trial but based on reports, the seeds contain the monosaccharides glucose, fructose, xylose and rhamnose, the disaccharide sucrose, and the trisaccharide planteose. Polysaccharides extracted from the seeds with cold water are composed of 61% xylose, 13.2% arabinose and 24% galacturonic acid, and hot water extract of the residue contains 78% xylose, 13.2% arabinose, 3% galactose and 6.2% galacturonic acid (Samuelsen, 2000). Fatty acids in the seeds include myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, behenic acid, lignoceric acid; flavonoids include apigenin, luteolin, baicalein, hispidulin, plantagin, scutellarein; phenolic compounds include caffeic acid, plantamajoside, acteoside; vitamins include ascorbic acid, carotenoids, vitamin k; and organic acids include fumaric, vanillic, benzoic acid, and cinnamic acid (Samuelsen, 2000). Other researchers reported that the *P. major* L. seeds contain 15.1% crude protein, 1.45% fat, 7.14% ash, 26.9% crude fibre,

45.87% total carbohydrate, 57.74% unsaturated fatty acid, 42.26% saturated fatty acid, 3.87% total essential amino acids, 6.69% total non-essential amino acids, as well as phenol (total 7.43 mg gallic/g), flavonoid (total 3.03 mg Quercetin/g), and tannins (2.43 mg Catechine/g) (Kobeasy *et al.*, 2011).

Each experimental group was divided into four replicates consisting of ten chicks in each. No synthetic enzymes or anticoccidials were included into the diets. Each floor pen was equipped with a feeding trough and nipple

drinkers and birds had free access to feed and water. During the study, the birds received a lighting regime of 24 hrs from 1 to 7 d of age and thereafter 23-h light: 1-h darkness until 42 d of age. Temperature was initially set at 32°C for the first 3 d of age and decreased by 3°C per week until the temperature 23°C was reached and then it was consistent until the end of the experiment. The humidity was 60-65% during the first week and decreased to 55-60% during the rest of the experiment.

Table 1. Composition of starter, grower, and finisher diets

Ingredient (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	53.38	54.82	60.63
Soybean meal	38.45	36.23	30.84
Vegetable oil	3.49	5.00	4.85
Limestone	1.54	1.35	1.30
Dicalcium phosphate	1.64	1.29	1.21
Vitamin premix ¹	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25
Salt (NaCl)	0.29	0.29	0.29
L-Lysine	0.32	0.23	0.13
DL-Methionine	0.39	0.29	0.25
<i>Nutrients composition</i>			
ME (Kcal/kg)	3025	3150	3200
Crude protein (%)	22	21	19
Lysine (%)	1.43	1.30	1.09
Methionine + Cystine (%)	1.07	0.95	0.86
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42

¹ Vitamin premix provided per kilogram of diet: retinyl palmitate, 10000 IU; cholecalciferol, 3500 IU; dl- α -tocopheryl acetate, 60 mg; vitamin K₃, 3 mg; vitamin B12, 0.1 mg; thiamine mononitrate, 3 mg; riboflavin, 6 mg; niacin, 40 mg; pyridoxine, 5 mg; pantothenic acid, 11 mg; folic acid, 1 mg; and biotin, 0.15 mg; cholin chloride, 500 mg; etoxycoin, 150 mg;

² Mineral premix provided per kilogram of diet: Fe, 60 mg (as FeSO₄, 7H₂O); Zn, 60 mg (as ZnSO₄); Mn, 100 mg (as MnO₂); Cu, 10 mg (as CuSO₄, 5H₂O); I, 1.6 mg (as KI); and Se, 0.15 mg (as Na₂SeO₃, 5H₂O).

Sample collection and measurements

Body weight gain (BWG) and feed intake (FI) of birds were measured per pen at the end of each period. Mortality and the weight of birds that died or were culled during the experiment were recorded. Corrected FCR was calculated based on mortality for these periods.

To evaluate anti-SRBC (sheep red blood cells) antibody titers, two birds per replicate were selected and were intramuscularly injected with 1 mL/chick of SRBC (15% suspension in phosphate buffered saline) at days 28 and 35. SRBC were used as an antigen to quantify the antibody response. Blood samples were collected at days 35 and 42. The serum from each sample was collected, heat inactivated at 56°C for 30 min and then analyzed for total anti-SRBC antibodies as described by Cheema *et al.* (2003).

The SRBCs used for inoculation and antibody titration were obtained from the same donor sheep.

At the end of the experiment, two birds per replicate were randomly selected and blood samples were taken from the brachial vein to obtain serum. Blood samples were immediately centrifuged at 1000 × g for 15 min at 4°C. The levels of serum glucose, total cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by an auto-analyzer using the protocol provided by the kit manufacturer (Zist-Shimi, Tehran, Iran). To obtain plasma, blood samples were taken from two SRBC-injected birds in heparin-containing syringes to avoid blood clot

formation. Blood smears were prepared on slides and painted by Giemsa method. The white and red blood cells counts were determined by SysmexK-1000 apparatus (Japan). One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope and then heterophil to lymphocyte ratio (H/L) was calculated and recorded.

The birds from which blood was collected but without SRBC injection were killed by cervical dislocation and then assessed for carcass characteristics (g/g of live body weight) and ileal microbial population. The ileum was assigned from Meckel's diverticulum to ileo-cecal junction. The ilea sections were excised and contents were pushed by gentle fingers into tubes. Digesta were pooled with a replicate and put on ice until they were transported to the laboratory for enumeration of the microbial population. One gram of ileal contents was homogenized in 9 mL sterile water. Each sample was serially diluted. Using these diluted subsamples, lactic acid bacteria population was enumerated on De Man-Rogosa-Sharpe (MRS) agar and *E. coli* was counted on Mac Conkey (MC) agar after incubation at 37°C in an anaerobic chamber for 48 hrs and in an aerobic chamber for 24 hrs, respectively (Guban *et al.*, 2006).

Statistical analysis

Prior to analysis, all percentage data were normalized by subjecting to arcsine transformation. As the pen represented the experimental unit, the effect of different growth promoters on performance, carcass characteristics, serum parameters, immune response and the ileal microbial population of broilers was statistically analyzed in a completely randomized design using the GLM procedure of SAS (2001). Means were compared for significant differences using the Tukey multiple range test ($P < 0.05$).

Results

Performance

We compared the effects of antibiotic virginiamycin (Vm), probiotic Protexin® (Pro) and *Plantago major* L. (plantain) on feed intake, body weight gain and feed conversion ratio of broilers at different periods (Table 2). In comparison to the control group, BWG increased by Pro and plantain treatments in the starter period, but by all treatments in grower, finisher and total period ($P < 0.01$). Dietary supplementation of Vm increased ($P < 0.01$) FI in starter and Vm and 0.5% plantain increased FI in finisher and total periods, respectively, but FCR was improved ($P < 0.05$) in finisher and total period by Vm and 1% plantain respectively.

Table 2. Comparison of the effects of antibiotic virginiamycin (Vm), probiotic Protexin® (Pro) and *Plantago major* L. (plantain) on feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) of broilers at different periods

	Control (con)	Con+Vm	Con+Pro	Con+0.5% plantain	Con+1% plantain	SEM	P-value
FI (g/b)							
0-10 d	189 ^b	191 ^b	199 ^{ab}	206 ^a	209 ^a	2.55	0.0001
11-25 d	843	866	860	889	853	16.07	0.377
26-42 d	1872 ^b	1935 ^a	1880 ^b	1896 ^b	1885 ^b	5.620	<0.0001
0-42 d	2904 ^b	2992 ^a	2940 ^{ab}	2991 ^a	2948 ^{ab}	19.819	0.032
BWG (g/b)							
0-10 d	140 ^c	142 ^{bc}	147 ^{ab}	146 ^{ab}	149 ^a	1.317	0.001
11-25 d	489 ^b	512 ^a	497 ^{ab}	502 ^{ab}	511 ^a	4.352	0.010
26-42 d	1037 ^b	1147 ^a	1089 ^{ab}	1088 ^{ab}	1105 ^a	14.360	0.002
0-42 d	1666 ^c	1802 ^a	1733 ^b	1736 ^b	1766 ^{ab}	14.562	0.0002
FCR							
0-10 d	1.35	1.34	1.35	1.40	1.40	0.021	0.164
11-25 d	1.72	1.69	1.73	1.77	1.67	0.031	0.230
26-42 d	1.81 ^a	1.69 ^b	1.73 ^{ab}	1.74 ^{ab}	1.70 ^{ab}	0.024	0.032
0-42 d	1.74 ^a	1.66 ^b	1.69 ^{ab}	1.72 ^{ab}	1.67 ^b	0.014	0.0058

^{a-c}Means within the same column with uncommon superscript differ significantly ($P < 0.05$).

Carcass characteristics

The effects of Vm, Pro, and plantain were assessed on the relative weight of carcass cuts and internal or immune organs of broilers at 42 d of age (Table 3). Although none of the

treatments had a significant effect on breast, thigh and liver masses, there was an elevated relative weight of these tissues to carcass and bursa ($P < 0.01$). Plantain also increased relative weight of the spleen ($P < 0.01$).

Table 3. Comparison of the effects of antibiotic virginiamycin (Vm), probiotic Protexin® (Pro) and *Plantago major* L. (plantain) on relative weight of carcass cuts and internal or immune organs of broilers at 42 d of age

Treatments	(g/kg of live body weight)					
	Carcass	Breast	Thigh	Liver	Spleen	Bursa
Control (Con)	85.16 ^b	24.09	19.79	2.23	0.15 ^b	0.08 ^b
Con+Vm	88.88 ^a	24.82	20.73	2.61	0.18 ^{ab}	0.11 ^a
Con+Pro	88.12 ^a	24.17	20.05	2.46	0.18 ^{ab}	0.11 ^a
Con+0.5%plantain	88.33 ^a	24.64	20.24	2.38	0.19 ^a	0.12 ^a
Con+1%plantain	88.27 ^a	24.82	20.53	2.41	0.19 ^a	0.11 ^a
SEM	0.644	0.444	0.335	0.122	0.008	0.006
P-value	0.007	0.643	0.336	0.310	0.009	0.009

^{a,b}Means within the same column with uncommon superscript differ significantly ($P < 0.05$).

Serum metabolites

The effects of Vm, Pro and plantain on serum glucose and lipids of broilers at 42 d of age is shown in Table 4. All treatments demonstrated a

hypcholesterolemic effect ($P < 0.01$) but only the higher level of plantain (1%) decreased ($P < 0.05$) serum glucose, triglyceride and LDL-C.

Table 4. Comparison of the effect of antibiotic virginiamycin (Vm), probiotic Protexin® (Pro), and *Plantago major* L. (plantain) on serum glucose and lipids of broilers at 42 d of age

Treatments	(mg/dL)				
	Glucose	Triglyceride	Cholesterol	LDL-C ¹	HDL-C ²
Control (Con)	216 ^b	106 ^a	123 ^a	23 ^a	70
Con+Vm	215 ^a	98 ^{ab}	115 ^b	21 ^{ab}	72
Con+Pro	216 ^a	103 ^a	114 ^b	20 ^{ab}	71
Con+0.5%plantain	210 ^{ab}	94 ^b	114 ^b	21 ^{ab}	72
Con+1%plantain	194 ^b	93 ^b	113 ^b	19 ^b	74
SEM	1.671	3.021	1.634	0.742	1.058
P-value	<0.0001	0.033	0.004	0.041	0.99

^{a,b}Means within the same column with uncommon superscript differ significantly ($P < 0.05$).

¹low-density lipoprotein cholesterol. ²high-density lipoprotein cholesterol.

Immune response

The effects of Vm, Pro and plantain on blood cells counts and first and second anti-SRBC immune response of broilers at 42 d of age are shown in Table 5. The inclusion of Pro and plantain to diets

enhanced immune system by increasing white and red blood cells, second immune response, and reducing H/L ratio ($P < 0.05$).

Table 5. Comparison of the effects of antibiotic virginiamycin (Vm), probiotic Protexin® (Pro) and *Plantago major* L. (plantain) on blood cells counts and first and second anti-SRBC immune response of broilers at 42 d of age

Treatments	Blood cells counts			anti-SRBC ¹	
	WBC ² ($\times 10^3/\mu\text{L}$)	RBC ³ ($\times 10^6/\mu\text{L}$)	H/L ⁴	1 st response (day 35)	2 nd response (day 42)
Control (Con)	218.07 ^b	2.15 ^c	0.29 ^a	5.25 ^b	5.50 ^c
Con+Vm	227.47 ^{ab}	2.31 ^b	0.26 ^{ab}	7.00 ^{ab}	7.25 ^b
Con+Pro	229.97 ^a	2.33 ^b	0.23 ^b	6.25 ^{ab}	8.00 ^{ab}
Con+0.5%plantain	231.02 ^a	2.44 ^{ab}	0.25 ^{ab}	6.00 ^b	8.50 ^{ab}
Con+1%plantain	236.32 ^a	2.55 ^a	0.23 ^b	8.00 ^a	8.75 ^a
SEM	2.570	0.034	0.011	0.598	0.438
P-value	0.002	<0.0001	0.013	0.050	0.0007

^{a-c}Means within the same column with uncommon superscript differ significantly ($P < 0.05$)

¹ antibody production based on log₁₀.

² white blood cells, ³ red blood cells, ⁴ heterophil to lymphocyte ratio.

Ileal microflora

The effects of Vm, Pro, and plantain on ileal microflora of broilers at 42 d of age are shown in Table 6. The results showed that Vm decreased

the ileal microbial population of *Lactobacillus* while Pro and plantain increased it ($P < 0.01$). Meanwhile, 1% plantain suppressed ileal *E. coli* counts.

Table 6. Comparison of the effects of antibiotic virginiamycin (Vm), probiotic Protexin® (Pro), and *Plantago major* L. (plantain) on ileal microbial population of broilers at 42 d of age

Treatments	Ileal microbial population	
	<i>Lactobacillus</i> (Log ₁₀ CFU/gr digesta) ¹	<i>E.coli</i> (Log ₁₀ CFU/gr digesta) ¹
Control (Con)	7.14 ^{ab}	7.02 ^a
Con+Vm	5.63 ^b	6.28 ^{ab}
Con+Pro	8.25 ^a	6.49 ^{ab}
Con+0.5%plantain	7.73 ^a	5.78 ^{ab}
Con+1%plantain	8.05 ^a	5.23 ^b
SEM	0.327	0.349
P-value	0.001	0.039

^{a,b}Means within the same column with uncommon superscript differ significantly ($P < 0.05$).

¹ logarithm of colony forming unit per gram of digesta.

Discussion

Performance

Antibiotic virginiamycin increased BWG in the grower and finisher periods and improved FCR in the finisher period ($P < 0.05$). Probiotic Protexin® and 0.5% medicinal plant *Plantago major* L. (plantain) increased BWG in the starter period, and 1% plantain increased BW in all the three periods. This coincides with the findings of many experiments showing that the supplementation of virginiamycin (Miles *et al.*, 2006), Protexin® (Kabir *et al.* 2004) and *Plantago major* L. (Chacrabati *et al.*, 2013) in broiler diets improved performance. It has been suggested that antibiotics improve nutrient utilization and decrease toxin secretion by the microorganism of the small intestine which led to improving growth performance of animals (O'Connor-Dennie, 2004). Probiotics have previously been shown to improve performance *via* greater retention of dietary fat in the intestine, consequently increasing AME_n and absorption of nutrients, such as protein (Kabir *et al.*, 2004). Lee (2002) reported that the active ingredients of medicinal plants increase nutrient digestibility, balance the gut microbial ecosystem, and increase the secretion of endogenous digestive enzymes, which may be the reason underlying increased feed intake in chickens fed plantain in our study. Improvement of FCR by Vm in this study is due to its inhibition of certain intestinal microflora that produces toxins or compete with the host for available nutrients (Dibner and Richards, 2005).

Carcass characteristics

Treated birds had a higher relative weight of carcass and bursa, and 1% plantain increased the relative weight of the spleen ($P < 0.01$). These

results are in agreement with the reports by Belay and Teeter (1996) which observed that adding virginiamycin to the diet of broilers increased the relative weight of carcass. This increasing may be related to the reduction in relative weight of internal organs, especially intestines *via* reduction of negative microflora populations and higher fat deposition in the carcass (Woodward *et al.*, 1988). Kabir *et al.*, (2004) found that 2 grams of probiotic Protexin® per liter of drinking water increased relative weight of the bursa in broilers possibly by raising nutrient availability and lowering abdominal fat (Habibi *et al.*, 2013). However, Bingöl *et al.* (2010) reported that addition of *Plantago major* L. extract at differing levels in broiler diet did not affect animal performance and carcass parameters, but Chacrabati *et al.* (2013) showed positive effects of *P. major* L. on carcass characteristics. *P. major* L. is not only a source of bioactivity but also a good source of protein and minerals which causes more protein retention and availability leading to better performance and yielded carcass in sheep (Al-Mamun *et al.*, 2008).

Serum metabolites

All treatments demonstrated a hypocholesterolemic effect ($P < 0.01$) in this experiment, similar to previous studies (Craig, 1999; Kalavathy *et al.*, 2003). It has been shown that these feed additives can decrease blood cholesterol *via* three mechanisms including: 1) inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a key enzyme involved in cholesterol synthesis regulation (Lee, 2002); 2) inhibition of farnesyl pyrophosphate, a key molecule in cholesterol synthesis (Hood *et al.* 1978); and 3) consumption

of cholesterol in gut digesta by probiotic microorganisms (Mohan *et al.*, 1995).

Immune response

The inclusion of Protexin® and *Plantago major* L. to diets enhanced immune system, reflected by increased white and red blood cells, second anti-SRBC immune response, and reduced H/L ratio ($P < 0.05$). In fact, when microorganisms of antibiotic, probiotic, and/or the active components of a phytochemical are entered into the gut in high doses, they are recognized as an external agent by the host animal defense system which alerts the immune system resulting in increased white blood cells and other immune feedbacks. Regarding the achieved second anti-SRBC immune response, we found that 1% plantain induced a stronger response than antibiotic ($P < 0.01$). The main mode of action of virginiamycin is defeating intestinal gram-positive bacteria which are immunoglobulin producers (Miles *et al.*, 2006). Kabir *et al.* (2004) evaluated the dynamics of probiotic Protexin® on the anti-SRBC immune response of broilers. They reported significantly higher antibody production in experimental birds *vs.* control ones. Overall, the enhancement of the immune system by probiotic is attributed to the increased macrophage activity and higher antibody production on the mucosal surface of some tissues such as the intestine wall. *P. major* L. showed a remarkable potential in stimulating the secretory metabolism of macrophages (Gomez-Flores *et al.*, 2000). Active compounds isolated from *P. major* L. have been reported to be antioxidant and anti-inflammatory which lead to immune system enhancement (Wang *et al.*, 1996; Marchesan *et al.*, 1998). Heterophil to lymphocyte ratio has been widely used as a physiological indicator for different forms of stress (Maxwell, 1993) and reduction of H/L in our study by Pro and 1% plantain indicated a reduction of heat stress in broilers that enhance the immune system. It has been showed that *P.*

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major L. stimulated immune activity on human lymphocyte proliferation (Chiang *et al.*, 2003)

Ileal microbial population

In this study, virginiamycin decreased ileal microbial population of lactic acid bacteria while the two other growth promoters increased it ($P < 0.01$). These results are inconsistent with previous research that showed a positive change in the intestinal microbial population of broilers fed antibiotics or probiotics (Parks *et al.*, 2001; Fioramonti *et al.*, 2003; Mountzouris *et al.*, 2011). Antibiotic alternatives, such as probiotics and medicinal plants can inhibit the growth of pathogenic bacteria and improve the population of non-pathogenic bacteria like *Lactobacillus* by decreasing pH of the gastrointestinal tract. Metiner *et al.* (2012) observed that the active components of *P. major* L. (Plantamajoside and acteoside) are responsible for the antimicrobial activity of this plant (Samuelson, 2000).

Conclusion

Our results indicated that 1% *Plantago major* L. performed better than the antibiotic virginiamycin and the probiotic Protexin® because it leads to increased body and carcass weight, lower serum cholesterol and TG, reduced H/L, and improved immune response and ileal microflora. However, it should be considered that response of broilers to growth promoters are inconsistent, which could be related to diet composition, dose of growth promoter, age of the bird, flock health and density, and environmental and management conditions, justifying the need for additional research in this area.

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