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Full Length Research Paper

Evaluation of St John's Wort (*Hypericum perforatum L.*) as an antibiotic growth promoter substitution on performance, carcass characteristics, some of the immune responses, and serum biochemical parameters of broiler chicks

Landy N.[1]*, Ghalamkari G. H.[2] and Toghyani M.[2]

[1]Young Researchers Club, Khorasgan Branch, Islamic Azad University, Isfahan, Iran.
[2]Department of Animal Science, Khorasgan Branch, Islamic Azad University, Isfahan, Iran.

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This experiment was conducted to examine the effect of dried aerial parts of the herb *Hypericum perforatum L.* (HP) as an antibiotic growth promoter substitution on performance, carcass traits, immune responses, and serum biochemical parameters of broiler chicks. In this study, 192 one-day old chicks (Ross 308) were allocated to four treatments with four replicates based on a completely randomized design. Dietary treatments included control, antibiotic (flavophospholipol), and 5 and 10 g HP/kg diet. Dietary supplementation of flavophospholipol increased final body weight of broilers at day 42 compared with those fed diets supplemented with 5 and 10 g HP/kg (P<0.05). Birds fed 5 and 10 g HP/kg in the diet had the highest feed conversion ratio at day 42, compared with other treatments (P<0.05). Antibody titer against avian influenza virus (AIV) increased in the group treated with 10 g HP/kg diet compared with those fed basal diet and basal diet supplemented with antibiotic (P<0.05). Total and high-density lipoprotein cholesterol (HDL-C) levels in antibiotic group were significantly higher than those fed basal diet or basal diet supplemented with 5 g HP/kg (P<0.05). In conclusion, the result of this study showed that addition of St. John's Wort powder seem not to have a positive influence on growth performance and it could not be considered as a antibiotic growth promoter substitution for broiler chicks.

Key words: Broilers, *Hypericum perforatum L.,* performance, immune responses, biochemical parameters, carcass characteristics.

INTRODUCTION

During the last decades, Sub-therapeutic dosages of antibiotics have been used extensively as growth promoters in livestock feeds. They have been reported to increase growth rate and feed efficiency of poultry and other livestock as a result of improved gut health, resulting in better nutrients utilization and improved feed conversion (Visek, 1978). However, issues such as loss of antibiotic efficiency along time and risk of residues in food of animal origin, with the possible development of bacterial resistance in humans, have concerned consumers (Thomke and Elwinger, 1998), creating a significant problem for poultry production. A complete ban on antibiotics in poultry feeds was brought in to force on January 1th by European Union (EU); thus, all of the antibiotics used at sub-therapeutic doses for growth promotion were withdrawn (Nollet, 2005; Cervantes, 2006; Michard, 2008; Toghyani et al., 2010). Now, As a

*Corresponding author. E-mail: n_landy1984@yahoo.com. Tel: +989132070979. Fax: +983115354038.

result of this ban in EU, increasing demand for organic animal products, and growing pressure on livestock producers in other parts of the world, alternative substances and strategies for animal growth promotion and disease prevention required to create a safety margin in animal production against unexpected hazards and stressful conditions (Nasir and Grashorn, 2006).

Phytogenic feed additives which also called as phytobiotics are plant derived products, used in animal feeding to improve performance through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (Toghyani et al., 2011).

Hypericum genus (St John's Wort), originating in Europe, West Asia and North Africa, is one of the oldest and the best experimentally and clinically examined herbal remedies in various cultures and medical systems. Different species of the genus Hypericum (Hypericaceae) have been used in traditional medicine to prevention or treatment of various diseases. *Hypericum perforatum* (HP) *L.* possess hypericin, hyperforin, flavonoids, xanthones and volatile oil. *H. perforatum L.* and other species of the genus Hypericum possess anxiolytic, antiviral, antimicrobial, analgesic, anti-inflammatory and antioxidant properties, while recently, interest has been directed towards the antidepressant activity of this plant extract (Axarlis, 1998; Flausino, 2002; Mattacec et al., 2002; Brenner, 2000; Rabanal, 2005). Also according to Kamel (2000), *H. perforatum L.* may be beneficial for animals due to active principles of this plant. Despite these findings, there has been a dearth of information on the possible performance enhancer effects of *H. perforatum* on broiler chickens. The present study was designed to compare the efficacy of different levels of dried aerial part powder of *H. perforatum* an antibiotic growth promoter on growth performance, carcass characteristics, immune responses, and some serum biochemical parameters in broiler chickens when used as supplements in the diet.

MATERIALS AND METHODS

Birds, diets and management

One hundred and ninety two one-day-old mixed sex broiler chicks (Ross 308) were purchased from a local hatchery. On arrival, birds were weighed and randomly assigned to one of four treatments with four replicates of 12 birds based on a completely randomized design. The dietary treatments consisted of the basal diet as control, antibiotic group receiving 4.5 mg/kg flavophospholipol, 5 and 10 g/kg *H. perforatum* powder added to the basal diet in inclusion of corn. The *H. perforatum* was cultivated in Esfahan City (Iran). Fresh aerial parts of *H. perforatum* were used for this experiment were collected during the flowering season and dried under standard conditions (45°C). The dried aerial parts were added to experimental diets of broilers after careful grinding. Table 1 lists the basal diet formulated to meet or exceed the nutrient requirements of broilers provided by Ross Broiler Manual (2007). The birds were fed a starter diet from day 0 to 14, a grower diet from day 14 to 28, and finisher diet from day 28 to 42. Chicks were raised on floor pens (120 × 120 × 80 cm) for 6 weeks and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness. The ambient temperature in experimental house was maintained at 32°C during the first week and gradually decreased by 3°C in the second and third weeks, and finally fixed at 22°C thereafter. Birds were allowed to free access to feed and water during the 42 day of growth period. The commercially available oil-adjuvant injectable emulsion against Newcastle disease virus (NDV) and avian influenza virus (AIV) were used (H9N2 subtype) for vaccinating broiler chicks, and they were injected subcutaneously with 0.2 ml per chick at 9 days of age.

Data collection

Body weights of broilers were determined at day 1, 14, 28 and 42 of age. Feed intake and weight gain were recorded in different periods and feed conversion ratio (FCR) was calculated. Mortality was recorded as it occurred and was used to adjust the total number of birds to determine the total feed intake per bird and FCR. At day 42, two male broilers per replicate were randomly selected, based on the average weight of the group and sacrificed. Carcass yield was calculated by dividing eviscerated weight by live weight. abdominal fat, gizzard, proventriculus, small intestine, cecum, were collected, weighed and calculated as a percentage of live body weight and also carefully examined to detect any pathological lesion or damages. Small intestine length and cecum length was measured.

At day 28, 2 male broilers from each pen were randomly selected, and blood samples were taken by puncture of the brachial vein for analysis of antibody titers against avian influenza virus. Serum antibody titers against AIV were measured by the hemagglutination inhibition test. At day 42, two male broilers per replicate were selected and blood samples were collected by syringes containing heparin to avoid blood clot formation. Blood smears were prepared using May–Greenwald–Giemsa stain. One hundred leukocytes per samples were counted by heterophil to lymphocyte separation under an optical microscope (Nikon, Japan) with 100× oil immersion lens, then heterophil to lymphocyte (H/L) ratio was calculated and recorded (Gross and Sigel, 1983). In order to determine albumin to globulin ratio (A/G), blood samples (the same 2 birds per replicate) were collected at day 42 and after serum separation albumin and protein concentration was determined using spectrophotometer and the kit package (Pars Azmoon Co; Tehran, Iran). Globulin concentration in serum was computed by subtracting albumin concentration from proteins.

After 12 h of fasting, blood samples were collected in non-heparinised tubes at day 42 from 8 birds in each treatment by puncturing the brachial vein and the blood was centrifuged at 2000 × g for 15 min to obtain serum (SIGMA 4-15 Lab Centrifuge, Germany). Individual serum samples were analyzed for total protein, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglyceride using the kit package (Pars Azmoon Co; Tehran, Iran).

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (1997). Means were compared using Duncan’s multiple range tests. Statements of statistical significance are based on $P<0.05$.

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Y_{ij} = individual observation, μ = overall mean, α_i = effect of treatment and e_{ij} represents the random error.

Table 1. The ingredient and chemical composition of basal starter, grower and finisher diets.

Ingredients (g/kg)	Starter	Grower	Finisher
Corn	559.4	567.8	593.3
Soybean meal	379.0	371.4	346.1
Soybean oil	16.7	24.4	26.7
DCP	19.0	16.2	14.7
$Caco_3$	12.0	9.7	9.5
NaCl	3.2	3.2	3.0
Mineral-Premix[1]	2.5	2.5	2.5
Vitamin-Premix[2]	2.5	2.5	2.5
DL-Methionine	3.6	2.3	1.5
L-Lysine	2.1	-	-
Calculated composition			
M. energy (kcal/kg)	2870	2950	3000
Crude protein (g/kg)	208.7	206.0	196.8
Calcium (g/kg)	9.96	8.40	7.96
Av. phosphorus (g/kg)	4.7	4.2	3.9
Meth.+ cysteine (g/kg)	10.1	8.8	7.7
Lysine (g/kg)	13.5	11.6	11.0
Sodium (g/kg)	1.5	1.5	1.5

[1]To provide the following per kg of diet: Vit A 10,000 IU, vitamin D3 2000 IU, vitamin E 5 IU, vitamin K 2 mg, riboflavin 4. 20 mg; vitamin B12 0.01 mg; pantothenic acid 5 mg; nicotinic acid 20 mg; folic acid, 0.5 mg. [2]To provide the following per kg of diet ; choline 3 mg; Mg 56 mg; Fe 20 mg; Cu 10 mg; Zn 50 mg; Co 125 mg; Iodine 0.8 mg.

RESULTS AND DISCUSSION

Performance and carcass traits

Data on performance indices are summarized in Table 2. Antibiotic supplementation increased body weight at different periods compared to other groups, but it was not significant at day 14 of age. The highest body weight at day 28 was observed in the group receiving antibiotic, but it was not different from broilers fed the control or 10 g HP/kg diet ($p<0.05$). At day 42 of age the body weight obtained in birds fed the diet containing 4.5 mg flavophospholipol/kg significantly ($p<0.05$) was higher than those fed 5 or 10 g HP/kg diet, but it was not different from broilers fed the basal diet. Broilers receiving 10 g HP/kg had higher feed intake compared to other groups during starter period, but not significantly ($P>0.05$). Antibiotic supplementation significantly ($P<0.05$) increased feed intake during growth period than those fed diet containing 10 g HP/kg diet. Treatments failed to induce any marked effect on feed intake during finisher period and entire experimental period. Supplementing the diet with antibiotic resulted in an improvement of FCR compared to other groups during starter and grower periods, but not significantly ($P>0.05$). Broilers receiving flavophospholipol or basal diet had lower FCR compared to broilers receiving 5 or 10 g HP/kg during finisher and entire experimental periods ($P<0.05$). Antibiotics may control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in chicks gut (Ferket, 2004). A more balanced biota population in gut would benefit animals by nutrient sparing, control of clinical diseases, protective effect against the production of toxins in the gastro-intestinal tract, and metabolic effects (Mellor, 2000). It is believed that the simultaneous action of some of these mechanisms generate the performance benefits observed in broilers and other domestic animals (Bedford, 2000). The improvement in body weight of broilers achieved with flavophospholipol could be attributed to its positive effect on nutrient digestibility.

Similarly, other trial use of flavophospholipol resulted in an enhanced growth and improved FCR (Landy et al., 2011a). The lowest body weight observed in chicks receiving St John's Wort could partially be due to the presence of some anti-nutritional factors in St John's Wort such as tannins. Tannins can bind proteins in digestive tracts and reduce protein absorption, thus resulting in decreased growth performance. Consistent with our results, Toghyani et al. (2011) reported that feeding yarrow to broiler chicks did not result in any significant improvement of productive traits due to the presence of some anti-nutritional factors in yarrow such as tannins, hydrocyanic acid, and linalool, and birds fed diets containing no yarrow tended to have higher feed intakes and growth rates. Also, use of high level of St John's Wort in the diet may have had an adverse effect on some beneficial microbial populations such as

Table 2. Effect of experimental diets on performance indices of broilers at different ages.

Variable	Dietary treatments				
	Control	Flavophospholipol	5 g St. John's Wort/kg	10 g St. John's Wort/kg	SEM[4]
DFI[1] (days)					
0-14	23.90	24.90	24.82	32.25	0.72
14-28	92.95[a]	94.27[a]	90.05[ab]	87.32[b]	1.50
28-42	165.85	162.58	165.38	161.28	161.28
0-42	90.79	92.67	90.52	90.53	1.31
FCR[2]					
0-14	1.41	1.39	1.45	1.44	0.03
14-28	1.88	1.74	1.89	1.79	0.04
28-42	2.09[b]	2.11[b]	2.35[a]	2.36[a]	0.05
0-42	1.84[b]	1.83[b]	1.99[a]	1.98[a]	0.03
BW[3] (g)					
14	261.5	274.5	264.7	269.7	7.62
28	1004.0[ab]	1085.2[a]	977.2[b]	996.5[ab]	28.29
42	2109.2[ab]	2162.7[a]	1939.2[c]	1950.5[c]	44.85

[a-c]Mean values followed by the same letters in the row do not differ according to Duncan test, [1]Daily feed intake (g per bird per day). [2]Feed conversation ratio (g/g). [3]Body weight. [4]Standard error of mean.

Table 3. Effect of experimental diets on carcass yield and internal relative organ weight of broilers at 42 days.

Relative organ weight	Dietary treatments				
	Control	Flavophospholipol	5 g St. John's Wort/kg	10 g St. John's Wort/kg	SEM[1]
Carcass (%)	73.8	73.3	74.3	73.6	0.63
Abdominal fat (%)	2.03	1.88	2.00	2.32	0.24
Gizzard (%)	3.42	3.02	2.89	3.18	0.27
Proventriculus (%)	0.71	0.60	0.57	0.56	0.11
Small intestine (%)	5.41	4.73	3.91	4.51	0.96
Cecum (%)	1.00	0.79	0.84	1.05	0.17
Small intestine (cm)	195	195	188	180	8.32
Cecum (cm)	39.4	41.6	39.2	40.8	1.3

[1]Standard error of mean.

lactobacillus, preventing the herb from exhibiting its positive influence on performance and resulting in a poorer FCR. In accord with our results, Toghyani et al. (2010) reported that use of high dosage of thyme had an adverse effect on growth performance of broiler chicks. Thus, it is probably the body weight and FCR in broiler fed diet containing St John's wort suppressed due to the levels of additives applied in our study. Unfortunately, no reports are available on the effects of St John's wort on bird growth performance for compare with result of this experiment.

Table 3 shows relative weight means (as a percentage of live weight at slaughter) of digestive and non-digestive organs, and absolute small intestine length as a function of treatments. The highest relative weight of abdominal fat obtained in broilers fed diet containing 10 g HP/kg diet, but the different was not significant (P>0.05). Tannins can bind proteins in digestive tracts and reduce protein absorption, thus resulting in deposition of fat in tissue. Carcass yield, gizzard, proventriculus, cecum and small intestine weights, or small intestine and ceca lengths were not markedly affected by dietary treatments. These results are consistent with those observed by Hernández et al. (2004) who did not find any differences among the control treatment and those containing antibiotic or mixtures of plant extracts for organ weight of 42-day-old broilers. Also, in other trial use of *Echinacea purpurea L*. had no significant effect on relative weight of carcass yield, abdominal fat pad, liver, gizzard, heart, and proventriculus.

Table 4. Effect of experimental diets on antibody titers against influenza viruse at day 28 and heterophil to lymphocyte and albumin to globulin ratios at day 42.

Variable	Dietary treatments				
	Control	Flavophospholipol	5 g St. John's Wort/kg	10 g St. John's Wort/kg	SEM[1]
Influenza (log_2)	3.42[c]	4.37[b]	5.14[ab]	6.57[a]	0.33
Heterophil to lymphocyte ratio	0.64	0.62	0.58	0.55	0.05
Albumin to globulin ratio	0.65[b]	1.01[a]	0.75[b]	0.72[b]	0.09

[a,c]Values in the same row not sharing a common superscript differ significantly (P<0.05). [1]Standard error of mean.

Table 5. Effect of experimental diets on serum biochemical parameters of broilers at day 42.

Biochemical parameter	Dietary treatments				
	Control	Flavophospholipol	5 g St. John's Wort/kg	10 g St. John's Wort/kg	SEM[2]
Protein[1]	4.30	4.11	4.25	4.17	0.93
Albumin[1]	1.70	1.93	1.83	1.76	0.40
Total cholesterol[1]	108[b]	125[a]	101[b]	114[ab]	7.42
LDL-cholesterol[1]	39.10	37.75	39.08	39.36	3.14
HDL-cholesterol[1]	120[bc]	143[a]	115[c]	133[ab]	8.42
Triglyceride[1]	118	100	113	114	19.43

Values in the same row not sharing a common superscript differ significantly (P<0.05). [1](mg/100 ml). [2].Standard error of mean.

Immune responses

The effect of experimental diets on antibody titers against Influenza virus at day 28 and heterophil to lymphocyte and albumin to globulin ratios at day 42 are presented in Table 4. Antibody titer against AIV increased in the group treated with 10 g HP/kg diet compared with those fed basal diet and basal diet supplemented with antibiotic (P<0.05). The highest albumin to globulin ratio obtained in broilers fed diets contains antibiotic. Use of St John's wort failed to have any effect on albumin to globulin ratio compare with control group. As St John's wort has been reported to have antibacterial, antiviral, and antioxidant activities (Axarlis, 1998; Flausino, 2002; Mattace et al., 2002; Brenner, 2000; Rabanal, 2005), an increase in immune responses of chicks was anticipated. Consistent with our finding in other trial oral gavage administrations of *H. perforatum L*. extract to mice infected with the influenza A virus (H_1N_1) was highly effective in preventing death, slowing the decline of arterial oxygen saturation, inhibiting lung consolidation and reducing lung virus titers (Xiu-ying et al., 2009). Also, Evstifeeva and Sibiriak (1996) reported immunostimulating activity of polyphenol fraction of John's Wort with respect to the system of mononuclear phagocyte system, cellular and humoral immunity.

Heterophil to lymphocyte ratio did not differ significantly (P>0.05) among treatments, though it tended to decrease in broilers fed diets containing 5 or 10 g HP/kg. The reliability of H/L ratio as a biological index of stress in birds is also, well documented (Maxwell, 1993; Bedanova et al., 2007). The lower H/L ratio observed in broilers fed diet containing 5 or 10 g HP/kg diet implies the positive influence of St John's Wort on reducing stress in broilers.

Blood biochemical parameters

Table 5 summarizes data obtained on the effect of experimental diets on serum biochemical parameters of broilers at day 42. No significant influence of experimental diets on protein, albumin, triglyceride, and LDL- cholesterol was observed (P>0.05). The feeding of the broilers with antibiotic resulted in a marked (P<0.05) increase in total and HDL- cholesterol concentration compared to those fed basal diet, or basal diet supplemented with 5 g HP/kg diet. The differences in triglyceride concentration did not reach statistical significance but it tended to decrease in serum of broilers fed diet containing antibiotic. The feeding of the broilers with St John's Wort had not any marked (P>0.05) effects in HDL- cholesterol concentration, but it tended to increase in broilers fed diets containing 10 g HP/kg diet. Serum biochemistry is a labile biochemical system which can reflect the condition of the organism and the changes happening to it under influence of internal and external factors (Toghyani et al., 2010). Similarly, in other trial in women, the use of St John's Wort and black cohosh had not any significant effect on serum lipid profile except for HDL- cholesterol concentration that increased in treatment group (Chung et al., 2007). In this trial increase, HDL- cholesterol is unclear and further studies are needed to elucidate its possible beneficial role in lipid metabolism. In conclusion, result of this study showed that addition of St. John's Wort powder seem not to have a positive influence on growth performance and it could not be considered as a antibiotic growth promoter substitution for broiler chicks, though result of this experiment indicated immunopotentiating effect of 10 g HP/kg diet.

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