INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-4, Issue-3, July-Sept-2014 ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 4th May-2014 *Revised: 24th May-2014* *Accepted: 4th June-2014*

Research article

THYMUS VULGARIS L., *GLYCYRRHIZA GLABRA*, AND COMBO ENZYME IN CORN OR BARLEY-BASAL DIETS IN BROILER CHICKENS

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ABSTRACT: This study was carried out to determine the effect of supplementation of *Thymus vulgaris* L. or *Glycyrrhiza glabra* in corn-soybean meal diets and the inclusion of exogenous enzyme of Combo® in barley-soybean meal diets beside of mentioned medicinal plants on carcass characteristics, some blood parameters, and broiler chicken performance. A total of 270 unsexed 1-d-old broiler chickens (Ross 308) were randomly allocated to 6 treatments with 3 replications of 15 birds. Dietary experiments were including of control (T1), the inclusion of *T. vulgaris*, *G. glabra*, their mixture (equal amount), and enzyme (Combo®) supplementation (T2, T3, T4, and T5, respectively) based on corn-soybean meal diets or enzyme supplementation plus equal amount of tested medicinal plants (T6) based on barley-soybean meal diets. Medicinal plants and enzyme supplementation were included in diets at level 0.5% and 0.2% of diets, respectively. The highest feed intake was found by T5 and T6 groups, while the highest weight gain was related to T2, T5, and T6 ($P<0.05$). Also, the lowest FCR was achieved by T6 ($P<0.05$). Diets contained medicinal plants (T2, T3, and T4) led to significantly decreases in carcass, breast, and liver weights ($P<0.05$). All diets led to significant decreases in abdominal fat, pancreas, and bursa weights as well as blood cholesterol content rather control ($P<0.05$). The lowest content of glucose was obtained by T4 ($P<0.05$). Diets contained *G. glabra* caused significantly increases in total protein content of blood ($P<0.05$). These results elucidate that effect of tested medicinal plants is depended on basal diets. The use of *T. vulgaris* L. or *G. glabra* together with enzyme supplementation in barley-based diets show better mien rather corn-basal diets without enzyme.
Key words: Barley, broiler, carcass, enzyme, medicinal plant.

INTRODUCTION

Phytogenic feed additives have attracted increasing interest as alternative feeding strategy to replace antibiotic growth promoters [1, 2, 3]. The presence of a number of pharmacologically active substances in phytogenic compounds possesses a potential for growth enhancement of poultry production [4]. Therefore, the potentials of many medicinal plants were study as antibiotic alternation in poultry production. Two medicinal plants of *Glycyrrhiza glabra* and *Thymus vulgaris* L. have beneficial effects in medicine. *G. glabra* have been consumed for the past 6000 years as a medicinal product in Asian countries [5]. It showed anti-microbial [6], anti-helicobacter [7], anti-atherosclerotic [8], anti-oxidative [9], antifungal [10], anti-viral [11], anti-infective [12], and immunity stimulator effects [13]. Also, *T. vulgaris* L. has anti-bacterial [14, 15, 16], anti-fungi [17], anti-virus [18], spasmolytic [19], anti-oxidant [20], and anti-parasites effects [20]. In previous studies, effect of variety of medicinal plants has demonstrated in corn-soybean meal diets in poultry [1, 2, 3, 21, 22, 23]. However, little is known about their effects on other basal diet grains in broiler production. On the other hand, replace of corn with other grains in diets lead to some problem in poultry [24] which the inclusion of exogenous enzymes could help to cope with such a problems [25, 26]. Therefore, this study was conducted to investigate the effect of medicinal plants *T. vulgaris* L. and *G. glabra* in corn-soybean meal diets on carcass characteristics, blood parameters, and broiler chicken performance. In addition, effect of inclusion of an exogenous enzyme, i.e., Combo®, in diets base on barley-soybean meal diet with mentioned medicinal plants was evaluated.

MATERIALS AND METHODS

The Animal Ethics Committee of the Agricultural Research Center of Qom-Iran was approved all procedure of the experiment.

Experimental Design and Birds

A total of 270 unsexed 1-d-old broiler chickens (Ross 308) were randomly divided to 6 treatments with 3 replications of 15 birds in each. Diets were formulated as starter (1-21 days of age) and grower (22-42 days of age) to meet their requirements [27]. Dietary experiments were including of control (T1), the inclusion of *T. vulgaris*, *G. glabra*, their mixture (equal amount), and enzyme (Combo®) supplementation (T2, T3, T4, and T5, respectively) based on corn-soybean meal, or enzyme supplementation plus equal amount of tested medicinal plants (T6) based on barley-soybean meal diet (Table 1). Medicinal plants and enzyme supplementation were included in diets at level 0.5% and 0.2%, respectively. Combo® contained 1000 unit phytase and 180 unit multiglucanase activities. Feed and water were offered *ad libitum* in all period of experiment. Water was changed daily and weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) was measured weekly. The lighting schedule was 23 h light / 1 h darkness at 32°C the first day. This was subsequently reduced 3°C each week until third week.

Carcass Characteristics

On 42 days of age, final body weights of broiler chickens were measured, 2 birds from each replicate were randomly selected, tagged, and were fasted for 8h (no limitation of water access). Birds were weighted and slaughtered by serving both of the right and left carotid artery and jugular vein in a single cut and bled for 180s. Carcass weights were measured after removal of feather, head, legs and abdominal contents. The breast, thighs, backside, neck, wings, and abdominal fat weights were recorded and calculated as the percentage of carcass weight. In addition, liver, heart, spleen, pancreas, and bursa weights as well as, intestine length were recorded.

Blood Parameters

On 42 days of ages, 2 chickens from each replicate were randomly selected and blood samples were taken from wing vein. Blood samples were transferred to vial tubes containing sodium heparin. The tubes were centrifuged at 5,000 × *g* for 20 min, and the supernatant was discarded. Glucose (GLU), cholesterol (CHO), triglyceride (TG), total protein (TP), albumin (Al), and globulin (GL) were determined by use of specific kits (Biosystem Company, Spain).

Statistical Analyses

A completely randomized design was employed. Pen was used as the experimental unit and data were analyzed by GLM procedure of SAS [28]. Duncan's multiple range test were used for comparison of means (P<0.05).

RESULTS

The effects of diets on broiler chicken performance are presented at Table 2. The results showed that T5 and T6 had the highest FI, and T2, T5, and T6 had the highest WG rather other diets (P<0.05).

Table 1: Diets formulation and composition (%) at starter (1 to 21 days) and grower (22 to 42 days).[1,2]

Item	Starter		Grower	
	T1-T5	T6	T1-T5	T6
Corn grain	53.8	45	60.7	42.58
Soybean meal	38.7	34.05	32.2	29.3
Barley	-	15	-	20
Soybean oil	3	2	3	3.47
Calcium carbonate	1.63	1	2.03	1
Dicalcium phosphate	1.72	2	1.13	2.5
Mineral and vitamin premix[3]	0.5	0.5	0.5	0.5
Common salt	0.44	0.3	0.23	0.3
Methionine	0.14	0.15	0.06	0.25
Lysine	0.07	-	0.05	0.1
Total	100	100	100	100
Metabolizable energy (Kcal/kg)	3000	2974.5	3055	3000
Crude protein	21.54	21.16	19.09	19.0
Calcium	0.93	0.92	0.85	1.02
Available phosphorus	0.45	0.40	0.33	0.49
Calcium: phosphorus	2.07	2.3	2.57	2.08
Energy: protein	139.27	140.57	160.03	157.89
Na+K-Cl (meq/kg)	230.50	230.70	231.75	230.44

[1]T1, T2, T3, T4, and T5 are control and diets contained 0.5% *Thymus vulgaris*, *Glycyrrhiza glabra*, their mixture (equal amount), or Combo® enzyme (at level 0.2%), and T6 is diet contained enzyme plus equal amount of tested medicinal plants.
[2]Enzyme supplement contained 1000 unit phytase 180 unit multiglucanase.
[3]Supplied the following per kilogram of diet: Vitamin A (retinyl acetate), 8,400 IU; vitamin D_3 (cholecalciferol), 3,000 IU ; vitamin E (DL-alpha-tocopheryl acetate), 25 IU ; K_3, 24 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; pantothenic acid, 15 mg; pyridoxine (pyridoxine_HCl), 4 mg; riboflavin, 10 mg; and thiamin, 3 mg (thiamin mononitrate). Supplied the following per kilogram of diet: 60 mg of copper ($CuSO_4$); 1.0 mg of iodine Ca (IO_3); 300 mg of iron ($FeSO_4$_H2O); 600 mg of manganese ($MnSO_4$_H_2O); 0.15 mg of selenium ($NaSeO_3$); 480 mg of zinc ($ZnSO_4$_H_2O); and 1.5 mg of cobalt ($CoSO_4$).

In addition, T6 led to the lowest FCR (P<0.05). The effects of dietary treatments on the carcass and organ characteristics of broiler chickens are illustrated at Tables 3 and 4, respectively. Diets contained medicinal plants (T2, T3, and T4) led to significantly decreases in carcass and breast weights (P<0.05). All diets led to significantly decreases in abdominal fat rather control (P<0.05). Diets contained medicinal plants (T2, T3, and T4) led to significantly decreases in liver weights (P<0.05). All diets led to significantly reduction in pancreas and bursa weights rather control (P<0.05). Adversely, all diets increased spleen weights rather control (P<0.05). Table 5 showed that the lowest content of GLU was obtained by T4 (P<0.05). All diets led to significantly drop in CHO content rather control (P<0.05). Control, T5, and T6 were significantly increased TG content rather other diets (P<0.05). Diets contained *G. glabra* caused significantly increases in TP content (P<0.05). The lowest content of Al was obtained by T5 and T6 (P<0.05). Also, the highest content of GL was related to T3 (P<0.05).

Table 2: Effect of treatments on broiler chicken performance at 1-42 days of age.

	FI[1] (g)	WG[3] (g)	FCR[3]
T1	4969.4[b]	2911.0[c]	1.71[ab]
T2	4948.0[b]	3012.3[a]	1.64[b]
T3	4864.0[c]	2879.8[c]	1.69[ab]
T4	4902.9[c]	2964.7[b]	1.75[a]
T5	5179.4[a]	2998.5[a]	1.63[b]
T6	5181.4[a]	3018.7[a]	1.52[c]
SEM	13.58	6.13	0.05
P-value	0.046	0.035	0.011

Means with common letters in the same columns are not significantly different (P<0.05). SEM: Standard error of the means. [1]Feed intake, [2]Weight gain, and [3]Feed conversion ratio.

T1, T2, T3, T4, and T5 are control and diets contained 0.5% *Thymus vulgaris*, *Glycyrrhiza glabra*, their mixture (equal amount), or Combo® enzyme (at level 0.2%) based on corn-soybean meal, and T6 is diet contained enzyme plus equal amount of tested medicinal plants based on barley-basal diets.

Table 3: Dietary experiment effects on carcass characteristics at 42 days of age (% of × carcass weight).

	Carcass	Breast	Thighs	Backs+Neck	Wings	Abdominal Fat
T1	70.7[a]	43.6[a]	30.3	21.4	9.3	2.7[a]
T2	78.7[b]	41.6[b]	30.4	19.7	8.9	2.4[b]
T3	68.2[b]	41.7[b]	30.1	19.9	9.4	2.6[ab]
T4	67.6[b]	40.9[c]	30.4	18.9	9.6	1.9[c]
T5	70.5[a]	44.1[a]	30.9	20.6	9.5	2.0[c]
T6	70.02[a]	43.5[a]	29.6	20.5	8.8	2.2[bc]
SEM	1.45	1.16	0.95	0.62	0.34	0.04
P-value	0.031	0.019	0.043	0.066	0.064	0.012

Means with common letters in the same columns are not significantly different (P<0.05). SEM: Standard error of the means.

T1, T2, T3, T4, and T5 are control and diets contained 0.5% *Thymus vulgaris*, *Glycyrrhiza glabra*, their mixture (equal amount), or Combo® enzyme (at level 0.2%) based on corn-soybean meal, and T6 is diet contained enzyme plus equal amount of tested medicinal plants based on barley-basal diets.

Table 4: The effect of dietary treatments on the organ weights (% of × live weight) and intestine length at 42 days of age.

	Liver	**Pancreas**	**Spleen**	**Bursa**	**Intestine (cm)**
T1	4.07[a]	0.17[a]	0.15[c]	0.15[a]	115.16
T2	3.46[bc]	0.16[b]	0.17[b]	0.13[c]	118.81
T3	3.60[b]	0.16[b]	0.18[b]	0.12[c]	117.37
T4	3.24[c]	0.16[b]	0.19[a]	0.13[b]	114.98
T5	3.99[a]	0.16[b]	0.19[a]	0.14[b]	110.62
T6	3.87[a]	0.16[b]	0.18[ab]	0.14[b]	117.72
SEM	0.151	0.008	0.011	0.007	2.391
P-value	0.008	0.036	0.001	0.033	0.188

Means with common letters in the same columns are not significantly different (P<0.05). SEM: Standard error of the means.

T1, T2, T3, T4, and T5 are control and diets contained 0.5% *Thymus vulgaris*, *Glycyrrhiza glabra*, their mixture (equal amount), or Combo® enzyme (at level 0.2%) based on corn-soybean meal, and T6 is diet contained enzyme plus equal amount of tested medicinal plants based on barley-basal diets.

Table 5: Effect of treatments on blood parameters (g/dl) at 42 days of age.

	GLU[1]	CHO[2]	TG[3]	TP[4]	Al[5]	GL[6]
T1	264.21[a]	148.16[a]	100.33[a]	40.75[bc]	19.83[a]	20.93[c]
T2	261.32[a]	140.67[b]	69.54[b]	43.58[b]	20.78[a]	22.80[b]
T3	257.17[b]	142.83[b]	69.17[b]	46.18[a]	19.98[a]	26.20[a]
T4	249.15[c]	125.85[c]	63.67[c]	38.97[c]	18.27[ab]	20.70[c]
T5	267.33[a]	143.43[b]	99.67[a]	40.25[bc]	17.73[b]	21.52[bc]
T6	262.74[a]	133.71[bc]	103.48[a]	43.14[b]	17.38[b]	27.63[bc]
SEM	3.13	1.13	6.66	1.13	0.85	0.64
P-value	0.012	0.002	0.001	0.038	0.061	0.018

Means with common letters in the same columns are not significantly different ($P<0.05$). SEM: Standard error of the means.
[1]Glucose, [2]Cholesterol, [3]Triglyceride, [4]Total protein, [5]Albumin, and [6]Globulin.
T1, T2, T3, T4, and T5 are control and diets contained 0.5% *Thymus vulgaris*, *Glycyrrhiza glabra*, their mixture (equal amount), or Combo® enzyme (at level 0.2%) based on corn-soybean meal, and T6 is diet contained enzyme plus equal amount of tested medicinal plants based on barley-basal diets.

DISCUSSION

Effect of medicinal plants was investigated mostly on corn-soybean meal diets (1, 2, 3) and focuses on their effects on other basal diets grain is scarce. Inclusion of exogenous enzyme in barley-soybean meal diet to investigate effect of medicinal plants is new issue in current study. Therefore, comprehensive comparison and explanation for obtained results may not be possible. Dietary supplementation of medicinal plants mixture with enzyme exhibited a significantly positive effect on FI, WG, and FCR. Results are similar to the finding of other study which the inclusion of licorice extracts in drinking water [3] led to the greatest FI and WG. They attributed to increased FI to changes in feed palatability and stimulated appetite. It is demonstrated that medicinal plants have appetite and digestion stimulating factors [29, 30]. On the other hand, cereal β-glucans have been shown to have prebiotic properties as they have the ability to pass undigested through the gastrointestinal tract, where they act as a substrate for microbial fermentation and selectively stimulate the growth and activity of a small number of beneficial bacteria [31]. In addition, exogenous enzyme supplementation can effectively degrade such β-glucans leading to a subsequent improvement in digestibility and nutrient utilization [32]. They might be main reasons for observed better FCR by treatments T5 and T6. Therefore, it seems that exogenous enzyme supplementation in barley-based diets (T5) and exogenous enzyme supplementation plus medicinal plants mixture (T6) brought about a beneficial effect on the gut health as these diets supported higher performance.

The use of exogenous enzyme supplementation degrade grain viscous component of diets [33] and reduce or eliminate the encapsulation effect of the cell wall polysaccharide leading consequently to an increase in nutrient digestion [34], thereby increase availability of nutrients. These reactions can improve nutrients absorption and increase carcass and its part weights. It is confirm by obtained results regarding carcass cuts in present study. No significant difference was observed in thighs, backs plus neck, and wings weights between diets. It is showed that the effect of diets on mentioned cuts are less pronounces. All diets caused decreases in abdominal fat weighs rather control. Feed additives may interact with fat digestion and absorption which can affect abdominal fat [1]. Pelicano et al. [35] and Demir et al. [36] reported that the inclusion of medicinal plants in diet had no effect on abdominal fat which was disagreement with observations of the present study. The effect of T5 and T6 on reduced abdominal fat deposition might be due to presence of viscose material in diets [37]. In presence of medicinal plants and fibrous grain, the bill acid secretions reduce, their digesta concentrations severely decrease, hepatic-intestinal circulation impress, and lipids emulsification strike. It results in the decrease of lipids absorption and lead to lower form of abdominal fat as showed in the present study. It is similar to other research [3]. Diets contained medicinal plants (T2, T3, and T4) caused a significant decrease in liver weights rather control. Decreases in lipid metabolism on liver by reduction of lipids uptake might result in lower liver weight. Diets based on barley caused a significant increase in liver weights. Liver increase its weight when is exposed to fiber sources in diets to improve bile secretion [36]. On the other hand, Debersac et al. [37] indicated that an herbal extract enhanced hepatic metabolism and increased liver weight in rats. All diets resulted in reduction in pancreas weight rather control. It is possible that none of treatments can change pancreas activity as well as pancreas weights. Observed reduction in bursa weights are in agreements with other study [3]. They had no clear explanation for this event.

The inclusion of *G. glabra* in diets (as single or mixed with *T. vulgaris* L.) could reduce blood glucose concentration. It can refer to strong anti-oxidant action and potential anti stress action of *G. glabra*. Some bacteria such as *Lactobacillus* and *Bifidobacteria* can reduce blood cholesterol.

The use of medicinal plants in broiler diets and fiber sources can induce *Lactobacillus* and *Bifidobacteria* growth in intestine [39] which they can interference with cholesterol metabolism and decrease blood cholesterol. This observation is the opposite of other research [3] who no significant difference was observed by supplementation of licorice extracts in drinking water. Differences in type and amount of used feed additives as well as differences in basal diets can explain observed inconsistency. The use of medicinal plant (T2, T3, and T4) in this study was associated with reduction in blood TG levels which agree with results of other [40]. They showed that the use of medicinal plant can reduce TG levels of blood. It is proposed that medicinal plants cause a reduction in liver 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) (key enzyme in cholesterol synthesis regulation) [41]. Spectrophotometric analysis of blood samples indicated that, during the whole experimental period, the inclusion of *G. glabra* in diets increased GL concentration which thereby led to increases in TP levels. Higher TP level suggested better ability of the hepatocytes of treated group to synthesize protein. Significantly higher serum GL level suggested that birds of treated group had potential for better humoral immune status, substantiated by the fact that higher liver weight was obtained in treated group. Lowered AL level, on the other hand, had no adverse effect on the colloidal state of blood as well as capillary permeability.

CONCLUSIONS

The findings of present study demonstrated that basal diets play important role to efficiency of inclusion of medicinal plants in broiler diets. The use of *T. vulgaris* L. or *G. glabra* together with enzyme supplementation in barley-based diets show better feature rather corn-basal diets without enzyme. However, tested medicinal plants have hypolipidemic activity and better potential to liver health.

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