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Thymus vulgaris L., *Glycyrrhiza glabra* or Combo® enzyme in corn *vs.* barley–based broiler diets

Majid Kalantar[1], Mostafa Rezaei[2], Jalal Salary[3], Hamid Reza Hemati Matin[4*]

[1]*Scientific Board Member of Agricultural Research Center of Qom, Iran*

[2]*Department of Animal Science, College of Agriculture, Saveh Branch, Islamic Azad University, Saveh, Iran*

[3]*Department of Animal Science, Faculty of Agriculture, Bu Ali Sina University, Hamedan, Iran*

[4]*School of Agriculture, Tarbiat Modares University, Tehran, Iran*

PEER REVIEW

ABSTRACT

Objective: To test the effect of supplementation of *Thymus vulgaris* L. (*T. vulgaris*) or *Glycyrrhiza glabra* (*G. glabra*) in corn–soybean meal diets as well as the inclusion of an exogenous enzyme *i.e.* Combo® in barley–soybean meal diets together with mentioned medicinal plants in broiler diets.
Methods: A total of 270 unsexed 1–day–old broiler chickens (Ross 308) was randomly assigned to 6 treatments with 3 replications of 15 birds in each. Diets were comprised of the control (T1), the inclusion of *T. vulgaris*, *G. glabra*, their mixture (equal amount), Combo® supplementation (T2, T3, T4 and T5, respectively) in diets based on corn–soybean meal diets and enzyme supplementation plus equal amount of tested medicinal plants (T6) based on barley–soybean meal diets. Medicinal plants and enzyme were included in diets at level 0.5% and 0.2% of diets, respectively.
Results: The highest feed intake was obtained by T1 at 1–21 d of age ($P<0.05$). All diets caused significant increases in weight gain and significant decreases in feed conversion ratio compared to control at this age ($P<0.05$). Significant reductions were acquired in feed intake by T3 and T6 at 22–42 d of age ($P<0.05$). All diets significantly decreased total number of aerobic bacteria, coliforms, Gram–negative bacteria and increased lactic acid bacteria compared to control ($P<0.05$).
Conclusions: The results showed that basal diet has vital character to effectiveness of medicinal plants in broiler diets. Beneficial effects on intestinal microflora were brought by use of *T. vulgaris* L. and *G. glabra* in corn–based diets or in barley–based diets together with enzyme. Thus, this capability can support growth performance of broiler chickens at lower age.

1. Introduction

Extensive attention has been paid to phytogenic feed additives as antibiotic replacement in poultry diets[1–3], because of their pharmacologically active substances[4]. Effects of antibiotics alternative are mediated by intestinal microflora[5]. They can manipulate gut functions and microbial habitat of domestic animals[6], as they prevent the growth of inward harmful bacteria in the gut[7], or promote the growth of baneful bacteria[1,8,9].

Glycyrrhiza glabra (*G. glabra*) and *Thymus vulgaris* L. (*T. vulgaris*) have shown beneficial effects in medicine. *G.

*Corresponding author: Hamid Reza Hemati Matin, School of Agriculture, Tarbiat Modares University, Tehran, Iran.
Tel: 00989183179379
E–mail: hamidhematti@yahoo.com

glabra showed antimicrobial[10], anti–*Helicobacter*[11], anti–atherosclerotic[12], antioxidative[13], anti–fungal[14], anti–viral[15], anti–infective[16], and immunity enhancement effects[17]. Herein, anti–bacterial[18–20], anti–fungi[21], anti–virus[22], spasmolytic[23], antioxidant, and anti–parasites effects of *T. vulgaris* L. was approved[24]. Therefore, it seems that quoted medicinal plants convince nutritionist to include them in diets as feed additive. However, effect of medicinal plants were mainly studied in corn–soybean meal diets in poultry[1–3,25–27], and little consideration was made regarding other basal grain–based diets in broiler production. Nevertheless, the inclusion of other grains (*e.g.* barley) in diets leads to some problem in poultry[28]. The supplementation of exogenous enzymes could solve to some extent problems[29,30]. Therefore, the present study was conducted to evaluate the effect of corn–soybean meal diets containing *T. vulgaris* L. and *G. glabra* on microflora population and broiler chicken growth performance. In addition, effect of inclusion of an exogenous enzyme, *i.e.*, Combo®, in diets based on barley–soybean meal with mentioned medicinal plants was assessed.

2. Materials and methods

2.1. Experimental design and birds

A total of 270 unsexed 1–day–old broiler chickens (Ross 308) were randomly allotted to 6 treatments with 3 replications of 15 birds in each. Diets were formulated as starter (1–21 d of age) and grower (22–42 d of age) to meet their requirements[31]. Dietary experiments comprised the control (based on corn–soybean meal, T1) and the inclusion of *T. vulgaris*, *G. glabra*, their mixture (equal amount), and enzyme (Combo®) (T2, T3, T4, and T5, respectively) in such diets, or enzyme plus equal amount of tested medicinal plants based on barley–soybean meal (T6) in diets (Table 1). Medicinal plants and enzyme were included in diets at level 0.5% and 0.2%, respectively. Combo® contained 1 000 IU phytase and 180 IU multi glucanase. 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 at the first day. The temperature was subsequently reduced by 3 °C each week until the third week.

2.2. Microbial sampling and incubation

On Day 42 of the experiment, 2 birds from each replicate were slaughtered by cervical dislocation and ileum contents were collected. Contents were gently removed into sterile sampling tubes and immediately transferred on ice to the laboratory. Serial dilutions of 1 g sample (10^{-4} to 10^{-7}) were made. Selective media of plate count agar, MacConkey agar, MRS agar plus 0.1% of Tween 80, and eosin–methylene blue agar were inoculated to detect the total number of aerobic bacteria, coliforms, lactic acid bacteria, and Gram–negative bacteria, respectively. Total number of aerobic bacteria and coliforms were counted after aerobic incubation for 24 h at 37 °C. The numbers of lactic acid bacteria were calculated after incubation in an anaerobic chamber at 37 °C for 48 h and Gram–negative bacteria were counted after incubation for 48 h at 37 °C[2,32].

Table 1

Diets formulation and composition (%) for starter (1–21 d) and grower (22–42 d).

Item	Starter		Grower	
	T1–T5	T6	T1–T5	T6
Corn grain	53.80	45.00	60.70	42.58
Soybean meal	38.70	34.05	32.20	29.30
Barley	–	15.00	–	20.00
Soybean oil	3.00	2.00	3.00	3.47
Calcium carbonate	1.63	1.00	2.03	1.00
Dicalcium phosphate	1.72	2.00	1.13	2.50
Mineral and vitamin premix[1]	0.50	0.50	0.50	0.50
Common salt	0.44	0.30	0.23	0.30
Methionine	0.14	0.15	0.06	0.25
Lysine	0.07	–	0.05	0.10
Total	100.00	100.00	100.00	100.00
Metabolizable energy (kcal/kg)	3 000.00	2 974.50	3 055.00	3 000.00
Crude protein	21.54	21.16	19.09	19.00
Calcium	0.93	0.92	0.85	1.02
Available phosphorus	0.45	0.40	0.33	0.49
Calcium: phosphorus	2.07	2.30	2.57	2.08
Energy: protein (kcal/%)	139.27	140.57	160.03	157.89
Na+K–Cl (meq/kg)	230.50	230.70	231.75	230.44

[1]: Vitamin and mineral provided/kilogram of diet including vitamin A (8 400 IU), vitamin D_3 (3 000 IU), vitamin E (25 IU), potassium 3 (24 mg), vitamin B_{12} (0.02 mg), biotin (0.10 mg), folacin (1.00 mg), niacin (50.00 mg), pantothenic acid (15.00 mg), pyridoxine (4.00 mg), riboflavin (10.00 mg), thiamin (3.00 mg), copper (60.00 mg), iodine calcium (1.00 mg), iron (300.00 mg), manganese (600.00 mg), selenium (0.15 mg), zinc (480.00 mg) and cobalt (1.50 mg).

2.3. Intestinal pH and viscosity

Two birds from each replicate were randomly selected and sacrificed by cervical dislocation at 42 d of age. The ileum content was gently removed and the pH values were immediately measured. The digesta diluted nine–fold (w/v) with distilled water were stirred for 5 min and the pH of the suspensions were measured using a calibrated pH meter (Metrohm, Germany). The removed digesta samplings were freezed at −20 °C until analyzing. Ileum digesta viscosity was measured by a viscometer (DV–II þ LV, Brookfield, Stroughton, MA, USA) previously calibrated with a silicone standard[2,33].

2.4. Statistical analyses

A completely randomized design was employed. Pen was used as the experimental unit. Experimental data were subjected to general linear models procedure of statistics analysis system[34]. Logarithmic (Log$_{10}$) transformation was applied for microbial colony forming unit (CFU). Duncan′s multiple range test was used for comparison of means at $P<0.05$.

3. Results

The effects of diets on broiler chicken growth performance are presented in Table 2. The results indicated that T1 showed the highest FI at 1–21 d of age ($P<0.05$). All diets caused significant increases in WG and significant decreases in FCR compared to control at this age ($P<0.05$). Significant reductions of FI were found in T3 and T6 at 22–42 d of age ($P<0.05$). Diets supplemented with *G. glabra* (T3) decreased WG in chickens ($P<0.05$). The lowest FCR were found in T6 at this age ($P<0.05$). The effects of dietary treatments on ileum microflora counts of broiler chickens at 42 d of age are showed in Table 3. All diets significantly decreased total number of aerobic bacteria, coliforms, and Gram–negative bacteria compared to control ($P<0.05$). In the same vein, all diets led to higher lactic acid bacteria counts compared to the control, and the highest one was found in T3 and T6 ($P<0.05$). Table 4 showed that the pH values of ileum contents of T1, T3, and T5 were significantly higher than T2, T4, and T6 ($P<0.05$). Moreover, the viscosity of ileum contents of T1 and T6 was significantly higher than that of other treatments ($P<0.05$).

Table 2

Effect of diets on broiler chicken performance at different ages.

The age of broiler chicken		FI (g)	WG (g)	FCR
1–21 d of age	T1	62.240^a	44.020^b	1.410^a
	T2	60.610^{ab}	45.650^a	1.330^b
	T3	60.740^b	44.760^a	1.350^b
	T4	61.480^{ab}	45.930^a	1.330^b
	T5	61.560^{ab}	45.950^a	1.340^b
	T6	58.320^b	45.390^a	1.310^b
	SEM	1.210	0.240	0.040
	P value	0.017	0.021	0.008
22–42 d of age	T1	175.550^a	98.130^b	1.790^{ab}
	T2	174.400^a	99.740^a	1.740^{ab}
	T3	169.880^b	92.390^c	1.830^a
	T4	171.490^{ab}	95.420^b	1.790^a
	T5	170.280^{ab}	99.850^a	1.730^b
	T6	167.140^b	97.320^{ab}	1.710^c
	SEM	2.220	1.110	0.060
	P–value	0.003	0.013	0.001

Means with common letters in the same columns are not significantly different ($P<0.05$). FI: Feed intake; WG: Weight gain; FCR: Feed conversion ratio.

Table 3

Effects of diets on selected ileum microflora counts (Log_{10} CFU/g of digesta) at 42 d of age.

Treatment	Total number of aerobic bacteria	Coliforms	Gram–negative bacteria	Lactic acid bacteria
T1	7.130^a	6.320^a	6.330^a	3.870^d
T2	5.820^c	5.070^d	5.610^b	4.900^b
T3	6.660^b	5.210^c	5.240^c	5.190^a
T4	6.660^b	5.270^c	5.210^c	4.900^b
T5	6.140^{bc}	5.270^c	5.610^b	4.120^c
T6	6.070^c	5.710^b	5.280^c	5.070^a
SEM	0.199	0.022	0.031	0.057
P value	0.002	0.022	0.001	0.044

Means with common letters in the same columns are not significantly different ($P<0.05$).

Table 4

The values of pH and viscosity of ileum contents in response to diets at 42 d of age.

	pH	Viscosity (cP)
T1	6.220^a	1.970^a
T2	5.270^b	1.620^b
T3	6.110^a	1.600^b
T4	5.360^b	1.630^b
T5	6.270^a	1.590^b
T6	5.310^b	1.890^a
SEM	0.080	0.040
P value	0.001	0.001

Means with common letters in the same columns are not significantly different ($P<0.05$).

4. Discussion

In previous studies medicinal plants were mainly included in corn–soybean meal diets[1–3], and effect of type of basal diets unheard. In present study, inclusion of exogenous enzyme in barley–soybean meal diet to investigate effect of medicinal plants is a new issue. Thus, it is possible that widespread details do not exist for comparison and explanation of obtained results. All diets whether based on corn or based on barely exhibited a significantly positive effect on WG and FCR compared to the control at 21 d of age. These results are similar to the findings of other researchers[1,3], in which the use of medicinal plants increased growth performance. It is demonstrated that medicinal plants are of appetite and digestion stimulating potentials[35,36], which thereby increase WG and results in better FCR. It is showed that β–glucans of cereal have prebiotic properties through the gastrointestinal tract, where they help to settle intestinal microflora[37], in favor of host. Degration of such β–glucans with exogenous enzymes lead to a subsequent improvement in digestibility and nutrient utilization[38]. These events could lead to performance improvement. On 22–42 d of age, observed results regarding growth performance parameters were different from those obtained at lower age. This finding inclitated that broilers might be adapted to tested diets with aging[3]. But what is clear is that T3 (*G. glabra* in corn–basal diet) led to the lowest WG. It's observed that the use of *G. glabra* must be limited to lower ages.

In this study, dietary treatments changed selected microbial populations in ileum segment. Medicinal plants have antimicrobial properties[1,2,39]. It is proposed that plant antibacterial properties are related to their lipophilic characters[40]. The major mechanism of medicinal plants is adhesion and thrust of bacterial membrane which inhibits bacterial enzyme activation[41,42]. Furthermore, β–glucans of cereal have been shown to have prebiotic properties through the gastrointestinal tract, where they act as a substrate for microbial fermentation and selectively stimulate the growth and activity of beneficial bacteria[37]. As the diet containing barley had higher proportion of fiber components, there was more substrate available for microbial growth. These reactions can reduce pathogenic populations in the intestine which was also seen in the present study by reducing total number of aerobic bacteria, coliforms, and Gram–negative

bacteria in ileum by tested diets compared to control. The results are in agreement with other study by use of fiber sources[32,43,44], and medicinal plants in diets[1,2].

Little information is available about pH values of intestinal contents by inclusion of medicinal plants in broiler diets. However, the inclusion of *T. vulgaris* L. (as single or mixed with *G. glabra*) in corn and barley–based diets led to decreases in pH values of ileum. It is possible that this reduction has main effect on decreases of harmful bacteria as observed in current study. The results showed that tested medicinal plants are capable to reduce digesta ileum viscosity. In addition, exogenous enzyme supplementation can effectively degrade β–glucans in barley[38], leading to dwindle in digesta viscosity. This principle was observed in T5 (inclusion of enzyme in barley–based diet). The lack of such observation in supplementation of enzyme and medicinal plants in barley–based diet is unclear.

Based on the results of current study it can be concluded that basal diets has determinative role to efficiency of inclusion of medicinal plants in broiler diets. The use of *T. vulgaris* L. and *G. glabra* in corn–based diets or barley–based diets together with tested enzyme have dissuasive effects in settling harmful bacteria in intestine. This ability encourages growth performance of broiler chickens especially in lower age.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Comments

Background

Medicinal plants with multi–effects induce nutritionist to include them in poultry diets. Little consideration was made regarding basal diets differences. Even so, the problem inclusion of some grains (*e.g.* barley) in diets can be solved with the addition of exogenous enzymes. Therefore, it is needed to test role of other basal diets to explain medicinal plant effects.

Research frontiers

The present research work depicts that basal diet has vital character in effectiveness of medicinal plants of *T. vulgaris* L. and *G. glabra* in broiler diets. Their beneficial effects on intestinal microflora are obvious by use of them in corn–based diets or together with enzyme, Combo® in barley–based diets.

Related reports

T. vulgaris L. and *G. glabra* have multi–beneficial effects in medicine. The differences in basal diets influence the effects of mentioned medicinal plants in broiler diets.

Innovations and breakthroughs

Authors have proposed that effects of *T. vulgaris* L. and *G. glabra* depended upon used basal grain in broiler diets. Corn–based diets or barley–based diets together with Combo® have dissuasive effects in settling harmful bacteria in intestine.

Applications

It can be suggested that the use of *T. vulgaris* L. and *G. glabra* as feed additive in diets is effective in corn–based diets or in barley–based diets with enzyme.

Peer review

This is a helpful research in which authors have demonstrated that basal diets have determinative effects when medicinal plants are included in broiler diets. These are approved based on biochemical, microbial, and growth parameters.

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