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The effect of different levels of Fenugreek (*Trigonella foenum-graecum* L.) powder and extract on performance, egg quality, blood parameters and immune responses of laying hens in second production cycle

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Abstract

The present study was carried out to determine the effects of fenugreek powder (FP) and extract (FE) on performance, egg quality, blood parameters and immune responses of laying hens. One-hundred and fifty Leghorn laying hens were used in a completely randomized design with five treatments and five replicates for eight weeks. Treatments were various levels of FP and FE including zero (control; T1), 1.00% FP (T2), 2.00% FP (T3), 0.10% FE (T4) and 0.20% FE (T5). The results of this experiment showed that feed intake was increased linearly by the inclusion of FP compared to the control group. Supplementation of laying hens diet with 2.00% FP adversely affected feed conversion ratio (FCR). The FCR was decreased by 0.10% inclusion of FE compared to 0.20%. Egg yolk color was the highest when 1.00% FP added to laying hens diets compared to the other treatments. Serum metabolites and immune responses of laying hens were not affected significantly by fenugreek supplementation. From the results of the present study, it can be concluded that using 1.00% FP can improve feed intake by supporting FCR. Inclusion of 1.00% FP in laying hens diet enhanced egg yolk color of laying hens in the second production cycle.

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Introduction

During the last decade and after banning the use of antibiotic growth promoters (AGP) in poultry nutrition, plant secondary metabolites and their derivatives have attracted a lot of attention for their potential role as alternatives for AGP. Recent studies showed that herbs and their derivatives can enhance growth performance and production properties and modulate the immune system of farm animals under normal or stress conditions.[1]

Fenugreek (*Trigonella foenum-graecum L.*) a multi-functional herb belonging to the family of Fabacecae is known for its antifungal, antiviral, anticarcinogenic, anti-diabetic and antimicrobial properties.[2] This plant is one of the oldest medicinal plants with excellent medicinal and nutritional properties.[3] Fenugreek contains numerous bioactive constituents such as alkaloids, flavonoids, steroid and saponins.[4] Alkaloids of this plant include trigocoumarin, nicotinic acid, trimeth coumarin, and trigonelline.[5] Fenugreek is an excellent off-season fodder and animal food supplement, due to having a high proportion of protein, fatty acids, and total carbohydrates.[4] Some researchers have studied the use of fenugreek in poultry feeding and their results have suggested that feed conversion ratios (FCRs) are affected positively by inclusion fenugreek into laying hens diets.[6] In this regard, researchers have reported that fenugreek extract in drinking water can improve product performance and immune system of laying hens,[4] however, others have noticed, unlike the previous observations that the use of fenugreek in layer diets in amounts of 1.00% and 2.00% has a negative influence on the egg production.[7] The objective of the present study was to confirm previous work on dietary supplementation of fenugreek powder (FP) or extract (FE) on performance, egg quality, blood parameters and immune response of laying hens.

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Materials and Methods

Fenugreek was purchased from a local producer and the parts of the plant suitable for consumption were dried in shade and powdered. The voucher specimens were deposited at Khuzestan Agricultural Sciences and Natural Resources University Herbarium (KHAU), Ahvaz, Iran (voucher no. 262). For the preparation of extract, an adequate amount of fenugreek powdered material was macerated with ethanol (80.00%) in a proportion of 1:5 (w/v) and left for 72 hr at room temperature. The extract was then shacked, filtered and evaporated in a rotating evaporator until the solvent was disappeared and gave semi-solid mass yielded about 10.00% w/w.[8] Prior to feeding trial, the chemical composition of FP was determined by the Association of Official Analytical Chemists methods. Crude protein, total lipids, crude fiber, ash, and nitrogen-free extract content of fenugreek samples were 19.95, 7.50, 28.84, 13.83 and 19.22 of a percentage of dry matter, respectively. All procedures used in the current experiment were approved by the Committee on Poultry Research of the Agricultural Sciences and Natural Resources University of Khuzestan, Ahvaz, Iran (215-11.20.2016). One-hundred and fifty Leghorn (Hy-Line, W-36) laying hens in a second production cycle were used in a completely randomized design with five treatments and five replicates (n = 6) for eight weeks. Treatments were various levels of FP and FE including zero (control; T1), 1.00% FP (T2), 2.00% FP (T3), 0.10% FE (T4) and 0.20% FE (T5). The laying hens were fed a corn-soybean meal-based diet supplemented with FP by exposing of wheat bran or supplemented with FE on top of the basal diet (Table 1). Feed and water were offered *ad libitum* throughout the experimental periods. Egg weight (EW; g), egg mass (EM; g per hen daily), hen-day egg production (EP; %) and feed intake (FI; g) were measured daily and calculated for a whole experimental period. The FCR was also calculated as the ratio of FI per EM during the experiment. At the end of each week, 10 eggs were randomly collected from each treatment (two eggs per replicate) and individually weighted and external and internal egg quality traits were determined. The egg shape index was calculated by dividing egg width by egg length. Eggs Shell, yolk and albumen percentages were calculated by their weights. Egg shell thickness was measured at three different points (top, middle, and bottom) using a micrometer and the average shell thickness (mm) was obtained from the average values of these three parts. Eggshell strength was determined by mechanical resistive device (Karl Kolb GmbH & Co., Dreieich, Germany). Albumin height, Haugh unit, and yolk color were described by an automatic egg analysis machine (EMT-5200; Robotmation Co. Ltd., Tokyo, Japan). Blood samples were randomly collected from 10 birds per treatment from the wing vein into sterilized tubes. The tubes were centrifuged (Hk 36; Hermle, Wehingen, Germany) at 3,000 rpm for 15 min and separated serum was stored in a freezer at – 20 ˚C until the time of biochemical analysis. The sera were used for the colorimetric determination of the blood sugar, triglyceride and cholesterol using commercial kits (Pars Azmoon, Tehran, Iran) according to the manufacturer's protocols. For determination of immune response, at 5th and 7th weeks of the experiment, 0.50 mL 20.00% suspension of sheep red blood cells (SRBCs) was injected into breast muscle of two hens per replicate and blood samples were taken from wing vein one week after each injection. Serum was separated and evaluated for antibody titer against SRBCs by the hemagglutination method.

Table 1. Composition and calculated analyses of the basal diet T1, T2 and T3.*

Ingredients (%)	T1	T2	T3
Corn	62.22	62.22	62.22
Soybean meal (44.00% protein)	20.80	20.80	20.80
Vegetable oil	2.50	2.50	2.50
Wheat bran	2.00	1.00	0.00
Fenugreek powder	0.00	1.00	2.00
Oyster shell	5.50	5.50	5.50
Di-calcium phosphate	1.40	1.40	1.40
Limestone	4.57	4.57	4.57
Salt	0.22	0.22	0.22
Sodium bicarbonate	0.21	0.21	0.21
DL-Methionine	0.08	0.08	0.08
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
Calculated analysis			
Metabolizable energy (kcal kg^{-1})	2807.20	2807.20	2807.20
Crude protein (%)	14.80	14.80	14.80
Calcium (%)	4.20	4.20	4.20
Available phosphorus (%)	0.38	0.38	0.38
L- Lysine (%)	0.73	0.73	0.73
Methionine + Cystine (%)	0.58	0.58	0.58

* With supplementation different levels (0.10 and 0.20%) of fenugreek extract on top to the control diet provided T4 and T5.
[1] Vitamin premix provided per kg of diet: Vitamin A: 2.40 mg; Vitamin D3: 75.00 µg; Vitamin E: 5.00 mg; Vitamin K3: 2.20 mg; Vitamin B1: 1.50 mg; Vitamin B2: 4.00 mg; Vitamin B3: 8.00 mg; Vitamin B5: 35.00 mg; Vitamin B6: 2.50 mg; Vitamin B9: 0.50 mg; Vitamin B12: 10.00 µg; Vitamin H2: 0.15 mg; Choline: 468.70 mg.
[2] Mineral premix provided per kg of diet: Mn: 80.00 mg; Fe: 75.00 mg; Zn: 64.00 mg; Cu: 6.00 mg; Se: 0.30 mg.

Statistical analysis. The present experiment was based on a completely randomized design. All results were statistically analyzed by General Linear Models, one-way analysis of variance, using SAS software (version 8.0; SAS Institute, Cary, USA). Significance between means was tested using Duncan multiple range tests. A probability value of $p \leq 0.05$ indicated that the difference was statistically significant.

Results

The results of productive traits are given in Table 2. The data indicate that EW, EM, and EP were not affected significantly by supplementation of FP or FE to the diets.

Hens fed a diet supplemented with 0.10% of FE, numerically produced about 5.70% more egg rather than a control group. Feed intake was affected significantly by using FP in laying hens diet and it was the highest at 2.00%. In terms of FCR, it was increased in T3 birds as a result of increasing FI in this group. Table 3 shows that the diet had no significant effect on egg quality traits such as egg shape index, Haugh unit, shell strength (kg per cm^2), shell thickness (mm) and shell, albumen and yolk weights (%). Only, egg yolk color was increased significantly by supplementation of hen diets with 1.00% FP.

Inclusion of FP and FE in experimental diets did not significantly affect the serum parameters such as glucose, triglycerides, and cholesterol throughout the experimental period (Table 4), ($p > 0.05$). However, serum triglycerides and cholesterol were decreased numerically in T4 rather than the control group (respectively 19.30 and 25.30%).

The effects of supplemental FP and FE on antibody titer against SRBCs of laying hens have been shown in Table 4. Results from this table represented that the inclusion of FP and FE had no significant effect on primary and secondary antibodies titer against SRBCs ($p > 0.05$).

Discussion

The FI was increased significantly in T2 and T3, but FCR was improved only in T2. As mentioned above, FCR was calculated by dividing FI to EM. In this study, FCR was increased by increasing FI as a result of supplementation of FP to laying hens diets. In 1.00% inclusion of FP, FI was increased, but FCR was the same as the control group. It was indicated that the FP at 1.00% can increase FI without changing FCR, thus in the equation, increasing FI without changing FCR resulted in EM improvement. In this study, FI increased linearly by supplementation of FP to laying hens diet. In the first step, increasing animal production depends on increasing FI. In this regard, it was shown that fenugreek contains bioactive components including biotin, trimethylamine, and neurin which can stimulate FI by

Table 2. Effect of dietary fenugreek powder (FP) and extract (FE) on productive traits of laying hens.

Treatments	Feed intake (g per hen daily)	Egg weight (g)	Egg mass (g per hen daily)	Egg production (%)	Feed conversion ratio (g feed per g egg mass)
T1 (Control)	93.90[c]	61.30	42.88	71.08	2.17[bc]
T2 (1.00% FP)	99.08[b]	62.31	43.94	72.38	2.26[bc]
T3 (2.00% FP)	107.19[a]	60.01	42.57	72.74	2.52[a]
T4 (0.10% FE)	96.95[bc]	61.98	45.62	75.41	2.13[c]
T5 (0.20% FE)	96.07[bc]	61.96	41.76	68.91	2.30[b]
Standard errors of means	1.16	0.31	0.58	0.91	0.03
***p*-value**	< 0.0001	0.14	0.27	0.25	0.0002

[abc] Means in the same column with different superscripts differ significantly ($p \leq 0.05$).

Table 3. Effect of dietary fenugreek powder (FP) and extract (FE) on egg quality traits of laying hens.

Treatments	Haugh unit	Shell strength (kg per cm^2)	Shell thickness (mm)	Shell weight (%)	Yolk weight (%)	Albumen weight (%)	Yolk color	Egg shape index
T1 (Control)	86.08	1.60	0.38	9.18	25.28	66.49	5.15[b]	75.65
T2 (1.00% FP)	86.49	1.51	0.37	9.28	26.04	66.22	6.23[a]	75.95
T3 (2.00% FP)	86.92	1.76	0.38	9.26	26.34	64.92	5.57[b]	75.53
T4 (0.10% FE)	85.62	1.74	0.39	9.41	24.55	66.20	5.67[b]	75.51
T5 (0.20% FE)	86.68	1.69	0.38	9.45	25.16	67.26	5.56[b]	75.90
Standard errors of means	0.44	0.05	0.002	0.08	0.22	0.44	0.14	0.09
***p*-value**	0.90	0.49	0.07	0.81	0.08	0.59	0.003	0.78

[ab] Means in the same column with different superscripts differ significantly ($p \leq 0.05$).

Table 4. Effect of dietary fenugreek powder (FP) and extract (FE) on blood parameters and antibody titer against sheep red blood cells of laying hens.

Treatments	Triglyceride (mg dL^{-1})	Cholesterol (mg dL^{-1})	Glucose (mg dL^{-1})	Primary antibody response (log^2)	Secondary antibody response (log^2)
T1 (Control)	1553.30	149.67	212.80	4.75	5.78
T2 (1.00% FP)	1637.00	162.80	222.80	4.62	6.10
T3 (2.00% FP)	1640.30	153.00	222.20	4.37	6.30
T4 (0.10% FE)	1252.80	111.80	217.60	4.50	6.00
T5 (0.20% FE)	1300.00	134.60	224.40	4.50	5.55
Standard errors of means	73.92	7.28	2.98	0.08	0.14
***p*-value**	0.25	0.15	0.76	0.72	0.56

No significant differences were observed among treatments ($p > 0.05$).

their action on the nervous system.[1,4,9] Increasing hens FI by inclusion FP may result from the action of FP on digestive enzyme secretion.[1] In line with present results, other researchers have reported that inclusion plant secondary metabolites (essential oils and oleoresins) in diet can increase the pancreatic digestive enzymes.[1]

Platel and Srinivasan have shown that fenugreek can increase pancreatic lipase activity in rats.[10] El-Mallah *et al.* have noted that increasing fenugreek seeds to turkey chicks' diet significantly increases nitrogen-free extract (NFE) digestibility.[11] El-Kaiaty *et al.* have reported that inclusion 0.50% fenugreek in laying hens diet has no significant effect on feed consumption compared to the control group.[12] In terms of EP and EM, the results of the current study were in agreement with El-Kaiaty *et al.* findings and in contrast with Abdalla *et al.* results.[12,13] Researchers have indicated that EP, EW, and EM of laying hens fed diets supplemented with fenugreek, cinnamon, fennel, and anise or their mixture are increased significantly.[13] These researchers have indicated that increases in EP, EW, and EM of laying hens may be due to the presence of unknown factors in herbs mixtures which have been considered essential for egg production.[13] Bayatizadeh *et al.* have reported that fenugreek as a good source of dietary protein and lipids can stimulate production parameters and improve FCR of laying hens.[4] Others have reported that FI and water consumption are not affected by addition of fenugreek seed aqueous extract to broiler water, but the weight of breast, thigh, and leg of broiler are significantly increased.[14] These researchers have concluded that the improvement in muscle weight in broiler may be due to antioxidant property of this plant which increases digestive enzymes and decreases bacterial activity.[14] Supplementation layer diet with FP and FE had no significant effect on egg quality except egg yolk color. Egg yolk color is one of the important egg quality characteristics and can affect egg marketing. A significant increase in egg yolk color by FP (T2) may be associated with the presence of carotenoids such as beta carotene in FP which can be deposited in egg yolk.[15] The enhancement of egg yolk color by medical plants has been observed in previous studies.[16, 17] Although FI of laying hens was the highest in T3, but egg yolk color was not affected in this treatment. It has been demonstrated that dietary fibers (particularly soluble fibers) may increase digesta viscosity, disrupt lipid absorption and also impair bile acid reabsorption.[18] In the present study, the fiber content of T3 was the highest compared to T1 and T2 (crud fibers were 3.04, 3.22 and 3.40 respectively in T1, T2, and T3) and this may affect lipid (and its constituents such as carotenoids) digestion and absorption. Although, in the present study hens serum lipid constituents were not affected by different levels of FP. This inconsistency needs to be more investigated. El-Kloub has detected a numerical increase in egg shape index, yolk index and shell thickness when white laying hens were fed diets supplemented with 0.15% fenugreek from 40 to 59 weeks of age, but the differences were not significant, however, a significant decrease in Haugh units was detected.[19] Results of the present study were not in agreement with the findings of El-Kaiaty *et al*. reporting that fenugreek has a significant effect on yolk and albumen weights.[12] In the present study, it was shown that hen's serum constituents were not affected by fenugreek supplementation. Blood biochemical parameters represent the nutritional and physiological status and variation of these parameters usually reflects the health of an animal.[20] The results of this study are in contrast with others reporting a decrease in laying hens serum total cholesterol concentration and an increase in high-density lipoprotein cholesterol concentration duo to fenugreek seed extract.[17] Also, El-Kaiaty *et al.* indicated that fenugreek seeds extract contains steroid saponins and reduce serum cholesterol.[12] The results of this study are in agreement with Weerasingha and Atapattu reporting that serum cholesterol levels are not affected by the dietary fenugreek seed powder in broiler chicken.[21] Previous findings have indicated that fenugreek contains bioactive components such as minerals, vitamins, lecithin and choline that help to dissolve cholesterol and fatty substances.[22] The previous study has indicated that fenugreek seed and its extract can reduce blood glucose levels.[23] The results from this study represented that the inclusion of FP and FE had no effect on antibody titer against SRBCs. In contrast with this results of Bayatizadeh *et al.* indicating that supplementation of FE to laying hens drinking water improves immune response (total antibody, immunoglobulin M and immunoglobulin G) of laying hens.[4]

According to the results of the present study, dietary inclusion of FP and FE had no significant effect on performance parameters of laying hens, although, egg yolk color increased effectively by adding 1.00% of FP to the diet via transferring carotenoids from diet to egg yolk.

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Conflict of interest

The authors declare that there is no conflict of interest.

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