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Effect of Different Levels of Fenugreek Powder Seeds on Performance, Egg Quality Characteristics, Serum and Egg Yolkcholesterolin Laying Hens

Seyed Mohammad Motamedi
Department of Animal Science, Islamic Azad University,
Science and Research branch of Tehran, Iran

Abstract: A study was conducted to evaluate the effects of ground fenugreek seeds given to laying hens at 0 (FS0), 0.5 (FS0.5), 1 (FS1) or 2 (FS2) g/hen/d on laying performance, egg quality characteristics, serum and egg yolk cholesterol concentrations. An experiment was performed as completely randomized layout with four treatments and four replications. One hundred and ninety-two, 49-week-old, (bovans strain) laying hens (48 hens per treatment) were fed for 12 weeks 100 g days^{-1} of a basal diet in addition to the specified ground fenugreek seeds amounts. While FS2 showed highest body weight loss at the end ofthe experiment (112.6 for FS2 vs. 29.7, 27.5and 99.10 g/hen for FS0, FS0.5 and FS1, respectively) there were no differences amongtreatment groups for daily feed intake (98.2, 101.4, 102.4 and 103.1 g/hen for FS0, FS0.5, FS1 and FS2, respectively), hen-day layingrate (81.17% for FS0 to 78.60% for SF2), eggweight (82.04 g for FS1 to 79.82 g for FS0.5), eggmass (49.13 g/hen/d for FS2 to 51.21 g/hen/dfor FS0), feed efficiency (feed intake/eggmass, 1.95 for FS0 to 2.19 for FS2) and egg quality characteristics. Shell weight variedfrom 8.20 g for FS0 to 8.46 g for FS1. Shell thickness varied from 0.97 mm for FS2 to 0.99mm for FS0.5. Yolk weight ranged from 16.8 gfor FS2 to 16.74 G for FS0. Albumen weight ranged from 37.28 g for FS1 to 38.90 g for FS0.5. Inclusion of ground fenugreek seeds reduced blood serum cholesterol from 104.3-84.7, 93.7 and 87.2 mg dL^{-1} for FS0.5, FS1 and FS2, respectively. It did not affect egg yolk cholesterol (21.75 mg g^{-1} for FS2 to 22.88 mg g^{-1} for FS0). It can be concluded that groundfenugreek seeds given to bovanslaying hens at up to 2 g/hen/d had no effect onfeed intake, laying production performance andegg quality but reduced hen's serum.

Key words: Fenugreek seed, laying hens, egg qualitative traits, yolk cholesterol, seeds

INTRODUCTION

Eggs are considered to have high cholesterol content. Due to the known relationships between cholesterol and coronary heart disease, many attempts have been made to lower hen egg cholesterol content. Attempts involved them anipulation of dietary levels of fiber (Menge *et al*., 1974), fat (Aida *et al*., 2005), copper (Pestie and Miller, 1998; Miller, 1994), addition of plant sterols (Clarenburg *et al*., 1971), natural plant products like garlic (Rahardja *et al*., 2010) and fenugreek seeds (Awadein *et al*., 2010; Safaa, 2007). Despite many medicinal properties attributed to fenugreek seed (*Trigonella foenum graecum* L.) (Basu, 2006; Acharya *et al*., 2006, 2008) there isscanty documented literature on its use tolower egg yolk cholesterol. Fenugreek has 45-60% carbohydrate (mostly Galactomannan mucilage), 20-30% protein enriched by Lysine tryptophan, 5-10% lipid, considerable amounts of pyridine-like alkaloid (including 0.2-0.36% Trigonelline, 0.5%), flavonoids, free amino acids (arginine, histidine and lysine and 0.09% hydroxyisoleucine), calciumand iron, 0.6-1.7% saponins, glycosides, cholesterol and citostrol, vitamins (A, B1, C) and 0.015% volatile oils (American Botanical Council, 2000). Fenugreek seeds used at low dietary levels (0.1-2%) reportedly showed egg yolk cholesterol reduction around 7% (Nasra *et al*., 2010; Safaa, 2007). It has been widely used as aspice and herbal remedy for lowering blood glucose and cholesterol levels in experimental animals and human (Basu, 2006; Basu *et al*., 2007). The hypoglycemic effect is thought to be associated with the fiber fraction whereas

the cholesterol lowering effect is attributed to the saponins components (Sidhu and Okenfull, 1986) which were documented to be about 5-6% and of steroidal nature with diosgenin as main sapogenin (Sauvaire *et al*., 1996). This study was conducted with the main objective of investigating the effect of different dietary-amounts of ground fenugreek seeds on laying performance and cholesterol content in egg-yolk of laying hens.

MATERIALS AND METHODS

Experimental design: This study was conducted in the research farm at the Animal Science Research Institute (ASRI) of Iran. One hundred and ninety-two (bovans strain) laying hens aged 49 weeks were divided randomly into four treatment groups with 48 birds each. They were allocated each group to one of the preformulated four dietary treatments viz: control basal diet (FS0), basal diet +0.5 g ground fenugreek seeds, basal diet+1 g ground fenugreek seeds and basal diet+2 g ground fenugreek seeds. These treatments were referred to as FS0, FS0.5, FS1 or FS2, respectively. Each hen was daily fed the set quantity of ground fenugreek seeds blended with 100 g of basal diet. The composition of the ground fenugreek seeds is shown in the footnotes of Table 1. The basal diet or control diet was a commercial feed that contained yellow corn (370 g kg^{-1}), Wheat (300 g kg^{-1}), soybean meal (250 g kg^{-1}), calcium carbonate (30 g kg^{-1}) and a mineral-vitamin mixture (50 g kg^{-1}). Because of the lack of available suited in gredients and to avoid oil incorporation which has been reported to affect egg cholesterol (Aida *et al*., 2005), the four mixtures were neither made isocaloric norisonitrogenus. The hens were housed in individual cages with individual feed trough and common water-trough in a room with ambient temperature of about 20°C and a photo period of 16 h light: 8 h darkness cycle. Water was provided *ad libitum* intake throughout the trial period which comprised an adaptation sub-period (8 days) during which all birds received the basal diet and a test sub-period (84 days) during which birds received their respective experimental diets.

Data collection: All the birds were weighed individually at the start and at the end of the experiment to determine the live weight changes. Feed consumption was measured weekly and feed efficiency (feed intake/(number of eggs×egg weight)) was calculated. Egg production was recorded daily and hen-day laying rate (%) was calculated as the ratio between the number of laid eggs and the number of feeding days. The eggs laid during days 44-45 of experiment were used for analysis of egg qualities (egg weight, shell weight, egg shell thickness, yolk weight and yolk cholesterol). Blood samples (4 mL) were collected at the end of the experiment (day 84) from the brachialwing vein using sterilized syringes and needles and used for serum cholesterol determination after centrifugation at 2000 rpm for 10 min.

Table 1: Ingredients and calculated offered components of experimental diets (g/hen/d)

Ingredients	Treatments*			
Ground fenugreek seed$^{\gamma}$	FS0 (0)	FS0.5 (0.5)	FS1 (1)	FS2 (2)
Yellow corn	37.00	37.00	37.00	37.00
Wheat	30.00	30.00	30.00	30.00
Soybean meal	25.00	25.00	25.00	25.00
Calcium carbonate	3.00	3.00	3.00	3.00
Mineral and vitamin mixture	5.00	5.00	5.00	5.00
Total	100.00	100.50	101.00	102.00
Calculated offered components$^{£}$				
Dry matter	1.22	92.10	95.14	95.67
Organic matter	89.00	90.12	91.16	92.29
Crude proteins	17.11	17.80	18.44	19.00
Ether extract	2.12	2.33	2.52	2.76
NDF	12.20	13.01	14.52	14.91
Saponins	1.02	1.05	1.07	1.12
Cholestero	0.10	0.11	0.11	0.12

*Treatments: 100 g basal diet+0 (FS0), 0.5 (FS0.5), 1 (FS1) or 2g GFS (FS2); γ Ground fenugreek seeds contained per 100 g:dry matter, 89.32 g; organic matter, 96.9 g; crude proteins, 26.97 g; ether extract, 5.28 g; NDF, 37.98 g; saponins, 1.59 g and cholesterol 0.31 g; §Basal diet (FS0) provided following nutrients per 100 g: Ca,4.3 g; P,0.6 g; Na, 0.14 g; Cl,0.23 g; Fe, 4 mg; Zn, 40 mg; Mn, 7 mg; Cu, 0.3 mg; I, 0.08 mg; Se, 0.01 mg; Co,0.02; methionine, 0.39 g; methionine+cysteine, 0.69 g; lysine, 0.89 g; Retinol, 800IU; Cholecalciferol, 220IU; α-tocopherol, 1.1IU; Thiamin, 0.33IU; Nicotinic acid, 909IU.; £ Components calculated using the daily offered amounts of FS0 and ground fenugreek seeds and their chemical analysis

Chemical analysis: Dry Matter of the diets (DM) was determined at 104°C for 24 h while all other analyses were done on samples dried at 65°C and ground in a mill to pass through a 0.5 mm screen. Ash content was determined by igniting the ground sample at 550°C in a muffle furnace for 12 h. The Association of Official Analytical Chemists method (AOAC, 1984) was used for Crude Proteins (CP) determination. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were determined as described by Van Soest *et al*. (1991) but sodium sulphite and alpha amylase were omitted from the NDF procedure. Anenzymatic method (cholesterol enzymatic colorimetric test, CHOD-PAP, Biomaghreb, Tunisia) was used for cholesterol in serum and in egg yolk solubilized in 2% (w/v) NaCl solution (Pasin *et al*., 1998). Cholesterol in feed was determined by a spectro photometric method employing ferric chloride in glacialacetic acid on lipid extract (Osman and Chin, 2006). Saponins content was measured with the vanillin-perchloric acid colorimetric method as described

by Wang *et al*. (2007) with some modifications. A volume of 0.1 or 0.075 mL of the diluted methanol crude extract of 0.2 g defatted samples was dried at 70°C in a water bath after which 0.1 mL of 5% (w/v) vanillinglacialacetic acid solution and 0.4 mL perchloric acid were added. The tube containing the mixture was vortex stirred to ensure complete distribution, plagued with glass marble and transferred to a water bath at 70°C for 15 min. It was removed there after and placed in ice-waterto cool. Following this, 2.5 mL glacialacetic acid was added to each tube. The solution was mixed well and the absorbance measured against a freshly prepared blank reagent at 540 nm. A standard calibration plot was generated using known concentrations of saponins (cat No: 8047-15-2, Riedel de Haen).

Statistical analysis: In order to adjust experimental diets, standard tables of feed requirements for laying hens were used and to compute chemical compounds and energy of the diets' feed also standard tables were used (NRC, 1994). In order to adjust the diets, UFFDA Software was used (Peste and Miller, 1994). The experiment was a completely randomized design with each hen used or egg tested as the experimental unit. Collected data were subjected to analysis of variance using the GLM procedure of SAS software (2002). Orthogonal contrasts and multiple comparison tests were performed among treatments when variables were significant.

RESULTS AND DISCUSSION

Laying performance: The effect of ground fenugreek seeds in corporation in the hens' diet on feed consumption, body weight change, egg production rate, egg weight and feed conversion ratio is shown in Table 2. Although, each hen was given 100 g of the control diet per day and either 0 (FS0), 0.5 (FS0.5), 1 (FS1) or 2 g (FS2) of ground fenugreek seeds, the actual mean in takes were only 98.25, 101.4, 102.4 and 103.1 g/hen/d, respectively (Table 2). The corresponding feed refusals were small and were not different ($p>0.05$).

With respect to feed consumption, Nasra *et al*. (2010) reported that while local Mandarah strain hens fed diets supplemented with 0.5% ground fenugreek seeds during their 16-28 weeks of age period had their feed consumption significantly decreased after 8 weeks and increased after 12 weeks of treatment those on 0.1% ground fenugreek seeds had their feed consumption increased after these both periods. The average feed consumption by hens on 0.5% ground fenugreek seeds diet was significantly reduced and that by hens on 0.1% ground fenugreek seeds increased. Abaza (2007) reported

Table 2: Dietary effects of ground fenugreek seed on the laying hen performances

Treatments*	Statistics					
Performances	FS0	FS0.5	FS1	FS2	SEMγ	p-values
Feed intake (g/d)	98.25^{a}	101.04^{b}	102.04^{c}	103.01^{d}	0.17	0.0001
Body weight change (g)	-29.07^{a}	-27.05^{a}	-99.10^{ab}	-112.06^{b}	22.05	0.0290
Hen-day laying rate (%)	81.17	79.82	82.04	78.60	3.01	0.6800
Egg weight (g)	62.19	64.00	60.70	61.12	1.21	0.7500
Egg mass (g/hen)	51.21	51.01	50.11	49.13	1.41	0.5500
Feed efficiency	1.95	2.05	2.07	2.19	0.08	0.0700

*Treatments: 100 g basal diet+0 (FS0), 0.5 (FS0.5), 1 (FS1) or 2 g ground fenugreek seeds (FS2); a-c: Mean values in the same row having different superscripts are significantly different ($p<0.05$); γ SEM = Standard Error of the Mean

similar results for decreasing feed consumption when laying hens of the same breed at age of 32 weeks were fed 0.5% fenugreek. El-Kloub (2006) indicated that fenugreek at levels 0.05, 0.1 and 0.15% did not affect feed consumption by Hy-Line White laying hens during 40-59 weeks of age. Abdalla *et al*. (2011) indicated that there was no effect of supplemented fenugreek at 1% level on feed consumption for Gimmizah laying hens. In the present study because of there striction on feed distribution, the hens showed slight loss of body weight throughout the 49 d-experimental period. Those on FS2 showed the greatest body weight loss: 112.6 vs. 29.7 to 99.10 g/hen. These results did not agree with the findings by Awadein *et al*. (2010) and Moustafa (2006) who found significant increase in live body weight gain due to 0.1 or 0.5 and 0.05% fenugreek seed levels, respectively. This difference in results may due to the differences in the amounts of feed offered and in hens' strains and ages. Despite the loss of their body weight, all hens irrespective of their ground fenugreek seeds treatment, maintained their weekly egg production rate unchanged throughout the 8 week experimental period. The overall mean egg production rate was not affected by ground fenugreek seeds levels ($p>0.07$) and ranged from 78.6 (FS2) to 81.17% (FS0). Such results may suggest that egg production was prior to body weight maintenance. Again, ground fenugreek seeds at the rate of 2 g/hen/d tended to reduce egg production even though it was associated with the highest feed in take. Ground fenugreek seeds showed also no significant effect on egg weight and egg mass which tended to be the lowest for FS2. Again, these results did not agree with those reported by Awadein *et al*. (2010) and El-Kloub (2006). The former reported increased egg production rate and egg mass for hens on 0.1 or 0.5% ground fenugreek seeds and decreased egg weight for hens on 0.5% ground fenugreek seeds. The latter found significant increase in egg production rate and egg mass with 0.05 or 0.15% fenugreek seed levels while egg weight was increased only by the 0.05% fenugreek

inclusion level. The results found here in showed that while feed conversion ratio (feed intake/egg mass) was not affected (p>0.07) by ground fenugreek seeds inclusion, it was the highest for FS2: 2.19 vs 2.07, 2.05 and 1.95 for FS0, SF0.5 and FS1, respectively. Such results would suggest that fenugreek seeds at high levels may exercise an adverse effect by mechanisms that cannot yet be explained with certitude. However, it is tempting to relate this to the saponins components even thought calculated saponins intakes were almost the same (1.01-1.09 g/hen/d). In this regard, fenugreek crude seed saponins administrated for 21-7 days old chicks at levels of 10 or 50 mg kg^{-1} BW or in drinking water at 500 mg kg^{-1} BW depressed body weights and caused some pathological changes among which liverfatty cytoplasmic vacuolation and necrosis of hepatocytes, epithelial degeneration of renal tubules and varying degrees of hemorrh age in the thigh and breast (Nakhla *et al*. 1991). Some of other adverse effects a scribed to several properties of saponins are: Reduction in intestinal motility (Klita *et al*., 1996), damage to the intestinal membrane, inhibition of nutrient transport and production of active metabolites (Wakabayashi *et al*., 1998). In contrast to such finding, Rao *et al*. (1996) found that there were no significant hematological, hepatic or histopathological changes in wean ling rats fed fenugreek seeds for 90 days.

Egg quality characteristics: Effects of ground fenugreek seeds on egg quality characteristics are presented in Table 3. These data show that there was no significant effect on yolk weigh, albumen weight and on shell thickness and weight. Results reported herein were in agreement with those reported by Abdalla *et al*. (2011) but not with those by Awadein *et al*. (2010) who found that the yolk percent was decreased significantly by 0.5% fenugreek compared to control treatment. In contrast, El-Kaiaty *et al*. (2002) indicated that fenugreek had a significant increase in yolk and albumen weights. Abaza (2007) indicated that hens fed diet supplemented with fenugreek had their egg shell thicker and albumen heavier than non supplemented hens.

Serum and egg yolk cholesterol: The results of serum and egg yolk cholesterol are shown in Table 4. There was a decrease in serum cholesterol due to added ground fenugreek seeds but there was no dose-response effect. Serum cholesterol concentrations were reduced from 107.3 for FS0-84.73, 93.75 and 87.20 mg dL^{-1} for FS0.5, FS1 and FS2, respectively. This serum cholesterol concentrations reduction which varied independently of added ground fenugreek seeds from 12.8-19.3%, occurred even thought calculated cholesterol intakes were 0.10 g/hen/d for FS0 and 0.109-0.118 g/hen/d for ground fenugreek seeds treatments. Since calculated daily saponins intake in the control diet (FS0, 1.02 g/hen/d) was slightly lower than those in ground fenugreek seeds containing diets (1.03-1.09 g/hen/d), the positive effect of ground fenugreek seeds on serum cholesterol concentrations would be due the particular composition of the saponins in fenugreek seed or to an unknown synergetic effect of saponins and other bioactive compounds in fenugreek seed. Saponins in the control treatment (FS0) would be provided in the soybean meal fraction. A decrease in serum cholesterol due to fenugreek seed was also report by Abdallal *et al*. (2011) in laying hens and Abaza (2007) in 32 weeks-old laying hens of local strain (Matrouh) oncorn-soybean meal diet containing 0.5% fenugreek seed powder. In the latter study, serum cholesterol concentration was reduced from 143.4-122.5 mg 100 mL^{-1}. Also, El-Kaiaty *et al*. (2002) found that afenu greek seeds extract containing steroidsaponins induced hypocholesterolaemia. Such an effect may result from an increased conversion of hepatic cholesterol to bile salts and loss in the feces of complexes of these substances with fenugreek fiber and saponins.

In the present study, contrary to the effect of ground fenugreek seeds on hens serum cholesterol concentrations, there were no reduction in yolk cholesterol contents nor on the amounts of cholesterol excreted daily in eggs as a

Table 3: Dietary effects of ground fenugreek seed on egg quality characteristics

Treatments*	Statistics					
Egg quality characteristics	FS0	FS0.5	FS1	FS2	SEMγ	p-value
Shell weight (g)	8.20	8.41	8.46	8.36	0.22	0.81
Shell thickness (mm)	0.98	0.99	0.98	0.97	0.05	0.75
Yolk weight (g)	16.74	17.01	17.22	16.80	0.35	0.53
Albumen weight (g)	37.11	37.90	37.28	37.39	1.12	0.85

*Treatments: 100 g basal diet+0 (FS0), 0.5 (FS0.5), 1 (FS1) or 2 g ground fenugreek seeds (FS2); a-c: Mean values in the same row having different superscripts are significantly different (p<0.05); γ SEM = Standard Error of the Mean

Table 4: Dietary effects of ground fenugreek seed on serum and egg yolk cholesterol

Treatments*	Statistics					
Egg yolk cholesterol	FS0	FS0.5	FS1	FS2	SEMγ	p-value
Serum cholesterol (mg dL^{-1})	107.38^{a}	84.73^{b}	93.75^{b}	87.20^{b}	4.01	0.0008
Yolk cholesterol (mg g^{-1})	22.50	22.36	21.74	21.75	0.60	0.1900
Cholesterol excretion (mg/hen/d)	318.61	311.51	312.02	275.23	15.50	0.3100
Cholesterol per egg (mg)	391.68	367.50	371.75	349.06	16.01	0.4900

*Treatments: 100 g basal diet+0 (FS0), 0.5 (FS0.5), 1 (FS1) or 2 g ground fenugreek seeds (FS2); a-c: Mean values in the same row having different superscripts are significantly different (p<0.05); γ SEM = Standard Error of the Mean

result of ground fenugreek seeds inclusion. These ranged from 22.50 (FS0) to 21.75 mg g^{-1} yolk (FS2) and 318.61 (FS0) to 275.2 mg/hen/d, respectively. The lack of effect of ground fenugreek seeds on yolk cholesterol did not agree with the results by Awadein *et al*. (2010) who reported a small but significant reduction in egg yolk cholesterol by feeding hens of local Mandarah strain diets containing 0.1 or 0.5% ground fenugreek seeds. Also, Safaa (2007) found that fenugreek at 2% level fed to 35-wk old Lohmann Brown laying hens reduced egg yolk cholesterol c oncentrations from 18.5-17.2 mg g^{-1} egg yolk. Moustafa (2006) observed a reduction in yolk total cholesterol concentration when Hy-Line White laying hens fed diets supplemented with 0.05, 0.1 or 0.15% fenugreek from 40-59 weeks of age. Consequently, discrepancies observed between the results and previous reports would be partially due to differences in the genotypes of fenugreek seed which would influence their saponins and other bio active compounds contents and to differences in hens' strains and ages. In regard to fenugreek seed genotype, Ciftic *et al*. (2011) found in nine fenugreek seed genotypes that contents of lipids ranged from 5.8-15.2%, total sterol varied from 14.203-18.833 mg kg^{-1} of lipids and cholesterol ranged from 270-1.281 mg kg^{-1} lipid. These researchers suggested that the amounts of cholesterol in fenugreek seeds were low and their absorption would be minimized by the excessive amounts of phytosterols. Taylor *et al*. (2002) reported levels of diosgenin in 10 fenugreek seed successions ranging from 0.24-0.92% depending on the accession and the year and location of cultivation. Saponins levels in fenugreek seed as high as 6% have been reported (Sauvaire *et al*., 1996). In the study, ground fenugreek seeds saponins and cholesterol contents were 1.59 and 0.31%, respectively. In the above men shined reports in which fenugreek seed reduced egg cholesterol, there were no data available on saponins and cholesterol in fenugreek seed nor in the diets. As to hens' strain effect, comparing the effects feeding three types of cereal grain and soyabean oil on the production, yolk cholesterol and yolk fatty acid concentrations of three strains of laying pullets, Shafey *et al*. (1992) found differences between strains of pullets in weight gain, food consumption, rate of lay, egg weight and yolk cholesterol. Thus, some hens genotypes seem particularly resistant to having their yolk cholesterol level changed.

CONCLUSION

Considering the data obtained here in, it can be concluded that ground fenugreek seed given to (bovans strain) laying hens at upto 2 g/hen/d had no effect on laying performance and egg quality but reduced hen's serum cholesterol. Further studies may be needed to sort out the differences observed among research groups. Some of them may compare various fenugreek seed genotypes and processing treatments.

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