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Effect of feeding hens with fenugreek seeds on Laying performance, egg quality characteristics, serum and egg yolk cholesterol

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Summary - A study was conducted to evaluate the effects of ground fenugreek seeds given to laying hens at 0 (FS0), 2 (FS2), 4 (FS4) or 6 (FS6) g/hen/d on laying performance, egg quality characteristics, serum and egg yolk cholesterol concentrations. Forty, 52-week-old, *Lohmann White* laying hens (10 hens per treatment) were fed for 7 weeks 100 g/d of a basal diet in addition to the specified ground fenugreek seeds amounts. While FS6 showed highest body weight loss at the end of the experiment (115.3 for FS6 *vs* 31.8, 28.9 and 97.0 g/hen for FS0, FS2 and FS4, respectively), there were no differences among treatment groups for daily feed intake (99.3, 101.2, 103.2 and 105.2 g/hen for FS0, FS2, FS4 and FS6, respectively), hen-day laying rate (83.7% for FS0 to 79.4% for SF6), egg weight (61.7 g for FS4 to 63.1 g for FS2), egg mass (48.6 g /hen/d for FS6 to 51.8 g /hen/d for FS0), feed efficiency (feed intake/egg mass, 1.93 for FS0 to 2.17 for FS6) and egg quality characteristics. Shell weight varied from 8.15 g for FS0 to 8.5 g for FS2. Shell thickness varied from 0.97 mm for FS6 to 0.99 mm for FS2. Yolk weight ranged from 16.5 g for FS6 to 16.7 G for FS0. Albumen weight ranged from 37.2 g for FS4 to 38.1 g for FS2. Inclusion of ground fenugreek seeds reduced blood serum cholesterol from 106.4 mg/dl to 85.8, 92.7 and 86.2 mg/dl for FS2, FS4 and FS6, respectively. It did not affect egg yolk cholesterol (21.4 mg/g for FS6 to 22.9 mg/g for FS0). It can be concluded that ground fenugreek seeds given to *Lohmann White* laying hens at up to 6 g/hen/d had no effect on feed intake, laying production performance and egg quality but reduced hen's serum cholesterol.

Keys words : Fenugreek seed, hens, egg, cholesterol

1. 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 the manipulation of dietary levels of fiber (Menge et al.1974), fat (Aida et al. 2005), copper (Pesti and Bakalli, 1998), addition of plant sterols (Clarenburg et al. 1971), natural plant products like garlic (Rahardja et al. 2010) and fenugreek seeds (Nasra et al. 2010; Safaa 2007) .

Despite many medicinal properties attributed to fenugreek seed *(Trigonella foenum graecum L)* (Basu 2006; Acharya et al. 2006; Acharya et al. 2008), there is scanty documented literature on its use to lower egg yolk cholesterol. Fenugreek seeds used at low dietary levels (0.1 to 2%) reportedly showed egg yolk cholesterol reduction around 7% (Nasra et al. 2010; Safaa 2007). It has been widely used as a spice 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.

2. Materials and Methods

2.1. Experimental design

Forty *Lohmann White* laying hens aged 52 weeks were divided randomly into four treatment groups with 10 birds each. They were allocated each group to one of the preformulated four dietary treatments *viz*: control basal diet (FS0), basal diet +2 g ground fenugreek seeds, basal diet + 4 g ground fenugreek seeds and basal diet + 6 g ground fenugreek seeds. These treatments were referred to as FS0, FS2, FS4 or FS6, 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 (630g/kg), soybean meal (250g/kg), calcium carbonate (80g/kg) and a mineral-vitamin mixture (40g/kg). Because of the lack of available suited ingredients and to avoid oil incorporation which has been reported to affect egg cholesterol (Aida et al. 2005), the four mixtures were neither made isocaloric nor isonitrogenus. 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 photoperiod of 16 h light: 8h darkness cycle. Water was provided *ad libitum* intake throughout the trial period which comprised an adaptation sub-period (8days) during which all birds received the basal diet and a test sub-period (49 days) during which birds received their respective experimental diets.

Table1. Ingredients and calculated offered components of experimental diets (g/hen/d)

Ingredients	Treatments*			
	FS0	FS2	FS4	FS6
Ground fenugreek seed[γ]	0	2	4	6
Yellow corn	63	63	63	63
Soybean meal	25	25	25	25
Calcium carbonate	8	8	8	8
Mineral and vitamin mixture[§]	4	4	4	4
Total	100	102	104	106
Calculated offered components [£]				
Dry matter	90.21	92.00	93.78	95.57
Organic matter	87.08	89.02	90.96	92.89
Crude proteins	17.42	17.96	18.50	19.04
Ether extract	2.52	2.63	2.73	2.84
NDF	12.70	13.46	14.22	14.98
Saponins	1.01	1.04	1.07	1.11
Cholesterol	0.10	0.11	0.11	0.12

*treatments: 100 g basal diet+0 (FS0), 2 (FS2), 4 (FS4) or 6g GFS (FS6); [γ] Ground fenugreek seeds contained per 100g:dry matter, 89.32g; organic matter, 96.9g; crude proteins, 26.97g; ether extract, 5.28g; NDF, 37.98g; saponins, 1.59 g, and cholesterol 0.31g; [§]Basal diet (FS0) provided following nutrients per 100g: Ca,4.3g ; P,0.6g; Na, 0.14g; Cl,0.23g; Fe, 4 mg; Zn, 40mg; Mn,7 mg; Cu, 0.3 mg; I, 0.08 mg; Se, 0.01 mg; Co,0.02; methionine,0.39g; methionine+cysteine,0.69g; lysine,0.89g; 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.

2.2. 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 x 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 to 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 49) from the brachial wing vein using sterilized syringes and needles and used for serum cholesterol determination after centrifugation at 2000 rpm for 10 min.

2.3. 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 0.5mm 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. An enzymatic 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 spectrophotometric method employing ferric chloride in glacial acetic 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) vanillin-glacial acetic 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 thereafter and placed in ice-water to cool. Following this, 2.5 ml glacial acetic 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 №: 8047-15-2, Riedel de Haen).

2.4. Statistical analysis

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 (1989). Orthogonal contrasts and multiple comparison tests were performed among treatments when variables were significant.

3. Results and discussions

3.1. Laying Performance

The effect of ground fenugreek seeds incorporation 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), 2(FS2), 4(FS4) or 6 g (FS3) of ground fenugreek seeds, the actual mean intakes were only 99.34, 101.20, 103.25 and 105.25 g/hen/d, respectively (table 2). The corresponding feed refusals were small and were not different (P>0.05) (data not shown).

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

	Treatments*				Statistics	
	FS0	FS2	FS4	FS6	SEM ᵞ	P-value
Feed intake (g/d)	99.34^{a}	101.20^{b}	103.25^{c}	105.25^{d}	0.19	0.0001
Body weight change (g)	-31.8^{a}	-28.9^{a}	-97.0^{ab}	-115.3^{b}	24.41	0.031
Hen-day laying rate (%)	83.67	80.82	81.84	79.39	2.94	0.77
Egg weight (g)	62.23	63.10	61.70	61.42	1.29	0.89
Egg mass (g/hen/d)	51.85	50.59	50.31	48.63	1.40	0.45
Feed efficiency	1.93	2.01	2.06	2.17	0.09	0.07

*treatments: 100 g basal diet+0 (FS0), 2 (FS2), 4 (FS4) or 6g ground fenugreek seeds(FS6); a-c: Mean values in the same row having different superscripts are significantly different (P <0.05); ᵞ SEM=standard error of the mean

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 similar results for decreasing feed consumption when laying hens of the same breed at age of 32 weeks were fed 0.5% fenugreek. Moustafa (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 the restriction on feed distribution, the hens showed slight loss of body weight throughout the 49d-experimental period. Those on FS6 showed the greatest body weight loss: 115.3 vs 0.31 to 0.97g/ hen. These results did not agree with the findings of Nasra 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 7 wk-experimental period. The overall mean egg production rate was not affected by ground fenugreek seeds levels (P>0.07) and ranged from 79.4 (FS6) to 83.7% (FS0). Such results may suggest that egg production was prior to body weight maintenance. Again, ground fenugreek seeds at the rate of 6 g/hen/d tended to reduce egg production even though it was associated with the highest feed intake. Ground fenugreek seeds showed also no significant effect on egg weight and egg mass which tended to be the lowest for FS6**.** Again, these results did not agree with those reported by Nasra et al. (2010) and Moustafa (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 herein 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 FS6: 2.17 vs 1.93, 2.01 and 2.06 for FS0, SF2, and FS4, 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 to 1.09 g/hen/d). In this regard, fenugreek crude seed saponins administrated for 21 days to 7-d-old chicks at levels of 10 or 50 mg / kg BW or in drinking water at 500 mg /kg BW depressed body weights and caused some pathological changes among which liver fatty cytoplasmic vacuolation and necrosis of hepatocytes, epithelial degeneration of renal tubules, and varying degrees of hemorrhage in the thigh and breast (Nakhla et al. 1991). Some of other adverse effects ascribed 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 weanling rats fed fenugreek seeds for 90 days.

3.2. 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 of Nasra 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.

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

	Treatments*				Statistics	
	FS0	FS2	FS4	FS6	EM$^{\gamma}$	P-value
Shell weight (g)	8.15	8.49	8.44	8.39	25	0.78
Shell thickness (mm)	0.98	0.99	0.99	0.97	0.02	0.79
Yolk weight g)	16.73	16.96	17.26	16.50	0.45	0.68
Albumen weight (g)	37.21	38.09	37.18	37.49	1.11	0.92

*treatments: 100 g basal diet+0 (FS0), 2 (FS2), 4 (FS4) or 6g ground fenugreek seeds (FS6); $^{\gamma}$ SEM=standard error of the mean

3.3. 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 106.4 for FS0 to 85.8, 92.7, and 86.2 mg/dl for FS2, FS4, and FS6, respectively. This serum cholesterol concentrations reduction, which varied independently of added ground fenugreek seeds from 12.8 to 19.3%, occurred even thought calculated cholesterol intakes were 0.10 g/hen/d for FS0 and 0.109 to 0.118 g/hen/d for ground fenugreek seeds treatments. Since calculated daily saponins intake in the control diet (FS0, 1.01g/hen/d) was slightly lower than those in ground fenugreek seeds containing diets (1.03 to 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*) on corn-soybean meal diet containing 0.5 % fenugreek seed powder. In the latter study, serum cholesterol concentration was reduced from 143.4 to 122.5 mg/100ml. Also, El-Kaiaty et al. (2002) found that a fenugreek seeds extract containing steroid saponins 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.

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

	Treatments*				Statistics	
	FS0	FS2	FS4	FS6	SEM γ.	P-value
Serum cholesterol (mg/dl)	106.38[a]	85.83[b]	92.75[b]	86.20[b]	3.44	0.0008
Yolk cholesterol (mg/g)	22.88	22.20	21.63	21.45	0.50	0.18
Cholesterol excretion (mg/hen/d)	320.71	302.76	304.62	280.15	13.6	0.22
Cholesterol per egg (mg)	384.75	376.60	373.75	353.26	14.21	0.45

*treatments: 100 g basal diet+0 (FS0), 2 (FS2), 4 (FS4) or 6g ground fenugreek seeds (FS6); a, b:Mean values in the same row having different superscripts are significantly different (P <0.05).; γ SEM=standard error of the mean

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 result of ground fenugreek seeds inclusion. These ranged from 22.88 (FS0) to 21.45 mg/g yolk (FS6) and 320.7 (FS0) to 280.1 mg/hen/d, respectively. The lack of effect of ground fenugreek seeds on yolk cholesterol did not agree with the results of Nasra 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 concentrations from 18.5 to 17.2 mg/g 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 to 59 weeks of age. Consequently, discrepancies observed between our results and previous reports would be partially due to differences in the genotypes of fenugreek seed which would influence their saponins and other bioactive compounds contents, and to differences in hens' strains and ages. In regard to fenugreek seed genotype, Ozan et al. (2011) found in nine fenugreek seed genotypes that contents of lipids ranged from 5.8 to 15.2%, total sterol varied from 14,203 to 18,833 mg/kg of lipids and cholesterol ranged from 270 to 1,281 mg/kg lipid. These authors 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% to 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 our

study, ground fenugreek seeds saponins and cholesterol contents were 1.59 and 0.31%, respectively. In the above menshined 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.

4. Conclusion

Considering the data obtained herein, it can be concluded that ground fenugreek seed given to *Lohmann White* laying hens at up to 6 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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