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Effect of whole fenugreek seed before and after its maceration in water on hens'laying performance and egg cholesterol profile.

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Abstract - Given the hypocholesterolemic property of fenugreek seed, the purpose of this study was to evaluate the effect of whole fenugreek seeds (WFS) and its water and hexane insoluble fraction (WHIFWFS) given to layers on laying performance and egg quality. Thirty, 69-week-old, *Lohmann White* laying hens (10 hens/treatment) were fed for 41 days 100 g/d of a basal diet (control), basal diet +1.75 g WHIFWFS and basal diet + 2 g WFS. Hen-day laying rate was not affected (P>0.05) by fenugreek addition. Mean weight of eggs laid throughout the whole experimental period was higher (P<0.05) for WHIFWFS (64.79 g) than for the control (62.23 g) and WFS (62.41 g). However, this difference was without consequence on mean egg mass and feed efficiency which were not affected (P> 0.05) by fenugreek addition. Physical characteristics of eggs laid on days 39-41, except shell weight which was the highest (P<0.05) for WHIFWFS, were not affected (P>0.05) by fenugreek addition. Fenugreek supplementation did not affect (P>0.05) triglyceride, total cholesterol and HDL cholesterol per g yolk and per egg. It was concluded that whole fenugreek seed given to *Lohmann White* laying hens had no effect on their laying performance and egg physical quality traits and cholesterol profile.

Key words: Fenugreek seed / egg cholesterol / laying hens

1. Introduction

Research carried out on use of fenugreek seeds (*Trigonella foenum graecum*) to lower hens' egg yolk cholesterol has been very scarce and led to inconsistent results. In some studies (Nasra et al. 2010; Safaa 2007; Moustafa 2006), a cholesterol-lowering effect was observed for fenugreek seeds at levels as low as 0.05 to 2% of the diet. In another study (Abdouli et al. 2014), no such an effect was found for daily consumption of 2 to 6 g of ground fenugreek seeds. Although the mechanism of the positive action is still unclear, it could to be attributed mainly to the saponins component. As a matter of fact, it has been found that fenugreek seeds contained variable quantities of saponins *viz:* 1.59 % (Abdouli et al. 2014) to 5-6% (Sauvaire et al. 1996) and that dietary karaya saponin reduced egg yolk cholesterol (Afrose et al. 2010). In such case, saponins could complex with dietary cholesterol rendering it unavailable for absorption, reduce hepatic cholesterol synthesis and, consequently, reduce egg yolk cholesterol. However, it is worth noting that not all saponins can lower egg yolk cholesterol. This has been the case of sarsaponin which failed to lower the serum cholesterol of laying hen and that of the egg yolk even though it increased the excretion of cholesterol and decreased the transfer of dietary cholesterol to the eggs (Sim et al. 1984). Thus, the present study was conducted to evaluate the responsiveness of laying hens to a daily dose of 2 g whole fenugreek seed and of the water and hexane insoluble fraction in 2 g whole fenugreek seed. Such approach allowed testing directly the effect of whole fenugreek seed and indirectly of its water and hexane soluble matter which was assumed to rich in saponins. Fenugreek seed was not ground to slow its transit in the digestive tract and eventually allow better release of its water soluble bioactive constituents.

2. Material and methods

2.1. Water insoluble fraction of whole fenugreek seed preparation

Fresh fenugreek seeds were purchased from a regional producer located at Mateur, north of Tunisia, and carefully cleaned from foreign matter. A 1.5 kg sample was divided in equal amounts into two nylon bags of 42 µm pore size. Each bag was soaked 3 times for 4hrs and once for 12 hrs in 4L of distilled water and then squeezed to filter out the soluble matter. The obtained water insoluble material was then soaked in 2 L of hexane for 24 hrs, filtered, partially dried at 60°C, weighted and kept in a caped polypropylene container during the experimental period. The obtained partially died water and hexane insoluble fraction of whole fenugreek seeds (WHIFWFS) accounted for 87.51 % of the whole fenugreek seed (WFS). On dry matter basis, the maceration yield was of 92.96%.

2.2. Experimental design

Thirty *Lohmann White* laying hens aged 69 weeks were divided randomly into three treatment groups with 10 birds each. They were allocated each group to one of three dietary treatments *viz*: basal diet (control), basal diet +1.75 g water and hexane insoluble fraction of whole fenugreek seed (WHIFWFS) and basal diet + 2 g whole fenugreek seeds (WFS). The two experimental diets provided the same mounts of fenugreek dry water insoluble matter (1.62 g/d). Each hen was daily fed the set quantity of fenugreek seeds blended with 100 g of basal diet. The compositions of the diets are shown in Table 1. 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 lasted 41 days.

2.3. 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 was offered once daily at 7:30 am and refusal was measured on days 5, 12, 19, 26, 33 and 41 of the experiment. Egg production and weight were recorded daily. Daily feed consumption, hen-day laying rate (number of laid eggs x100/ number of feeding days) , and feed efficiency (feed consumption / (number of eggs x egg weight)) were calculated per period (P1-P6) corresponding to days1-5, 6-12, 13-19, 20-26, 27-33 and 34-41. The eggs laid during days 39 to 41 of the experiment were used for analysis of egg qualities (egg weight, shell weight, egg shell thickness, yolk weight and yolk cholesterol and triglycerides).

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

	Treatments		
	Control (Basal diet)[£]	**WHIFWFS**[γ]	**WFS**[€]
WFS[€]	0	0	2
WHIFWFS[γ]	0	1.75	0
Yellow corn	64	64	64
Soybean meal	24	24	24
Calcium carbonate	8	8	8
Mineral and vitamin mixture[§]	4	4	4
Total	100	101.75	102
Calculated offered components			
Dry matter	88,47	90,10	90,22
Organic matter	80,5	82,07	82,18
Crude proteins	15,2	15,67	15,66
Ether extract	5,09	5,17	5,20
NDF	9,95	10,67	10,65
Cell contents	90,05	90,95	91,10

[£] Chemical composition of basal diet (% dry matter): organic matter, 80.0; crude proteins,15.20; fat, 5.09; neutral detergent fiber, 9.95; [γ]WHIFWFS=water & hexane insoluble fraction of whole fenugreek seed; [€]WFS= whole fenugreek seed; [§] Mineral and vitamin mixture composition (/ kg): Ca,335g ; P,37g; Na, 34g; Mg, 4g; Fe, 2.14g; Zn, 1.53g; Mn,1.8g; Cu, 0.15g; I, 0.03g; Se, 0.005g; Co,0.006g; Vit A, 180000IU; Vit D, 49500IU; Vit E, 165mg.

2.4. 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. Kits from Biomaghreb in Tunisia (cholesterol enzymatic colorimetric CHOD-PAP; Cholesterol-HDL and Triglycerides enzymatic colorimetric GPO-PAP) were used for total cholesterol, HDL-cholesterol and triglycerides in 2 to 3-pooled egg yolk per hen solubilized in 2% (w/v) NaCl solution (Pasin et al. 1998). Total fat was determined by extraction with diethyl ether for 6 hrs in a soxhlet extractor. Phytochemical screening tests were carried out as described by Jansi Rani et al. (2013) on samples (3.3 g) of ground WHIFWFS and WFS extracted for 6 hrs in 250 ml of methanol or hexane using a soxhlet extractor and in hot water (60°C).

2.5. Statistical analysis

Collected data were subjected to analysis of variance using the GLM procedure of SAS (1989). Daily feed consumption, hen-daily laying rate, egg weight and mass and feed efficiency data were first tested for the diet, period and diet x period effects. Period and treatment x period affects were found to be insignificant (α=0.05) and, therefore, only treatment (diets) effect was retained and all data were subjected to a one-way analysis of variance. Means were compared by orthogonal contrast.

3. Results and discussion

3.1. Effect of water and hexane maceration on Fenugreek seed chemical composition

The chemical compositions of the whole fenugreek seed before and after its maceration in water and hexane are shown in Table 2. Such data suggested that the solubilized matter, which accounted for 7.04% of fenugreek DM, was made up mostly of cytoplasmic constituents (cell content) and of little fat. Thus, there was an increase of NDF and CP levels and a low decrease of fat level.

Although the effect of water and hexane maceration of fenugreek seed on its secondary metabolites was not quantified, the presence of terpenoids, alkaloids, coumarins, gum and mucilage, tannins, flavoinoids and saponins and the absence of starch and reducing sugars were revealed by phytochemical screening of distilled water, hexane and methanol extracts of both WFS and WHIFWFS (Table 3).

Table 2. Chemical compositions of whole fenugreek seed and of its water and hexane insoluble fraction (in %DM except DM; mean ± standard deviation)

Treatments	DM	OM	CP	FAT	NDF	ADF	Cell ntent
WFS[€]	87.46± 0.14	95.91± 0.03	26.38± 0.05	6.07± 0.17	39.88± 2.30	16.14± 0.34	60.12± 2.30
WHIFWFS[γ]	92.91± 0.14	96.65± 0.06	29.03± 0.07	5.01 0.08	44.44± 0.07	16.70± 0.15	55.56± 0.07

[γ] WHIFWFS= water & hexane insoluble fraction of whole fenugreek seed; [£] WFS= whole fenugreek seed; DM=dry matter, OM=organic matter, CP=crude proteins, NDF=neutral detergent fiber, ADF=acid detergent fiber, cell content=100-NDF

In this regard, Yadav et al. (2011) found that hot or cold extraction of powdered fenugreek seed in water was more effective than in organic solvents. They reported the presence of alkaloids, flavonoids, amino acid, tannins, protein, starch, mucilage and saponins in the extracts. Crude extracts of fenugreek with different solvents such as methanol, ethanol, acetone and hexane contained high yields of phenolic compounds and showed high antioxidant activity (Bukhari et al. 2008). It was, then, concluded that WHIFWFS contained much less secondary metabolites than WFS. Therefore, since both were offered to supply the same amounts of primary nutrients (NDF, CP, fat, Cell content; Table 1), any difference in hens laying production and egg traits data was ought to be due to difference in water and hexane soluble constituents of the macerated et non macerated fenugreek seed.

Table 3. Phytochemical screening of hexane, methanol and water extracts of fenugreek seed before and after maceration in water and hexane

	WFS[€]			WHIFWFS[γ]		
Solvents	**Hexane**	**Methanol**	**Water**	**Hexane**	**Methanol**	**Water**
Terpenoids	+	+	-	+	+	-
Alkaloïds	-	+	+	-	+	+
Coumarins	-	+	+	-	+	+
Gum and mucilage	-	-	+	-	-	+
Flavonoids	nd	+	nd	nd	+	nd
Tanins	-	+	+	-	+	+
Saponins	-	+	+	-	+	+
Starch	-	-	-	-	-	-
Reducing sugars	-	-	-	-	-	-

[£] WFS= whole fenugreek seed ; [γ] WHIFWFS= water & hexane insoluble fraction of whole fenugreek seed;
+= presence; -=absence, nd=not determined

3.2. Laying Performance

The effect of fenugreek seed incorporation in the hens' diet on feed consumption, body weight change, hen-day laying rate, egg weight, egg mass and feed efficiency is shown in Table 4. To ensure full fenugreek consumption, each hen was given 100 g of the control diet per day mixed with its corresponding amount and type of fenugreek seed. Therefore, feed refusals were small ranging from 0.6 to 1.3 g DM, free from fenugreek and not different ($P>0.05$) (data not shown). Consequently, feed consumption of the control treatment was the lowest ($P<0.05$): 87.5 vs 88.79 and 89.65 g DM/d for WHIFWFS and WFS, respectively. Such difference was due to the difference in the amounts of feed offered and unlikely a negative fenugreek seed effect. In this regard, Abdalla et al. (2011) indicated that fenugreek seed at 0.1% level was of no effect of on feed consumption by *Gimmizah* laying hens.

Likewise, Moustafa (2006) reported that fenugreek at levels of 0.05, 0.1 and 0.15% did not affect feed consumption by *Hy-Line White* laying hens during 40-59 weeks of age. Abdouli et al. (2014) found no negative effect on feed intake of 2 to 6 g ground fenugreek seed /day/hen. However, other studies did report decreasing feed consumption when laying hens were fed fenugreek at 0.5% of the diet (Abaza 2007; Nasra et al.2010).

In the present study, because of the restriction on feed distribution, the hens showed slight loss of body weight throughout the 41d-experimental period. The live body weight loss of the control tended to be higher than that of WHIFWFS which tended to be higher than that of WFS. Similar results were found when *Lohmann White* laying hens received 100 g basal diet/d without or with 2 g fenugreek seed for 49 days *viz*: -115.3 vs 31.8 g (Abdouli et al. 2014). Hen-day laying rate remained unchanged throughout the 41-experimental period and was not affected (P>0.05) by fenugreek addition. Mean weight of eggs laid throughout the whole experimental period was higher (P<0.05) for WHIFWFS (64.79 g) than for the control (62.23 g) and WFS (62.41 g). However, this difference was without consequence on mean egg mass and feed efficiency which were not affected (P> 0.05) by fenugreek addition. These results were in agreement with their homologous ones reported by Abdouli et al. (2014) but not with those reported by Nasra et al. (2010), Abdallah et al. (2011) and Moustafa (2006). Nasra et al. (2010) reported increased egg production rate and egg mass and decreased egg weight for hens on 0.5% ground fenugreek seeds. Abdallah et al. (2011) showed that at 0.1% level, fenugreek seed improved egg weight and mass, production rate and feed conversion ratio. Moustafa (2006) 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. Madian and Esa (2006) indicated that fenugreek seed meal given to *Matrouh* hens at levels of 0.5, 1 or 1.5 % of the diet decreased egg number, egg mass, egg production rate, while egg weight and hatchability percent were significantly increased. The cause of the observed positive effects only with fenugreek levels of 0.5% or less remains unknown.

Table 4. Hens body weight change, feed intake, hen-day laying rate, egg weight and mass, and feed efficiency of control and fenugreek-treated laying hens (overall 41 day-period; mean ± standard deviation)

	Treatments			Statistics	
	Control	**WHIFWFS[γ]**	**WFS**	**SEM [¥]**	**P-value**
Body weight change, g	-129.56±55.8	-105.8±65.98	-52.30±33.5	29.39	0.194
	87.50[a]±2.26	88.79[b]±4.04	89.65[b]±1.23	0.36	0.0002
Feed intake, g DM/d					
Hen-day laying rate, %	82.03±17.73	82.90±13.35	84.42±11.37	1.85	0.655
Egg weight, g	62.23[a]±3.13	64.79[b]±3.94	62.41[a]±3.95	0.48	0.0002
Egg mass, g/hen/d	50.93±10.95	53.53±8.25	52.59±7.25	1.15	0.277
Feed efficiency	1.94±1.37	1.70±0.30	1.74±0.25	0.10	0.15

[γ] WHIFWFS= water& hexane insoluble fraction of whole fenugreek seed; [£] WFS= whole fenugreek seed; [¥] SEM=standard error of the mean; [ab] line means with different superscripts are differ significantly (P < 0.05).

3.3. Physical egg characteristics

Mean weight and physical characteristics of eggs laid on days 39-41 are shown in table 5. All parameters, except shell weight which was the highest (P<0.05) for WHIFWFS, were not affected (P>0.05) by fenugreek addition. The observed higher shell weight for WHIFWFS compared to the control and WFS treatments may be explained by the higher egg weight for this treatment (Tables 4 and 5). Physical egg characteristics in the present study were partially in agreement with those reported by Abdalla et al. (2011) and Abaza (2007) and not with those of Nasra et al. (2010) and El-Kaiaty et al**.** (2002). Abaza (2007) found that hens fed diet supplemented with fenugreek had their egg shell thicker and albumen heavier than non supplemented hens. Nasra et al. (2010) 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.

Table 5. Physical characteristics of eggs laid on days 39-41 (mean ± standard deviation)

	Treatments			Statistics	
	Control	WHIFWFS[γ]	WFS	SEM[¥]	P-value
Egg weight, g	61.03±4.77	63.32±5.54	61.09±3.36	0.89	0.142
	7.56[a]±0.80	8.35[b]±0.72	7.95[ab]±0.74	0.14	0.0024
Shell weight, g					
Shell thickness, mm	0.84±0.071	0.81±0.092	0.80±0.13	0.02	0.471
Albumen weight,,g	36.19±3.51	37.32±3.73	34.28±6.69	0.95	0.087
Yolk weight, g	15.54±1.34	15.96±1.84	15.96±1.84	0.33	0.648

[γ]WHIFWFS= water and hexane insoluble fraction of whole fenugreek seed; [£] WFS= whole fenugreek seed; [¥] SEM=standard error of the mean; [ab] line means with different superscripts are differ significantly (P < 0.05).

3.4. Egg yolk triglyceride and cholesterol.

Egg yolk triglyceride and total and HDL-cholesterol data are shown in table 5. It was found that fenugreek supplementation did not affect (P>0.05) triglyceride, total cholesterol and HDL cholesterol per g yolk and per egg. All values were within the range of those reported by Marshall et al. (2009) for eggs from black *leghorn* hens fed control, black tea or garlic powder supplements. Their values ranged from 52.03 to 113.80; 17.94 to 79.33 and 9.06 to 39.44 mg/g egg yolk for total triglyceride, total cholesterol and HDL-cholesterol, respectively.

Table 6. Egg yolk triglyceride and total and HDL-cholesterol

	Treatments			Statistics	
	Control	WHIFWFS[γ]	WFS	SEM[¥]	P-value
Triglyceride, mg/g	248.94±13.46	260.81±17.18	257.99±17.93	5.16	0.253
	3.91±0.40	4.17±0.60	4.13±0.42	0.15	0.453
Triglyceride, g/egg					
Total cholesterol, mg/g yolk	23.12±1.92	23.74±1.90	24.03±1.17	0.54	0.481
Total cholesterol, mg/egg	363.58±40.23	379.01±44.20	386.88±51.20	14.37	0.515
HDL-cholesterol, mg/g yolk	15.28±2.01	13.48±2.36	13.82±2.02	0.68	0.156
HDL-cholesterol, mg/egg	241.68±46.36	216.15±49.58	223.51±48.36	15.21	0.484

[γ]WHIFWFS= water and hexane insoluble fraction of whole fenugreek seed; [£] WFS= whole fenugreek seed; [¥] SEM=standard error of the mean

In the present study, the lack of fenugreek seed effect was in line with the findings of Abdouli at al. (2014). In contrast, Nasra et al. (2010) 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. 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. Also, Safaa (2007) found that fenugreek at 2% level fed to 35-wk old *Lohmann Brown* laying hens reduced egg yolk cholesterol from 18.5 to 17.2 mg/g egg yolk. In these studies, egg yolk cholesterol reductions were statistically significant but of small magnitude and the mechanisms underlying such an affect could not be understood. Beneficial effects may be mediated via 4-hydroxylisoleucine, saponins and phenols in fenugreek seed. 4-hydroxyisoleucine isolated from fenugreek seed decreased significantly the plasma triglyceride levels by 33%, free fatty acids by 14% and total cholesterol by 22% and increased HDL–cholesterol/total cholesterol ratio by 39% in the dyslipidemic hamster model (Narender et al. 2006). When steroid saponins were extracted from fenugreek seeds and separated from all other constituents and administered to normal and streptozotocin diabetic rats mixed with food (12.5 mg/day per 300 g body weight), they decreased total plasma cholesterol without any change in triglycerides (Petit et al. 1995). Olfa et al. (2010) indicated that flavonoids in fenugreek ethyl acetate extract administered to Wistar rats significantly lowered the levels of plasma total cholesterol, triglycerides, and LDL-cholesterol and increased level of HDL-cholesterol.

These documented effects of 4-hydroxylisoleucine, saponins and flavonoids in fenugreek seed on cholesterol metabolism in mammals may not exist in egg-laying fowl. If such metabolites reduced hen's plasma cholesterol, their action on egg cholesterol might not have been the same. On this subject, Abdouli et al. (2014) indicated that ground fenugreek seeds given at 2 to 6g/d reduced hens' blood serum cholesterol from 106.4 to 85.8mg/dl but did not affect egg yolk cholesterol (21.4 to 22.9 mg/g). On the other hand, it has been found that not all saponins could lower egg yolk cholesterol. As an example, while karaya-saponin lowered serum (23·0%) and egg yolk cholesterol (Afrose et al. 2010) and digitonin at 0.025% reduced egg cholesterol but no that in serum (Tumova et al. 2004), dietary sarsaponins failed to lower the cholesterol content of egg yolk and that of the serum of laying hens (Sim et al. 1984).

Factors that influence the bioactive compounds levels in fenugreek seed like the genotype and cultivation conditions, the daily amounts of offered control diets and their ingredient compositions which influence the fat, cholesterol and bioactive compounds supply and hens' strains and ages may have caused the observed discrepancies among the various results.

4. Conclusion

Neither the whole fenugreek seed nor its water and hexane insoluble fraction given in small amounts as bioactive phytonutrients sources affected negatively or positively hens' feed intake, laying performance, feed efficiency and eggs physical characteristics and cholesterol profile. Consequently, use of fenugreek seed or its water extract to lower hens' egg yolk cholesterol appeared unsuccessful.

5. References

Abaza IM (2007) Effect of using fenugreek, chamomile and radish as feed additives on productive performance and digestibility coefficients of laying hens. Egypt Poult Sci 27:199-218.

Abdalla AA, Mona, Ahmed M, Abaza IM, Aly OM, Hassan EY (2011) Effect of using some medicinal plants and their mixtures on productive and reproductive performance of *gimmizah* strain 2-egg production period. Egypt Poult Sci 31:641-654.

Abdouli H, Haj-Ayed M, Belhouane S, Hcini E (2014) Effect of feeding hens with fenugreek seeds on Laying performance, egg quality characteristics, serum and egg yolk Cholesterol. J New Sci 3(1):1-9.

Afrose S, Hossain MS, Tsujii H (2010) Effect of dietary karaya saponin on serum and egg yolk cholesterol in laying hens. Br Poult Sci 51(6):797-804.

AOAC (1984). Official methods of analysis.10th Edition, Washington DC, USA.

Bukhari SB, Bhanger M.I, Memon S (2008) Antioxidative Activity of Extracts from Fenugreek Seeds (*Trigonella foenum-graecum*). Pak J Anal Environ Chem 9:78-83.

El-Kaiaty AM, Soliman AZ, Hassan MS (2002) The physiological and immunological effects of some natural feed additives in laver hen diets. Egypt Poult Sci 22:175-183.

Jansi Rani D, Rahini Devi R, VidyaShri M (2013) Phytochemical screening and antimicrobial activity of various solvent extracts of *Annona reticulate* leaves. Int J Sci Invent Today 2(5):347-358.

Madian AH, Esa NM (2006) Effect of feeding different levels of fenugreek seed meal on the performance and egg quality of *Matouh* hens under climatic conditions of upper Egypt. Minia J Agric Res Develop 26(2):207-218

Marshall AA, Kokoete EE (2009) Egg yolk cholesterol lowering effects of garlic and tea. J Med Plants Res 3(12):1113-1117.

Moustafa, Kout El-Kloub (2006) Effect of using commercial and natural growth promoters on the performance of commercial laying hens. Egypt poult Sci 26: 941-965

Narender T, Puri A, Shweta, Khaliq T, Saxena S, Bhatia G, Chandra R (2006) 4-Hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent. Bioorg Med Chem Lett 16(2):29329-6.

Nasra BA, Yahya ZE, Abd El-Ghany FA (2010) Effect of dietary supplementation with phytoestrogens sources before sexual maturity on productive performance of mandarah hens. Egypt Poult Sci 30:829-846.

Olfa Ha, Bouaziz M, Jamoussi K, El Feki A, Sayadi S, and Makni-Ayedi F (2010) Lipid Lowering and Antioxidant Effects of an Ethyl Acetate Extract of Fenugreek Seeds in High Cholesterol-Fed Rats. *J Agric Food Chem 58* (4):2116–2122

Pasin G, Smith GM, O'Mahony M (1998) Rapid determination of total cholesterol in egg yolk using commercial diagnostic cholesterol reagent. Food Chem 61:255-259.

Petit PR, Sauvaire YD, Hillaire-Buys DM, LeconteOM, Baissac YG, Ponsin GR, Ribes GR (1995) Steroid saponins from fenugreek seeds: Extraction, purification, and pharmacological investigation on feeding behavior and plasma cholestero. Steroids 60:674-680.

Safaa HM (2007) Effect of dietary garlic or fenugreek on cholesterol metabolism in laying hens. Egypt Poult Sci 27:1207-1221.

SAS Institute, Inc. (1989) Version 6. SAS Institute, Cary, NC.

Sauvaire Y, Baissac Y, Leconte O, Petit P, Ribes G (1996) Steroid saponins from fenugreek and some of their bio biological properties. Adva Exper Med & Biol 405:37-46.

Sim JS, Kitts WD, Bragg DB (1984) Effect of dietary saponin on egg cholesterol level and laying hen performance. Canad J Anim Sci 64:977–984

Tumova E, Hartlova H, Ledvinka Z, Fucikova A (2004) The effect of digitonin on egg quality, cholesterol content in eggs, biochemical and haematological parameters in laying hens. Czech J Anim Sci 49(1):33–37

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods of dietary fiber, neutral detergent fiber and non-starch carbohydrates in relation to animal nutrition. J Dairy Sci 74:3583-3597.

Yadav R, Tiwari R, Chowdhary P, Pradhan CK (2011) A pharmacognostical monograph of *Trigonella Foenum-graecum* seeds. Int J Pharm & Pharm Sci 3(5): 442-445