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Dietary Forage Legume *(Onobrychis altissima grossh.)* Supplementation on Serum/Yolk Cholesterol, Triglycerides and Egg Shell Characteristics in Laying Hens

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Abstract: In recent years, the consumer's desire for healthier foods has increased the demand for animal products containing low cholesterol and enriched with omega-3 fatty acids. In response to the perceived need, poultry researchers have focused on reducing egg yolk cholesterol to satisfy the health conscious of consumers. The present study was conducted to determine the effects of dietary forage legume supplementation on plasma triglyceride and cholesterol concentrations, egg yolk cholesterol contents and egg shell characteristics in indigenous laying hens at Mazandaran Native Fowls Breeding Station in north of Iran. A total of 60 laying hens were kept under commercial conditions from 35-45 weeks of age and were fed a commercial isocaloric and isonitrogenous corn-soybean meal diet. Birds were divided randomly into six treatment groups of ten birds each, and fed diets containing 0, 0.75, 1.25, 2.5, 5 and 10% added forage legume for 10 weeks. Ten eggs from each treatment groups were pooled in the last four-day of each period for the egg yolk cholesterol contents and egg shell breaking strength. The blood samples were taken from the wing-vein at the end of each period from all birds of each treatment groups for serum triglyceride and cholesterol contents. The results showed that there were no significant differences in final body weight, egg weight and yolk weight due to different forage legume treatments. Forage legume supplementation to the diet did not significantly affect plasma cholesterol and triglyceride concentration, while it has significantly ($p \leq 0.05$) reduced egg yolk cholesterol concentrations. Egg yolk cholesterol content was reduced from 16.41mg/g yolk in control group to 13.01mg/g yolk in 10% forage legume supplemented diet. Feeding the basal diet supplemented with any of 0, 0.75, 1.25, 2.5, 5 and 10% levels of forage legume had no significant effect on egg breaking strength. The results demonstrate that there is no co-linearity response on plasma cholesterol level and egg yolk cholesterol content to the dietary forage legume supplementation. It can be concluded that the incorporation of forage legume supplementation in layer diets could improve egg quality with no any negative effects on laying hen's performance.

Key words: Forage legume, yolk cholesterol, egg shell, plasma triglyceride

Introduction

From the consumer's point of view, fat is associated with poor dietetic quality and moreover, consumer awareness of the correlation between saturated fat consumption and obesity or coronary heart disease has stimulated the demand for low fat products of animal origins. Traditionally, for table consumption, almost exclusively used are chicken eggs. Recently, consumers limit their intake of eggs because of adverse publicity about saturated fats and cholesterol. Developed countries have shown a decrease in dietary fat and cholesterol consumption in recent years. This is largely the result of on-going massive public health campaign advocating a low fat, high carbohydrate diet as ideal to bring down blood cholesterol level to prevent heart disease and arthrosclerosis. Despite this effort, the number of people with elevated blood cholesterol continues to increase (Shafey *et al.,* 2003). Recently, most of the emphasis has focused on the enrichment of n-3 fatty acids in the egg yolk from dietary sources rich in C18: 3 and C22: 6 because of cholesterol-lowering effect of n-3 fatty acids.

Cholesterol balance in egg-laying hen differs greatly from that in mammals. Laying hens generally are not fed products of animal origin and usually meet their bodies needs for cholesterol entirely by *de novo* synthesis. In addition, most of the cholesterol in laying hen plasma resides in the very low-density lipoproteins (VLDL) fraction (Elkin *et al.,* 1999), whereas in normolipidic human, hamster, pigs, dogs and rabbits, low density lipoproteins (LDL) or high density lipoproteins (HDL) are the main carriers of cholesterol (Kieft *et al.,* 1991). Moreover, the major route of cholesterol excretion in laying hen is via the egg, which contains approximately two-thirds of the hen's typical daily cholesterol production of 300 mg. The liver and ovary are the primary sites of cholesterol biosynthesis in laying hens (Naber, 1983). However, there is little, if any, direct transfer of ovarian-synthesized cholesterol to developing oocytes *in vivo*. Thus, the contributions of the ovaries to egg

cholesterol levels are minimal at best (Shafey *et al.*, 1999). In contrast, cholesterol is readily transferred from the blood across the ovarian membranes to developing ova and therefore most, if not all, egg yolk cholesterol originates from blood cholesterol (MacLachlan *et al.*, 1996).

The literature contains limited data on the variations in the cholesterol content of eggs from commercial layers, but existing reports on chicken egg cholesterol values show considerable variation (Hatice and Ergul, 2005). But however, there is no breed and strains of chickens that lay eggs with significantly lower cholesterol than other chickens. The genetic programs as well as a vast array of dietary treatments have resulted in only slight reductions (generally 5% or less) in avian egg cholesterol content (Griffin, 1992). It has been hypothesized that cholesterol is essential for yolk formation and that egg production would cease when yolk cholesterol deposition was inadequate for embryonic survival (Hargis, 1988). Health professional suggests decreasing saturated fat intake is important. Consumption of polyunsaturated fatty acid has been reported to reduce the risk of atherosclerosis and stroke (Lada and Rudel, 2003). Monounsaturated and polyunsaturated fats may lower blood cholesterol levels when they replace saturated fat in the diet (Shafey *et al.*, 2003). It has been reported that saturated fat in the diet, not dietary cholesterol, is the most important factors what influences blood cholesterol levels (Howell *et al.*, 1997). The compositions of the fatty acids in the diet of laying hens are known to influence fatty acid profile of the egg yolk (Shafey and Dingle, 1992; Shafey *et al.*, 1999). It is well known that the fatty acid composition of animal products can be altered by nutrition. Thus, apart from genetic factors, especially the influences of feeding regimen on cholesterol content of laying hens need further investigation. One of the reasonable and simple ways is to manipulate and to enrich the egg with mono and polyunsaturated fatty acids through dietary change. Dietary fatty acids have a marked effect on fat and cholesterol metabolism (Shafey *et al.*, 2003). The presence of unsaturated fatty acids increases cholesterol and phospholipids synthesis, while saturated fatty acids had little effect (Weiss *et al.*, 1967). The manipulation of egg yolk cholesterol and fatty acids contents requires better understanding of factors that influence the deposition of cholesterol and fatty acids composition of cholesterol and fatty acids in egg yolk. This study was designed to assess the effects of dietary forage legume supplementation on serum/yolk cholesterol, triglycerides and egg shell characteristics in laying hens.

Materials and Methods

Layer hens management and diets: The local strain birds were obtained from Mazandaran Native Fowls Breeding Station in north of Iran. Studies were carried out at an experiment farm belonging to the same breeding station. A total of 60 laying hens were kept under commercial conditions from 35-45 weeks of age. They were fed a commercial isocaloric and isonitrogenous corn-soybean meal diet. Hens were divided randomly into six treatment groups of ten birds each, and fed diets containing 0, 0.75, 1.25, 2.5, 5 and 10% added the seed of forage legume *(Onobrychis altissima Grossh.)* for a 10 weeks period. Egg production was recorded daily.

Sample collection and chemical analysis: On the last four days of each 14-day period, eggs were collected for measuring egg weight, yolk weight, and shell thickness. Body weight changes were determined by weighing birds, individually, at the start, and, at the end of the study (weeks 10th of the experiment). A three ml heparinized blood sample was taken from the wing-vein at the end of each period from all birds of each treatment groups. The samples were centrifuged at 2300 g for 20 minutes and plasma was collected for further analysis. Duplicate aliquots from each sample were prepared for cholesterol and triglyceride analysis. Ten eggs from each treatment were pooled in the last four-day of each period for the cholesterol analysis. Egg yolk was extracted using 2:1 (vol/vol) chloroform: methanol using a modified Folch *et al.* (1957) method and then yolk cholesterol was determined by the method of Zlakis *et al.* (1953). Serum cholesterol and triglyceride was estimated using a cholesterol and triglyceride diagnostic kit (Ziestshimi Co., Iran). The egg compression tests were conducted on an egg shell intensity meter's machine (Ogawa Seiki Co. Ltd., Japan) for measuring breaking force. Breaking force was defined as the force in grams required fracturing the shell. When the shell failed, the directional movement of the compression plate was reversed and the egg removed. For measuring breaking force, the eggs were compressed between two flat plates with the major axis perpendicular to the compression surface. When the shell failed, the directional movement of the compression plate was reversed and the egg removed. The force applied to the egg during compression was recorded. Breaking force was defined as the force in kg/cm^2 to the shell surface required to fracture the shell.

Statistical analysis: Analysis of variance (ANOVA) was performed on all data using the General Linear Models procedure of the SAS Institute (1989). The significance of the fixed effect of the each dietary treatment in the ANOVA models was assessed using F-tests for the variance ratio. If a significant effect of variables was calculated, means were contrasted by Duncan's multiple range tests (Steel and Torrie, 1980).

Table 1: Effect of forage legume on mean serum cholesterol (mg/dL) in laying hens. Values are mean ±SEM

	Laying periods				
	week				
Diets	35-37	37-39	39-41	41-43	43-45
Control diet	178±8.2	193±6.3	199±5.4	171±6.2	159±7.3
0.75%	158±6.5	194±8.9	185±8.7	154±2.6	144±4.9
1.25%	156±9.1	180±7.6	180±6.1	167±8.6	145±6.1
2.50%	171±3.8	189±4.8	179±7.4	158±9.1	155±3.1
5.00%	177±4.6	166±9.6	188±7.1	174±3.8	163±9.2
10.0%	167±6.2	186±4.8	176±3.4	159±7.4	149±5.4

Table 2: Effect of forage legume on yolk cholesterol (mg/g) in laying hens. Values are mean±SEM

	Laying periods				
	week				
Diets	35-37	37-39	39-41	41-43	43-45
Control	16.21±0.3^{a}	16.41^{a}a±0.5	16.17^{a}±0.6	15.59^{a}±0.3	16.00^{a}±0.7
0.75%	15.60±0.6^{a}	15.21^{a}±0.3	16.00^{a}±0.7	15.41^{a}±0.5	16.01^{a}±0.8
1.25%	15.50±0.4^{a}	16.60^{a}±0.7	16.21^{a}±0.8	15.65^{a}±0.4	15.94^{a}±0.4
2.50%	15.41±0.8^{a}	15.80^{a}±0.3	15.41^{a}±0.3	14.41^{a}±0.3	15.83^{a}±0.5
5.00%	15.21±0.3^{a}	15.60^{a}±0.4	15.70^{a}±0.4	13.61^{ab}±0.4	13.96^{ab}±0.5
10.0%	14.10±0.4^{b}	13.01^{b}±0.3	14.09^{b}±0.5	13.21^{b}±0.5	13.09^{b}±0.3

a, b: Means within the same column with different superscript letters differ significantly ($p<0.05$).

Results

The results showed that there were no significant differences in final body weight, egg and yolk weight due to different levels of forage legume treatments (data not shown). The data for dietary forage legume supplement on the serum cholesterol levels is shown in Table 1.

Generally, the mean serum cholesterol contents numerically were lower in dietary forage legume supplementation groups in comparing to control group ($p>0.05$). The data on serum triglyceride levels is presented in Fig. 1.

Dietary forage legume supplementation did not significantly reduce serum triglyceride levels in laying hens ($p>0.05$). The results of the egg yolk cholesterol content are represented in Table 2. Dietary supplementation of forage legume significantly reduced ($p<0.05$) yolk cholesterol concentration. These differences are more pronounced at 10% forage legume supplemented diet. Egg yolk cholesterol content was reduced from 16.41 mg/g in control group to 13.01 mg/g in 10% forage legume supplemented diet (Table 2).

The means of egg breaking strength are summarized in Table 3. Feeding the basal diet supplemented with 0, 0.75, 1.25, 2.5, 5 and 10% forage legume had no significant effect on egg breaking strength.

Discussion

The role of animal products such as meat, eggs and milk is quite important in providing many essential nutrients for sufficient and balanced nutrition in humans. The high nutrient density of eggs relative to their caloric content makes them an excellent food for many people with special dietary needs (Hatice and Ergul, 2005). Despite the fact that scientific and technological advances in the poultry industry have made abundant and economic egg production possible, egg consumption has not noticeably increased in most countries (Nys, 2001). The main purpose of the present study was focused on, to evaluate the effects of forage legume dietary supplementation on egg yolk cholesterol. Results of this experiment showed that, the egg yolk cholesterol content significantly reduced at 10% of forage legume added to the diet. Although, there was a tendency for reducing egg yolk cholesterol content at 5% forage legume diet applied, but at 10% levels the reduction was well pronounced. This results are in agreement with the finding of Mottaghitalab and Taraz (2004), that the supplementation of garlic powder at 1% in layer's hen diet reduced egg cholesterol from 16.9mg/g yolk to 13.4 mg/g yolk. Shafey *et al.* (2003) investigated the relationship between type of grain and oil supplement on blood and egg yolk cholesterol and found that dietary type of grain and oil supplement altered fatty acid profile of egg yolk and composition of plasma lipoproteins without having a significant effect on the overall performance or egg yolk content of lipid and cholesterol. The similar results have been reported by other research groups (Cherian and Sim, 1991; Ahn *et al.*, 1995; Baucells *et al.*, 2000).

Table 3: Effect of forage legume on egg breaking strength (kg/cm^2) in laying hens. Values are mean±SEM

	Laying periods				
	week				
Diets	35-37	37-39	39-41	41-43	43-45
Control	2.61	3.10	2.12	3.04	2.40
0.75%	2.23	3.00	2.62	2.01	2.95
1.25%	3.01	2.92	3.09	2.11	3.00
2.50%	2.84	2.33	3.11	2.61	3.11
5.00%	2.21	2.00	2.92	2.01	2.33
10.0%	2.09	2.17	2.98	2.38	2.72

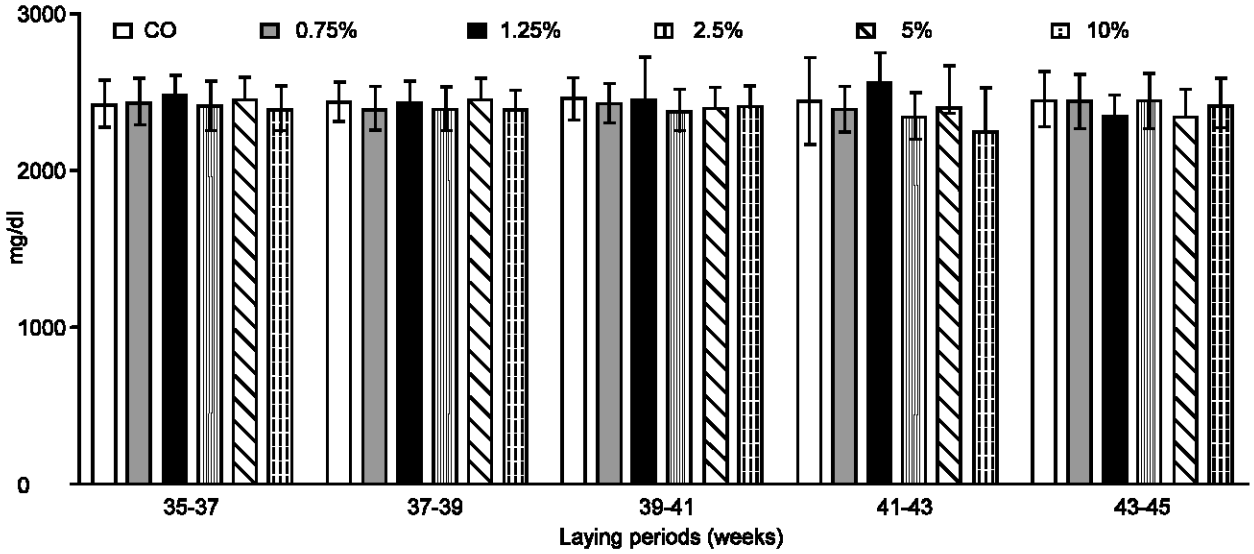

Fig. 1: Effect of forage legume on mean serum triglyceride (mg/dL) in laying hens. Values are mean ± SEM

Dietary supplementation of forage legume in this experiment did not affect the serum cholesterol and triglyceride concentrations of laying hens at the present study. These results are in agreement with the finding of Shafey *et al.* (2003), who showed that dietary type of grain and oil supplement did not alter plasma total concentration of triglyceride concentrations. It has been shown that the composition of egg yolk fatty acids is a reflection of the fatty acids synthesized by the liver of a laying hen on a standard diet since the amount of egg yolk fatty acids provided by adipose tissue is about 20% (Grimes *et al.*, 1996). The results of the present study demonstrate that there is no co-linearity response on plasma cholesterol levels and egg yolk cholesterol contents to the dietary forage legume supplementation. In a recent study, it has been shown that the egg and serum cholesterol contents can be manipulated by genotype and rearing system manipulation (Hatice and Ergul, 2005). They reported that egg yolk and serum cholesterol contents in white layers (Babcock-300) were lower than those in brown layers (IsaBrown). They have shown that the cholesterol contents of eggs from laying hens reared in floor pens were lower than that of those reared in cages.

In the present study, the different levels of dietary forage legume supplementation did not affect on egg breaking strength. Anderson *et al.* (2004) have shown a genetic difference in egg shell formation characteristics exist between strains. They indicated that the genetic selection has produced larger eggs that are rounder in shape, where have higher resistance to crushing forces. Hatice and Ergul (2005) reported that the effect of genotype on egg shell ratio and thickness was not significant. In conclusion, added dietary forage legume fed improved egg compositions by reducing the cholesterol contents of the egg. In addition, the supplemented forage legume had no any negative effects on layer hen's performance and egg breaking strength. Therefore, on the basis of the present study, supplementation of higher percentage of forage legume (more than 10%) in layer's ration is recommended for further investigation.

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