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Effect of Psyllium Husk Fiber on Growth Performance, Egg Quality Traits and Lipid Profile in Layers under High Ambient Temperature

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ABSTRACT

The present study was conducted to evaluate the impact of cholesterol lowering effect of Psyllium husk in egg yolk cholesterol through dietary supplementation in white Leghorn layers. A total of 180 commercial layer hens were randomly divided into four equal groups of 45 birds each to be allocated to four dietary regimes, consist of 0, 5, 10 and 20 g of Psyllium per kg diet, each group was subdivided into three replicate containing 15 layers per replicate. Dietary treatments had a non significant effect on weight gain, egg production, feed intake and mortality. There was a positive impact of dietary Psyllium levels on egg mass and shell thickness, however, haugh unit and egg shape index deteriorated with higher dietary inclusion of Psyllium. Cholesterol levels in yolk and blood reduced significantly ($P<0.05$) with an increasing level of dietary Psyllium. The results of the present study suggested that dietary inclusion of Psyllium can be an effective tool for the reduction of blood and egg yolk cholesterol levels.

Key Words: Psyllium, Egg traits, Yolk cholesterol, Egg production, Lipid profile.

INTRODUCTION

The egg is the important contributor towards a balanced and nutritious human diets, however, some health conscious are vary of its higher cholesterol contribution and therefore, can be reluctant to be benefited enough from this precious nutritious item. Many dietary products have been applied to reduce the cholesterol contents in eggs. One such dietary intervention is the soluble fiber which has been shown to be supportive to the cholesterol lowering effects of low-fat diets in individuals with mild to moderate Hypercholesterolemia (Anderson et al., 1992, Jenkin et al., 1993, Glore et al., 1994; Ahmedet al., 2010). One such source of soluble fiber is Psyllium which is a herb native to parts of Asia, the Mediterranean regions of Europe and North Africa, and is now widely cultivated in India and the American southwest. It is a good source of natural and concentrated soluble fiber. Psyllium fiber, also known as Ispaghula and Isapgol, is derived from the husk of blonde Psyllium seeds from *Plantago psyllium* which is commonly known as *Plantago ovate* (Kendall, 2004), which is recognized as a potent agent in lowering plasma cholesterol (Anderson et al., 2000a). Psyllium fiberis a gel forming mucilage that lowers blood lipid concentrations (Anderson et al, 2000b, Fischer et al., 2004). Moreover, it is well accepted as a safe and effective bulk laxative and is an adjunct to dietary intervention for individuals who do not adequately respond to a low-fat and low cholesterol diet (Anderson et al., 1988). The supplementation of Plantago Psyllium can lower total cholesterol (TC), and LDL-C (Anderson et al., 2000b). Levels of HDL-C, which isalso known as good cholesterol,were shown to increase by dietary Psyllium supplementation (Oson et al., 1997). The cholesterol-lowering effect of Psyllium has been reported in children (Davidson et al., 1996), as well as in adults (Florholmen et al., 1982). Additional benefits of Psyllium supplementation have also been reported to improved blood sugar

levels in some people with diabetes (Florholmen et al., 1982; Rodriguez-Moran et al., 1998; Anderson et al., 1999).

Previously, Garvin et al. (1965) showed that Psyllium when administered as a hydrophilic mucilloid resulted in a reduction of total cholesterol by 9% in the subject (human) within time period of five weeks. Anderson et al. (1998) also reported that Psyllium, when taken as part of a low-fat diet, resulted in a further of reduction of 3-6% of serum LDL cholesterol concentrations which was an additional of 5-9% relative to placebo, with no effect on serum HDL-cholesterol or triglyceride concentrations. Likewise, Psyllium has also been reported to reduce serum total cholesterol concentration 5-15% and serum LDL-cholesterol concentration 8-20% in hyper holesterolemic men consuming high-fat diets (Everson *et al.*, 1992). Several other studies on animals as well as in humans have also validated the claim of hypocholesterolemic properties of Psyllium (Everson et al., 1992; Turley et al., 1996). It was also reported that consumption of foods containing dietary fiber, may improve the long-term maintenance of low atherogenic LDL-cholesterol (Davidson et al., 1998). Psyllium (*Plantago Ovata*) seed husk fiber, a widely used soluble fiber, has been known to reduce serum total cholesterol and LDL cholesterol (Anderson et al., 2000b; Ganji and Betts, 1995).

So there is a mounting evidence to prove that the Psyllium fiber is a very useful tool in lowering serum total cholesterol or serum LDL-cholesterol concentrations in humans. Chicken eggs are an excellent source of protein and other nutrients and a regular and essential part of our breakfast and meals since long. On the other hand, chicken eggs have high contents of cholesterol (213-280mg) and health advisors recommend limiting or eliminating eggs from human diets in general and especial people with a high blood cholesterol level. So efforts are required to divert resources to develop methods of producing eggs having lower levels of cholesterol. Therefore, there is a need of biological evaluation of Psyllium husk addition in the egg laying poultry diets as a possible opportunity of lowering cholesterol in the blood serum as well as in the egg yolk.

MATERIALS AND METHODS

Ethical approval

Ethical approval of all procedures in this study has not been sought from ethical committee of the PMAS Arid Agriculture University, Pakistan.

Bird husbandry and dietary treatments

A total of 180 Hy-Line W-98 hens, 70 weeks old (50 weeks in to lay) with uniform body weight were collected from a commercial flock of Breeding and Incubation section, Poultry Research Institute, Rawalpindi. The experimental house was thoroughly cleaned and disinfected before the shifting of these hens. Hens were maintained in four major dietary treatment groups for 90 days. Each treatment group was subdivided in to three replicate groups of 15 birds each. A basal diet was formulated to meet the nutrient requirements set according to Hy-Line 2000 guidelines as presented in table 1. Four dietary treatments were then made by addition of Psyllium fiber on the top of basal diet at the inclusion levels of 0, 5, 10 and 20g/kg of diet, representing as T_1, T_2, T_3 and T_4, respectively.

Table 1. Composition of experimental basal diet

Ingredients	Composition (g/kg)
Corn	510.00
Rice Broken	71.00
Rice Polishing	80.00
Canola Meal	70.00
Guar Meal	40.00
Sunflower Meal	20.00
Soybean Meal	104.60
Fish Meal	44.00
Molasses	28.00
Bone Meal	14.00
Marble Chips	67.00
NaCl	0.50
L-Lysine	1.10
DL-Methionine	0.80
Vitamin Pre-mix[1]	2.50
Mineral Premix[2]	2.50
Total	1000.00
Calculate analysis	
Metabolisable Energy (ME Kcal/Kg)	2790.00
Crude Protein (g/kg)	162.00
Crude Fat (g/kg)	34.50
CrudeFiber(g/kg)	40.80
Total Ash (g/kg)	89.80
Calcium (g/kg)	38.00
Av. Phosphorus(g/kg)	3.60
Sodium	1.78
NaCl	3.75
Linolenic acid	14.60

[1]Provided the following per kilogram of diet: vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200 ICU; vitamin E (as dl-α-tocopheryl acetate), 8 IU; vitamin B12, 0.02 mg; riboflavin, 5.5 mg; d-calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B1 (thiamin mononitrate), 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg; vitamin K (menadione sodium bisulfate complex), 2 mg.[2]Provided the following per kilogram of diet: manganese, 65 mg; iodine, 1 mg; iron, 55 mg; copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg.

Growth performance

Birds were weighed at the beginning (Initial weight), and then biweekly up to the end of the experiment. Egg production was recorded daily at the same time of the day and was calculated on a hen-day basis. As follows: total number of eggs collected divided by total number of live hens per day in each group. Records of the feed intake were also taken on weekly basis.

Egg Quality Analysis

To determine egg quality traits, 30eggs from each treatment groups were used at 14-days interval throughout the experimental period for which a 2-d egg collection was saved during the week. Egg mass was calculated as a factor of egg weight and hen-day egg production. Measures of egg weight and specific gravity were also determined at that time. The short and long diameters of the eggs were measured by a digital caliper with a sensitivity of 0.001 mm and were used to determine the egg shape index. The eggs were then broken on individual basis on a flat surface, and a waiting period of 5 min was given to settle the egg contents naturally. The heights of the yolk and albumen, the long and short diameters of the albumen, and the diameter of the yolk were measured using the caliper. The yolks separated from the albumen and then weighed and recorded. From the values obtained, the following data were calculated using the formulas shown below (Yannakopoulos and Tserveni- Gousi, 1986):

Shape index = short edge/long edge × 100

Yolk index = yolk height/yolk diameter× 100

Haugh units were determined at the same time on same number of eggs (30 eggs from each treatment). Haugh units were calculated from the records of albumen height and egg weight using following equation:

Haugh unit = 100 × log (albumen height + 7.57 − 1.7 × egg weight$^{0.37}$ (Nesheim *et al.*, 1979).

The shells of the broken eggs were washed under gently flowing tap water to release albumen residues, and were then air-dried and weighed. Shell thickness was determined bi-weekly on above same eggs from each treatment group (without the shell membranes): the measure was carried out with a digital caliper with a sensitivity of 0.001 mm at three points of the egg shell (air cell, equator, and sharp end).

Determination of cholesterol concentration in the blood serum

On 14, 28, 42, 56 and 70 days of the experiment, blood samples were collected from the bronchial vein of 9 hens from each treatment (3 hens/ replicate) to determine the serum cholesterol.The collected blood samples were centrifuged at 3000 r.p.m. for 10 min and the serum was decanted into aseptically treated vials and stored in deep freezer at -20ºC up to analysis for total cholesterol. Serum cholesterol was measured by using diagnostic kit (RANDOX Diagnostics, Catalog No. CH 207, 56RANDOX Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY) and spectrophotometer apparatus. Atherogenic index (IA) was calculated using the following formula:

AI= log (TG/HDL-C) (Kanthe et al., 2012).

Statistical analysis

Data were analyzed by using the SPSS version 16 (SPSS, Cary, NC, USA) statistical analysis program. A randomized complete block design was used to analyse data by using analysis of variance (ANOVA). The mean values were considered significantly different when P-value was <0.05 whereas trends were reported at P=0.1. The comparison of means was made using Tukey's test. (Steel and Torrie, 1980)

RESULTS

Nutrients composition of Psyllium husk

The Psyllium husk (*Plantago ovate* - as reported in previous studies) are mainly composed of 8.6% moisture, 21.7% crude protein, (CP), 6.1% crude fiber, 29.5% ether extract, 4.5% total ash and 29.7% nitrogen-free extract. Macro-mineral content was 572 mg calcium/100 g; phosphorus was 540 mg/100 g; magnesium (264); sodium (17.8) and potassium (810). Further, micro-mineral content was as follows: copper (2.7), zinc (6.2), iron (9.7) and manganese (8.5).

Effect of Psyllium on layer performance

There were no treatment related mortalities recorded during the entire study period. Dietary treatments had no effect on weight gain (Table 2).

Effect of Psyllium on the internal and external egg quality traits

There was increase (P<0.05) in egg mass and shell thickness with highest being observed when diets contained highest levels of phyllium fibre. However, there was a deterioration (P<0.05) in haugh unit and egg shape index noted as the levels of dietary phyllium increased. The results showed that there was no significance (P>0.05) effect of dietary treatments on shell weights, albumen weights and yolk weight (Table 3).

Effect of Psyllium on blood lipid profile and yolk cholesterol

There was significant (P<0.05) reduction in egg yolk cholesterol levels noted with increasing levels of dietary Psyllium, the best reduction in egg cholesterol contents were noted when dietary Psyllium was added at rate of 20g/kg of diet (Table 4).Similarly a lowered levels (P<0.05) of blood serum cholesterol, triglyceride, low-density lipoprotein (LDL) and very low density lipoprotein contents were noted at higher levels of dietary Psyllium addition. Strangely the high-density lipoprotein (HDL) concentration in the blood serum increased (P<0.05) with increasing the dietary addition of Psyllium. IA is used as a predictor of atherosclerosis in biological system (Ackay et al., 2014) which was reduced with increased addition of dietary Psyllium. .

Table 2. Effect of various dietary treatments of Psyllium husk on body weight gain (grams), egg production and mortality (%) of layers at 70 weeks

Parameter	Psyllium Levels (g/kg diet)				SEM
	0 (T_1)	5 (T_2)	10 (T_3)	20 (T_4)	
Initial body weight (g/b)	1567	1558	1556	1581	0.389
Final body weight (g/b)	1625	1614	1599	1600	0.753
Body weight gain (grams during whole experiment)	58[b]	56[b]	43[b]	19[a]	0.007
Egg production (%, egg/hen/day)	62.05	63.10	64.01	63.85	0.238
Mortality (%)	0.00	0.00	0.00	0.00	--

*T1 = Control; T2= 5 gm Psyllium/ Kg Feed; T3= 10 gm Psyllium/ Kg Feed; T4= 20 gm Psyllium/ Kg Feed; [a,b] Means within a row without common superscripts differ significantly (P≤ 0.05).

Table 3. Effect of various dietary treatments of Psyllium husk on egg quality parameters in layers at 70 weeks

Parameter	Psyllium Levels (g/kg diet)				SEM
	0 (T_1)	5(T_2)	10(T_3)	20(T_4)	
Egg External Quality					
Egg weight (g)	60.99	61.06	60.06	63.09	0.561
Egg mass (g/d/hen)[†]	40.76[b]	42.83[ab]	43.80[ab]	45.66[a]	0.682
Egg shape index	76.80[b]	73.00[a]	71.33[a]	71.00[a]	0.781
Specific gravity	1.088	1.079	1.080	1.080	0.016
Egg Internal Quality					
Haugh units	76.06[a]	70.16[b]	74.83[a]	59.70[c]	2.910
Shell thickness (mm)	0.34[b]	0.35[b]	0.36[a]	0.36[a]	0.004
Shell weight (g)	7.50	7.73	7.06	7.56	0.164
Yolk weight (g)	16.16	15.33	15.00	16.40	0.403
Albumen weight (g)	37.33	38.01	38.00	39.13	0.313
Egg (%)					
Yolk	26.49	25.1	24.97	25.99	0.295
Albumen	61.20	62.23	63.27	62.02	0.399
Shell	12.29	12.65	11.75	11.98	0.210

*T1 = Control; T2= 5 gm Psyllium/ Kg Feed; T3= 10 gm Psyllium/ Kg Feed; T4= 20 gm Psyllium/ Kg Feed; [a,b] Means within a row without common superscripts differ significantly (P≤ 0.05).

Table 4. Effect of various dietary treatments of Psyllium husk on yolk and blood cholesterol in layers at 70 weeks

Cholesterol	Psyllium Levels (g/kg diet)				SEM
	0 (T1)	5 (T2)	10 (T3)	20 (T4)	
Total Cholesterol(mg/dl)	192.95 [a]	190.67 [a]	181.00 [b]	172.07 [b]	5.571
Triglyceride (mg/dl)	94.08 [a]	92.03 [a]	84.43 [b]	83.70 [b]	1.400
High Density Lipoprotein (mg/dl)	39.03 [b]	40.01 [b]	41.71 [a]	44.02 [a]	0.642
Low Density Lipoprotein (mg/dl)	135.11[a]	132.25[b]	122.41[c]	111.30[d]	2.829
Very Low Density Lipoprotein (mg/dl)	18.81 [a]	18.40 [a]	16.89 [b]	16.74 [b]	0.279
Atherogenic index (IA)	3.46 [a]	3.30 [b]	2.94 [c]	2.53 [d]	0.110
Yolk Cholesterol (mg/dl)	17.32[a]	17.26[a]	14.63[b]	14.02[b]	0.032

*T1 = Control; T2= 5 gm Psyllium/ Kg Feed; T3= 10 gm Psyllium/ Kg Feed; T4= 20 gm Psyllium/ Kg Feed; [a,b] Means within a row without common superscripts differ significantly ($P \leq 0.05$).

DISCUSSION

Effect of Psyllium on layer performance

Reduced weight gain in present study was in consistent with the early findings (Anonymous, 2010) this may be justified as Psyllium husk fiber reduces the energy intake under a restricted feeding regime. In addition to that there is another explanation for this phenomenon of reduced weight gain and that is as the dietary fiber level increase it resulted in a stabilization of insulin and glucose responses which then relates with poor nutrient utilisation (Leeuw et al., 2004) resultantly, it reduces the weight gain (Anonymous, 2010). No improvement in egg production in present study was in agreement with the findings of Roberts et al. (2007) who described that dietary fiber does not improve the egg production in layers.

Effect of Psyllium on internal and external egg quality parameters

Findings of the present study were in accordance with Roberts et al. (2007) who described that dietary fiber does not improve the egg weight, however, on the contrary to the previous findings egg mass founded to be higher with increasing levels of dietary fiber in the present study. These findings were indeed surprising as the egg mass increased even when weight gain went down due to lower energy intake in higher Psyllium fed birds this is beyond authors understanding at this stage and therefore, needs further investigation.

Effect of Psyllium on blood lipid profile and yolk cholesterol

Psyllium husk has a hypercholesterolemia effect in biological system (Anderson et al., 2000b) these findings are in consistent with the results of present study. Wolever *et al.* (1994) and Sprecher et al. (1993) reported that 10g Psyllium supplementation per day reduced 5-9% low density Lipid (LDL) cholesterol without altering HDL cholesterol or serum cholesterol or serum triglycerides concentrations (Levin et al. 1990; Stoy et al. 1993) largely by increasing their faecal excretion in animals (Arjmandi et al., 1992a; Turley et al., 1996 and Matheson and Story, 1996). Similarly, findings of Olson et al. (1997) are in agreement with present findings who reported that Psyllium reduce the 5% total cholesterol concentration than control diets. Likewise, Anderson et al. (2000a) reported that Psyllium decreased the serum's Low density lipoproteins cholesterol and total cholesterol concentration significantly. In another study, Ziai et al. (2004) reported that the Psyllium significantly decrease the High-density lipoproteins and reduces the Low-density lipids, lower cholesterol / high-density lipoproteins ratio and further reported that Psyllium improves the glycemic control. However, in present study data indicated an increase in the HDL in the blood serum which is contrary to the findings of Ziai et al. (2004). Likewise, Ganji and Kuo (2008) also reported that Psyllium fiber reduces the concentration of total cholesterol and decreases the high density lipoproteins cholesterol. In another study, Buhman et al. (1998)reported that Psyllium hydrocolloid significantly reduces the liver cholesterol in rats and further reported an increase in faecal excretions of total bile acid and steroid in high fiber fed rats compared to the controlled diet fed groups. Terpstra et al. (1998) reported that dietary addition of Psyllium in Hamsters reduces the plasma cholesterol ester transfer. Similar findings were reported and supported by Olson et al. (1997) who reported that dietary inclusion of cereal products enriched in Psyllium reduce

the total cholesterol, HDL cholesterol and LDL cholesterol levels in the blood this can be explained due to a possible improvement in the intestinal viscosity (Dikeman et al., 2006) and a lowering of blood glucose concentrations in higher fiber diets. Likewise, Terpstra et al. (2000) proposed that Psyllium lower the concentration of plasma cholesterol, very-low-density-lipoprotein cholesterol and triglyceride by reducing the energy intake and less a depression in hunger signals which ultimately resulted in reduction in the serum glucose and insulin concentration in animals (Pastors et al., 1991). Shrestha et al. (2007) reported that Psyllium lower down the concentrations of cholesterol and Low-density lipoproteins by reducing the activity of cholesteryl-ester-transfer-protein and by increasing the LDL receptor-mediated uptake and by modification in the intravascular processing of lipoproteins. Vega-López et al. (2003) reported that Psyllium as functional fiber reduces the plasma low density lipoprotein cholesterol in functional physiology of both sexes in human due to increase in HMG-CoA reductase gene expression in monocytes while the effect of Psyllium on plasma triglycerides are sex related property, it decreases in male but increases in females. In another study, Uehleke et al. (2008) reported that Psyllium reduce the cholesterol by minimizing the unfavourable effects on the Gastrointestinal Tract (GIT) and increase in the the GIT mass of many mammals (Cannon et al., 2010) in dairy cows and also increase the populations of bifido-bacteria and lactobacilli in the reticulo-rumen and fermentation in the colon. The finding of low egg yolk cholesterol in present trial was supported by McNaughton (1978) who reported that dietary fiber reduces the egg yolk cholesterol in layers. On contrary, Roberts et al. (2007) described that the dietary fiber has a non-significant effect on the egg yolk cholesterol in laying hens. Likewise, Weiss and Scott (1979) also investigated that the dietary fiber has non-significant result on the cholesterol contents of the eggs in laying hens.

CONCLUSION

Data from present research trial suggests that Psyllium can lower the blood serum and egg cholesterol contents without negatively affecting the egg mass and shell thickness. However, an increase in HDL contents and reduction in hugh units needs to be understood through a more refined study model.

Competing interests

The authors declare that they have no competing interests.

Author`s contributions

Mukhtar N., Mehmood R., Hassan Khan S. and Mehmood Ashrif N. deigned and performed the experiment. Waseem Mirza M. analysed data and wrote the paper.

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