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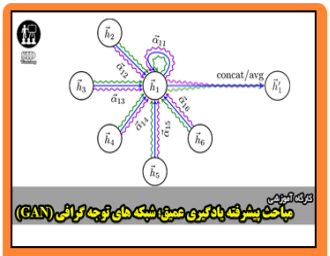


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# Egg Yolk Cholesterol of Hens Fed Barley Malt Rootlets

Research Article

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## ABSTRACT

This study aimed to decrease the cholesterol content of egg yolk of laying hens through inclusion of barley malt rootlets in the diets. Forty-two, 54-week-old, Lohman laying hens were fed for a 12 weeks laying period on 3 iso-caloric and iso-nitrogenous diets contained 0 (control), 25.5 or 51.1g barley malt rootlets/kg. Inclusion of barley malt rootlets at 25.5 or 51.1g/kg diet significantly decreased ($P<0.001$) concentrations of plasma cholesterol (by 54.9 and 33.3%, respectively) and triglycerides (by 26.4% and 27.3, respectively) compared with the control. Feeding barley malt rootlets at 25.5 g/kg diet tended to decrease ($P>0.05$) concentrations of total lipids, triglycerides, cholesterol, low-density lipoproteins and phospholipids in egg yolk by 4.7, 5.7, 5.2, 3.9 and 5.3% respectively, while its inclusion at 51.1 g/kg diet significantly ($P<0.05$) decreased the same respective parameters by 10.4, 10.4, 10.4, 10.6 and 11.22% respectively, compared with the control. It could be concluded that inclusion of barley malt rootlets in laying hen diets at 25.5 g/kg diet tended to decrease cholesterol and low-density lipoproteins in egg yolk (by 5.2 and 3.9%, respectively, while its inclusion at 51.1 g/kg diet decreased cholesterol and triglycerides concentrations in blood plasma and also decreased cholesterol and low-density lipoproteins in egg yolk (by 10.6 and 10.4%, respectively).

KEY WORDS barley malt rootlets, cholesterol, egg yolk, laying hen, lipids, plasma.

## INTRODUCTION

Although eggs possess protein of significant biological value and are an excellent source of vitamins and minerals, many people limit their consumption of eggs because they associated high cholesterol content (1 large egg, 50 g, contains 213 mg of cholesterol) with cardiovascular disease (Zeidler, 2002). Moreover, Butarbutar (2004) mentioned that elevated serum cholesterol in human has been strongly correlated with consuming greater amounts of cholesterol than normal.

Barley malt rootlets, also called malt culms or malt sprouts, a by-product of the brewing industry, consist of the plumule and radicle of barley (McDonald *et al.* 1995), and also may include some of the malt hulls. Barley malt rootlets contain 156 g/kg crude fiber and 22 g/kg ether extract, on a dry matter basis, (McDonald *et al.* 1995). Truswell (1999) reported that barley is high in the soluble fiber β-glucan. Wang *et al.* (1992) reported that β-glucans are effective polysaccharides for reducing plasma cholesterol concentration in chicks. El-Husseiny *et al.* (1997) found that barley malt rootlets inclusion in rabbit diets lowered plasma cholesterol. Jonker *et al.* (2010) showed that barley β-glucan lowers plasma cholesterol in rats.

Tocopherols (vitamin E) and tocotrienols, grouped as tocols, are a class of lipid-soluble antioxidants (Cavallero *et al.* 2004). Bonnely *et al.* (2000) reported that, oil of barley malt rootlets contains 20.6 and 4.2 microgram of alpha-tocopherol and gamma-tocopherol, respectively, per gram of dry rootlets. Sahin *et al.* (2002) reported that supplemental alpha-tocopherol acetate decreased serum cholesterol concentration of laying hens. Cavallero *et al.* (2004) re-

ported that, the concentration of tocotrienols in barley grain is higher than in most other grains, with a favorable distribution of the most biologically active homologues. Tocotrienols are reported to be capable of reducing serum LDL cholesterol in chickens (Qureshi *et al.* 1991a).

Therefore, the objective of this study was to investigate the effects of inclusion of barley malt rootlets in laying hen diets on plasma concentrations of cholesterol and triglycerides and on lipid composition of egg yolk.

## MATERIALS AND METHODS

Forty-two, 54-week-old, Lohman laying hens were randomly divided into three equal groups, each of 14 hens (7 experimental units, each consisted of 2 hens housed in one cage). These hen groups were assigned randomly to three experimental diets to evaluate the effects of barley malt rootlets (BMR) inclusion in the diets on plasma concentrations of cholesterol and triglycerides and on lipid composition of egg yolk. Diets were: diet 1 (control) contained 0 g BMR/kg, while diets 2 and 3 contained 25.5 and 51.1 g BMR, respectively/kg diet. Barley malt rootlets in diets 2 and 3 replaced only parts of both of soybean meal and rice polish of the control diet in order to maintain all diets having all and the same feed ingredients except the tested material (BMR), (Table 1). All diets were iso-caloric and iso-nitrogenous, covering the nutritional requirements of laying hens (NRC, 1994). Diets, in mash form, and fresh water were supplied *ad libitum*. Birds were reared at room temperature and were exposed to 18 h light/d. Hens were fed the experimental diets for a 12 weeks laying period. The diets were analyzed for proximate composition according to (AOAC, 1996) methods.

At the end of the experiment, heparinzed blood samples were collected, via wing vein, from 4 hens/treatment, chosen at random and plasma was separated by centrifugation and kept frozen at -20 $^{o}$C until analyzed for cholesterol and triglycerides. Plasma cholesterol concentration was determined according to a quantitative-enzymatic-colorimetric method for determination of total cholesterol in serum or plasma (Stein, 1986). Cholesterol esterase hydrolyzes cholesterol esters to free cholesterol and fatty acids. The free cholesterol so produced plus the preformed cholesterol are then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.

A quinoneimine chromogen, with absorption maxima at 500nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase (POD) with hydrogen peroxide. The intensity of the final red color is proportional to total cholesterol concentration. Plasma triglycerides concentration was determined according to a quantitative-enzymatic-colorimetric method for determination of triglycerides in serum or plasma (Scheletter and Nussel, 1975). Glycerol and fatty acids are first formed by lipase action on the triglycerides. Glycerol is then phosphorylated by adenosine-5[1]- triphosphate (ATP) to produce glycerol-3-phosphate and adenosine-5[1]- diphosphate in a reaction catalyzed by glycerol kinase. The glycerol-3-phosphate is oxidized by glycerylphosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Peroxide reacts with a- 4 aminoantipyrine and 4 -chlorophenol under the catalytic influence of peroxidase to form quinoneimine. The intensity of the color, which is proportional to triglycerides concentration, is read at 500 nm.

**Table 1** Feed ingredients and nutrients composition of the experimental diets

| | BMR, g/kg diet | | |
|---|---|---|---|
| Ingredient, g/kg | 0 (control) | 25.5 | 51.1 |
| Yellow corn | 570 | 570 | 570 |
| Soybean meal (44% CP) | 180 | 167.8 | 155.5 |
| Barley malt rootlets[1] | 0.00 | 25.5 | 51.1 |
| Rice polish | 100 | 86.7 | 73.4 |
| Fish meal | 50 | 50 | 50 |
| Bone meal | 21.5 | 21.5 | 21.5 |
| Limestone | 70 | 70 | 70 |
| Vitamin-mineral Premix[2] | 2.5 | 2.5 | 2.5 |
| Sodium chloride | 5.0 | 5.0 | 5.0 |
| Methionin | 1.0 | 1.0 | 1.0 |
| Nutrients composition | | | |
| Crude protein, g/kg | 176.1 | 177.0 | 177.9 |
| Crude fiber, g/kg | 30 | 33.8 | 38.9 |
| Ether extract, g/kg | 43.1 | 46.7 | 49.3 |
| ME, MJ/kg | 11.63 | 11.60 | 11.58 |

[1] 162 g/kg crude fiber and 21 g/kg ether extract, on DM basis, analytical values.
[2] Vitamin and mineral premix supplied per kg of diet: retinyl acetate 3.4 mg, cholecalciferol 0.075 mg, dl-alpha-tocopheryl acetate 10 mg, Vitamin K 2 mg, Vitamin $B_1$ I mg, Vitamin $B_2$ 4 mg, Vitamin $B_6$ 1.5 mg, Vitamin $B_{12}$ 0.001 mg, Pantothenic acid 10 mg, Niacin 20 mg, Folic acid 1 mg, Biotin 0.05 mg, Choline Chloride 500 mg, Fe 30 mg, Mn 40 mg, Cu 3 mg, I 3 mg, Cobalt 0.2 mg, Zn 45 mg and Se 0.1 mg.

Eggs were collected for chemical analysis during the last 3 days of the experimental period. Twelve eggs per each treatment were taken at random, and then were weighted, cracked and their yolks were separated. Then each 4 yolks were pooled and homogenized and considered one sample i.e. each treatment had 3 samples of these pooled egg yolks for chemical analysis.

These samples of the pooled yolks were freezed and stored at -20 $^{o}$C until the chemical analysis was performed. Egg yolk samples were analyzed for total lipids, triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and phospholipids. Total lipid content of egg yolk was determined gravimetrically after extraction with chloroform: methanol (2:1) according to (Folch *et al.* 1957). Triglycerides content of egg yolk was determined colorimetrically

according to the method of (Lowell *et al.* 1973). The developed color was read at 410 nm.

Cholesterol concentration in egg yolk was determined according to an enzymatic-colorimetric method for the determination of cholesterol in egg (Shen *et al.* 1982). Cholesterol is oxidized by cholesterol oxidase to cholestenone. In the presence of catalase, the hydrogen peroxide produced in this reaction oxidizes methanol to formaldehyde. The latter reacts with acetylacetone forming a yellow lutidine-dye in the presence of $Nh^{+}$ -ions$_4$. The concentration of the lutidine-dye (3,5-diacetyl-1,4-dihydrolutidine) formed is stoichiometric to the amount of cholesterol and is measured by the increase of light absorbance in the visible range at 405 nm. Accurately weighed 0.5 g yolk into a 50 mL volumetric flask and 1 g sea-sand was added (the volume displacement of 0.400 mL must be taken into account in the calculation formula); heated under a reflux condenser for 30 min with 20 mL *freshly prepared* methanolic potassium hydroxide solution (1.0 mol/L) and 10 mL isopropanol while stirring (magnetic stirrer). The turbid solution was allowed to cool, and filled up to the mark with isoproponol at room temperature after removal of the magnetic rod (rinse with iso-propanol); mixed, filtered through a fluted filter and the clear solution was used for the assay. Low density lipoprotein cholesterol was determined colorimetrically according to (Wieland and Seidel, 1983), while high density lipoprotein cholesterol was determined colorimetrically according to (Eckel, 1977).

Phospholipids concentration in egg yolk was determined after precipitation of the phospholipids in egg yolk according to the method of (Kates, 1972), which depends on the fact that precipitation of phosphatides from neutral lipid depends on the general insolubility in cold acetone of most phosphatides in salt form. An aliquot of neutral lipids phospholipids mixture was placed in a 15 mL centrifuge tube and the solvent was evaporated in nitrogen stream at 30°C to 0.2-0.3. Acetone (7.5 mL) plus Mg $C1_{2.}$ $6H_2o$ in methanol (0.15 mL, 10% w/v) were added then mixed on a Vortex mixer and cooled on an ice bath for 1 h. The precipitated phospholipids were separated by centrifugation at 5000 rpm for 5 min. The acetone supernatant was removed by Pasteur pipet. The precipitate was then washed twice by suspending it in 2 mL of cold acetone, cooling on ice and centrifuged as above .The precipitated phospholipids were freed of excess solvent in a stream of nitrogen and the dry residue was dissolved to a known concentration in redistilled chloroform (2 mL). Then the colorimetric method of Kaur *et al.* (1973) was employed for the quantitative determination of the phospholipids.

The effects of dietary treatments were examined using analysis of variance for completely randomized design experiments using SAS (1996), while differences among means were evaluated using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Plasma lipids

Inclusion of barley malt rootlets (BMR) in laying hen diets at 25.5 or 51.1 g/kg significantly (P<0.001) decreased concentrations of plasma cholesterol (by 54.9 and 33.3%, respectively) and triglycerides (by 26.4% and 27.3, respectively) compared with the control (Table 2).

**Table 2** Plasma cholesterol and triglycerides concentrations[1] of laying hens fed diets included barley malt rootlets (BMR) at different levels

| | BMR (g/kg diet) | | |
|---|---|---|---|
| Item | 0 (control) | 25.5 | 51.1 |
| Cholesterol, mg/dL | 129.3$^{a}$±11.95 | 58.3$^{b}$±5.17 | 86.3$^{b}$±8.18 |
| Triglycerides, mg/dL | 762$^{a}$±12.43 | 561$^{b}$±32.19 | 554$^{b}$±27.93 |

[1] Means±SEM; n= 4.
The means within the same row that have at leost one common letter, do not have significant difference (P>0.01).

Such effects could be attributed to fiber and oil contents of BMR. Barley malt rootlets used in the present study contained 162 g/kg crude fiber and 21 g/kg ether extract, on a dry matter basis, Table (1). El-Husseiny *et al.* (1997) found that barley malt rootlets inclusion in rabbit diets lowered blood plasma cholesterol. Dandey and Bobraszczyk (2001) reported that barley has one of the highest levels (up to 6%) of β-glucan, a water-soluble polysaccharide, nutritionally classified as soluble dietary fiber, while Sharma and Gujral (2010) reported that barley is an excellent source of β-glucan. It has been hypothesized that upon ingestion, β-glucans increase small intestinal viscosity resulting in reduced bile acid and cholesterol or triglyceride absorption thus lowering plasma cholesterol (Topping, 1991; Wang *et al.* 1992). The liver responds by taking up more LDL-cholesterol from the blood stream thereby lowering the concentration of LDL-cholesterol in the blood. Lopez *et al.* (1999) reported that soluble dietary fiber has the ability to interact with water, and is almost fully fermented by the large intestine microflora. Short chain fatty acids, which are products of fermentation of soluble fiber in the gut, may inhibit synthesis of cholesterol by the liver, thereby, reducing the concentration of blood cholesterol. Wang *et al.* (1992) reported that β-glucans are effective polysaccharides for reducing plasma cholesterol concentration in chicks. Moreover, Wang *et al.* (1997) found that total plasma cholesterol was lower in hamsters fed diets containing barley soluble dietary fiber (SDF) compared to those fed barley diets with SDF being removed or those fed insoluble die-

tary fiber. Jonker *et al*. (2010) showed that barley β-glucan lowers plasma cholesterol in rats. Razdan and Pettersson (1994) observed a reduction in total plasma cholesterol and triacylglycerols of broiler chicken fed diets with added chitosan which is a bioactive polymer obtained from marine crustaceans that can be classed as one of the dietary fibres of animal origin. Also, Razdan *et al*. (1997) reported that chitosan reduced total cholesterol concentration in plasma of broiler chicken.

Qureshi *et al*. (1989) reported that the concentration of tocotrienols in the barley grain is high, with a favorable distribution of the most biologically active homologues (**γ**-tocotrienol and **δ**-tocotrienol). Peterson (1994) reported that tocotrienols showed a relevant concentration in the hulls of barley. Sharma and Gujral (2010) reported that barley is a functional grain and an excellent source of tocotrienols and tocopherols. Bonnely *et al*. (2000) reported that, oil of barley malt rootlets contains 20.6 and 4.2 microgram of alpha-tocopherol and gamma-tocopherol, respectively, per gram of dry rootlets. Supplemental alpha-tocopherol acetate decreased serum cholesterol concentration of laying hens, (Sahin *et al*. 2002).

Qureshi *et al*. (1986) found that highly purified tocotrienols from barley oil inhibited the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (the rate-limiting enzyme of cholesterol biosynthesis) in rat and avian hepatocytes. Moreover, Qureshi *et al*. (1991a) found that supplementing chicken diets with brewer's grain, a by product of brewing industry, decreased serum total cholesterol and low-density lipoproteins (LDL)-cholesterol (by 10.8% and 22.7%, respectively) as a result of its content of tocotrienols that act as a cholesterol inhibitor, due to suppressing the enzymatic activity of HMG-CoA reductase and decreasing the enzymatic activity of HMG-CoA synthase. Moreover, Burger *et al*. (1984) and Wang *et al*. (1993) reported a cholesterol-lowering effect of barley oil and barley oil fractions for chicks.

**Egg yolk lipids**

Inclusion of barley malt rootlets in laying hen diets at 25.5 g/kg diet tended to decrease (P>0.05) concentrations of total lipids, triglycerides, cholesterol, low-density lipoproteins and Phospholipids in egg yolk by 4.7, 5.7, 5.2, 3.9 and 5.3% respectively, while its inclusion at 51.1 g/kg diet significantly (P<0.05) decreased the same respective parameters by 10.4, 10.4, 10.4, 10.6 and 11.22%, respectively compared to the control (Table 3). Such decreases could be attributed to the high fiber content of barley malt rootlets [162 g crude fiber/kg DM, Table (1)]. Hargis (1988) reported that dietary fiber affects cholesterol metabolism of laying hens by decreasing absorption of cholesterol, binding with bile salts in the intestinal tract, shortening intestinal transit time and increasing fecal sterol excretion.

**Table 3** Lipids composition (units/100 g yolk)[1] of laying hens fed diets included barley malt rootlets (BMR) at different levels

| | BMR, g/kg diet | | |
|---|---|---|---|
| Item | 0 (control) | 25.5 | 51.1 |
| Total lipids, g | 15.0$^{a}$±0.58 | 14.30$^{ab}$±0.35 | 13.44$^{b}$±0.23 |
| Triglyceride, g | 9.75 $^{a}$±0.38 | 9.19$^{ab}$±0.14 | 8.74$^{b}$±0.15 |
| Cholesterol, g | 1.35$^{a}$±0.05 | 1.28$^{ab}$±0.03 | 1.21$^{b}$±0.02 |
| LDL[2], mg | 0.180$^{a}$±0.006 | 0.173$^{ab}$±0.005 | 0.161$^{b}$±0.003 |
| HDL[3], mg | 0.00 | 0.00 | 0.00 |
| Phospholipids, g | 3.03$^{a}$±0.09 | 2.87$^{ab}$±0.08 | 2.69$^{b}$±0.04 |

[1] Means±SEM; n= 3.
[2] LDL: Low density lipoprotein.
[3] HDL: High density lipoprotein.
The means within the same row that have at leost one common letter, do not have significant difference (P>0.05).

Qureshi *et al*. (1984) reported that laying hens fed barley produced eggs with less cholesterol than eggs produced from corn-fed hens which is in agreement with the present findings.

Razdan *et al*. (1997) found that yolk cholesterol concentration decreased with addition of 20 or 30 g/kg chitosan to the diets of hens, reporting that chitosan may have a reductive effect on yolk cholesterol levels, similar to those of vegetable fibers.

In addition, a 20% reduction in cholesterol content was found in the yolks produced by hens fed 14, 21, and 28% chia diets (Ayerza and Coates, 2000), which was attributed to the high fiber content of the chia (60.9% total dietary fiber, on a dry basis, with 7% being soluble and 55.9% insoluble) (Weber *et al*. 1991).

Lipids are synthesized in the liver of a laying hen and transported to the ovary by lipoproteins. Lipoproteins serve as precursors of egg yolk lipid, and plasma very low-density lipoproteins (VLDL) are the major components of egg yolk (Chapman, 1980). Cholesterol is largely synthesized in the liver and like lipids, transported to the growing follicles primarily in the VLDL (McDonald and Shafey, 1989). Moreover, Gallaher *et al*. (1993) reported that the cholesterol-lowering effect of soluble fiber in hamsters was due to the reduction of VLDL cholesterol**.** Accordingly, the lowering effect of barley malt rootlets on cholesterol and lipids concentrations of egg yolk, in the present study is more likely a secondary consequence arising from its lowering effects on cholesterol and triglycerides in plasma (Table 2).

## CONCLUSION

It could be concluded that inclusion of barley malt rootlets in laying hen diets at 25.5 g/kg diet tended to decrease cholesterol and low-density lipoproteins in egg yolk (by 5.2 and 3.9%, respectively), while its inclusion at 51.1 g/kg decreased cholesterol and triglycerides concentrations in plasma and also decreased cholesterol and low-density lipoproteins in egg yolk (by 10.6 and 10.4%, respectively).

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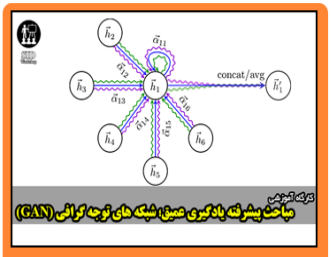


**مباحث پیشرفته یادگیری عمیق؛ شبکه های توجه گرافی (Graph Attention Networks)**