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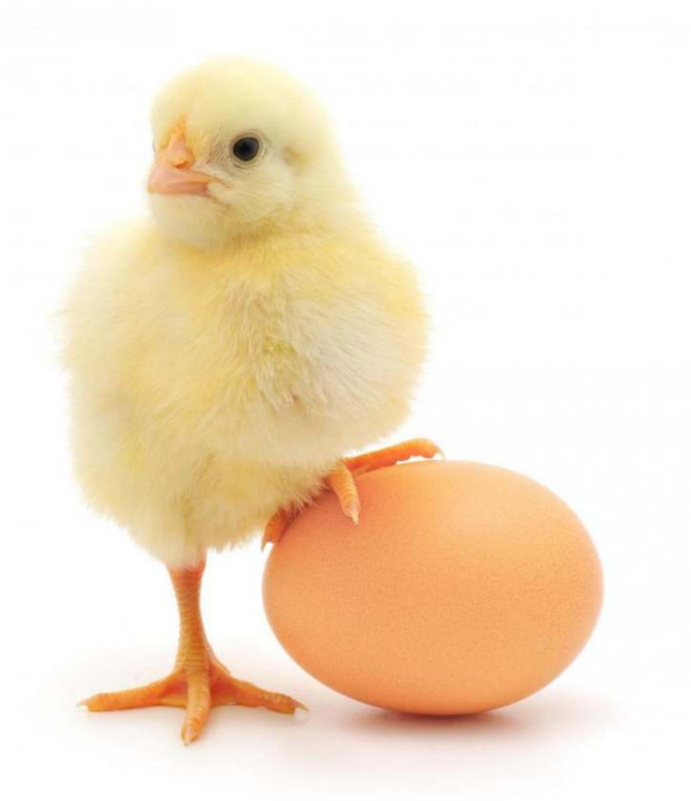

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Lipid Composition of Egg Yolk and Serum in Laying Hens Fed Diets Containing Black Cumin (*Nigella sativa*)

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Abstract: Laying hens were fed diets without or with 10 or 30 g of the whole seed of black cumin (*Nigella sativa*)/kg. The concentrations of total lipids, total cholesterol, phospholipids and triacylglycerols in serum and egg yolk were measured. Feeding of the diets with 1 and 3% black cumin seeds for a period of three months reduced egg yolk total cholesterol by 34 and 42%, respectively. Serum cholesterol concentrations averaged for the whole feeding period were lowered by 15 and 23% after feeding the diets with 1 and 3% black cumin seeds, respectively. Black cumin seeds in the diet of laying hens also caused a lowering of serum and egg-yolk concentrations of triacylglycerols and phospholipids. Inclusion of black cumin seeds in the diet caused a significant reduction in egg production, without any effect on egg width and length, while there was a significant increase in hen's body weight. The increase in body weight in the hens fed black cumin seeds is explained by the ingested feed energy not used for egg production. It is concluded that black cumin seeds and/or the active principle are of interest as potential egg-yolk cholesterol-lowering agents.

Key words: Laying hens, lipids, egg yolk, serum cholesterol

Introduction

Egg lipids are confined to the yolk and account for about 30% of the fresh weight of yolk and for 60% of the yolk dry matter (Leskanish and Noble, 1997). The fatty acid composition of egg lipids in laying hens can be influenced predictably by the fatty acid composition of the diet (Bavelaar and Beynen, 2004; Beynen, 2005). In contrast, attempts to manipulate the total amount of fat or the cholesterol content of eggs through diet change have been only marginally successful (Scott and Leo, 1993; Van Elswyk *et al*., 1992; Farran *et al*., 1995, Stewart, 1998). In a review of the literature, Stewart (1988) stated that breeding selection may reduce egg yolk cholesterol by up to 5% and that the possible range of reduction by diet change and drugs is in the order of 5-10%. In later work, the feeding of a synthetic HMG-CoA reductase inhibitor to laying hens has been demonstrated to lower egg-yolk cholesterol content by up to 30% (Elkin *et al*., 1993). High levels of serum cholesterol in man are associated with increased risk of coronary heart disease. It is also known that the intake of egg cholesterol contributes to an elevation of the level of serum cholesterol (Beynen and Katan, 1989). Thus, it is considered relevant to find ways to produce eggs low in cholesterol (Stewart, 1988).

The liver of the layer hen produces most of the lipids found in egg yolk, the lipids being transported to the ovary by serum lipoproteins (Elkin, 1997). Thus, a decrease in serum lipid concentrations might lead to a decrease in egg-yolk lipids. The oil component of black cumin (*Nigella sativa*) seeds, when administered orally to rats, has been shown to lower serum cholesterol and triacylglycerol concentrations (El-Dakhakhny *et al.,* 2000). The feeding of powdered black cumin seeds to laying hens has been shown to lower serum cholesterol and triacylglycerol concentrations, which indeed was associated with a decrease in egg yolk cholesterol contents (Akhtar *et al*., 2003). As far as we know, the publication of Akhtar *et al*. (2003) provides the only information in the literature on the effect of black cumin seeds on serum and egg-yolk lipids in laying hens. This prompted us to perform the present study on the effect of feeding diets either without or with full black cumin seeds on the concentrations total lipids, cholesterol, triacylglycerols and phospholipids in serum and egg yolk obtained from laying hens.

Materials and Methods

Sixty-three single-comb White Leghorn hens aged 68 weeks were used. They were kept in raised wire cages (30 x 46 cm). There were three birds per cage. The cages were placed in an open-sided house. Additional lighting was provided to obtain a photoperiod of about 16 hours light and 8 hours dark. The birds were allotted to one of three dietary treatments for a period of three months. Table 1 shows the ingredient composition of the control diet, which also had served as pre-experimental feed. The experimental diets were obtained by mixing the control diet with intact black cumin seeds in either a 99:1 or 97:3 (w/w) ratio. The

Table 1: Ingredient composition of the control diet

Ingredient	
Sorghum (g/kg)	510
Groundnut concentrate (g/kg)	240
Wheat bran (g/kg)	105
Oyster shell (g/kg)	90
Sodium chloride (g/kg)	5
Concentrate[1] (g/kg)	50

[1]Composition: crude protein, 560 g/kg; crude fat, 80 g/kg; crude fibre, 30 g/kg; ash, 160 g/kg.

Table 2: Calculated composition of the control diet without black cumin and that of the experimental diets

	Black cumin (g/kg diet)		
Macronutrient	0	10	30
Dry matter (g/kg)	862	863	882
Crude protein (g/kg)	202	202	206
Crude fat (g/kg)	26	29	36
Crude fiber (g/kg)	39	39	40
Ash (g/kg)	131	130	131

[1]The composition of the diets containing added black cumin was calculated using the ingredient composition of the control diet and the reported composition of black cumin seeds. The composition of the seeds is as follows (g/kg): dry matter, 945; crude protein, 213; crude fat, 355; crude fiber, 55; ash, 38 (Babayan *et al.*, 1978).

seeds were bought on a local market and their viability was confirmed. The diets were provided on a restricted basis, each cage being supplied with 300 g/day, i.e. on average 100 g/bird/day. Water was provided *ad libitum*. Eggs were collected each day at 15.00 hours during the entire experimental period. For chemical analysis, 10 eggs were randomly selected per treatment group at the beginning and at the end of the experiment. Blood samples were taken every two weeks, the first samples being collected at two weeks after the beginning of the experiment. On the days of sampling, 10 chickens per treatment group were randomly selected and bled from the wing vein. The blood was allowed to clot at room temperature for one hour and was then centrifuged at 2700xg for 15 minutes after which the serum was collected.

Total lipids in egg yolk and serum were determined according to Frings *et al.* (1972). Total cholesterol was measured by the method of Kim and Goldberg (1969). The weight of cholesterol was taken to be 387 g/mol. Phospholipids were determined as described by Connerty *et al.* (1961). To calculate the molar concentration of phospholipids in serum, the weight was assumed to be 775 g/mol. Triacylglycerols were analyzed with the use of a test combination (Biosystem Reagents and Instruments, Barcelona, Spain) based on the enzymatic method described by Fossati and Prencips (1982). The weight of triacylglycerols was taken to be 850 g/mol. Egg length and width were measured with a caliper.

For statistical evaluation, the data were analyzed by the General Linear Models (GLM) procedure using software of the SAS Institute Inc. (1982). $P < 0.05$ was pre-set a criterion of statistical significance.

Results

Table 2 shows the macronutrient composition of the diets. The addition of black cumin to the diet had no major impact on dietary composition except that the highest inclusion level markedly raised the fat content. Final body weights of the hens fed black cumin were about 10% higher than those of their counterparts fed the control diet (Table 3). The feeding of black cumin seeds caused a dose-dependent drop of egg production (Table 4). The length and width of the eggs were not affected by black cumin (Table 4).

The time courses of the concentrations of the various serum lipids were irregular, but the general picture was that dietary black cumin seeds produced a decrease in serum lipids. To enhance clarity, the mean values for each time point per treatment group were averaged. Table 5 shows that inclusion of black cumin seeds in the diet caused a decrease in all lipid classes, the decrease being dose-dependent for cholesterol and phospholipids. The dietary concentration of 3% black cumin lowered serum cholesterol by on average 23% and serum phospholipids by 30%.

Initial egg-yolk lipid concentrations were similar for the three treatment groups (Table 6). At the end of the experimental period, the feeding of black cumin seeds had produced dose-dependent decreases in egg-yolk concentrations of total lipids, cholesterol and triacylglycerols. The diet with 3% black cumin seeds had induced decreases in egg-yolk total lipids, cholesterol, phospholipids and triacylglycerols by 34, 45, 11 and 20%, respectively.

Discussion

Total lipids content of egg yolk in the control group was found to be 30% of the total egg yolk weight. This value corresponds with that reported by others (Ternes and Scholtyssek, 1994; Cunningham and Lee, 1978). Inclusion of black cumin in the diet at levels of 10 and 30 g/kg significantly reduced the content of egg yolk total lipids by 19 and 34%, respectively. Cholesterol concentration of egg yolk in the control group was similar to values reported by Van Elswyk *et al.* (1991) and Panada and Singh (1990), but lower values were found by Scott and Leo (1989) and Marks and Washburn (1977). Generally, with the Liebermann-Burchard method as used in this study, higher values are measured than with analysis based on gas-liquid chromatography (Scott and Leo, 1989). In the hens given the diets with 10 and 30 g black cumin seeds/kg for three months, cholesterol contents of egg yolk were lowered by 34 and 45%, respectively.

Thus, the feeding of black cumin seeds to laying hens

Table 3: Body weight of laying hens fed the experimental diets

	Black cumin (g/kg diet)		
	0	10	30
Initial (g)	1286±14	1300±7	1270±30
Final (g)	1260[a]±13	1384[b]±7	1376[b]±9

Means ± SEM; n = 21 hens.
[ab]Values within a line with different superscript letter are significantly different (P<0.01)

Table 4: Effect of feeding black cumin on egg production during the entire three-month experimental period

	Black cumin (g/kg diet)		
Egg characteristic	0	10	30
Hen-day egg production (%)	67[a]±1.9	61[b]±1.6	56[c]±1.0
Egg length (mm)	56.9±1.3	57.3±0.8	58.6±0.6
Egg width (mm)	43.3±0.1	42.5±0.4	42.9±0.4

Means ± SEM, n = 7 cages (egg production) or n = 10 eggs (egg length and width). [abc]Values with different superscript letter are significantly different (P<0.01).

was found to lower egg yolk cholesterol by about 35%. Would the use of low-cholesterol eggs as produced by hens fed black cumin have any impact on human health? The average daily cholesterol intake by adults is about 300 mg. When whole egg consumption equals three eggs/week and when a normal egg provides 250 mg of cholesterol, then the contribution of eggs to cholesterol intake is 107 mg/day. When low-cholesterol eggs providing 163 mg of cholesterol are consumed, daily cholesterol intake would be 263 mg. The expected decrease in serum cholesterol is 0.05 mmol/l which equals a 0.83% lowering at an initial serum cholesterol concentration of 6 mmol/l. As a rule of thumb, a 1% lowering in serum cholesterol may cause a 2% decrease in coronary heart disease in middle-aged men. Thus, a 0.83% lowering of serum cholesterol will decrease the risk of coronary heart disease in middle-aged men by 1.66% which, on a population level, has a considerable impact in westernized countries.

As to the mechanism by which black cumin seeds may lower egg yolk cholesterol, we can only speculate. Feeding the seeds to the laying hens reduced serum cholesterol concentrations by about 20%. It is reasonable to suggest that the decrease in egg yolk cholesterol is secondary to the decrease in serum cholesterol which is the precursor for egg yolk cholesterol. Why does black cumin seed lower serum cholesterol? The seeds may either inhibit *de novo* cholesterol synthesis or stimulate bile acid excretion. It is well-known that both effects would lead to a decrease in serum cholesterol (Beynen *et al.*, 1987). Further research is necessary to identify the mode of action of black cumin seeds.

The amount of triacylglycerols and phospholipids comprised about 55 and 30% of the egg yolk total lipid contents, which is similar to the values reported by Leskanish and Noble (1997). Feeding black cumin seeds caused significant reductions in the concentrations of egg yolk triacylglycerols and phospholipids. The seeds were also found to lower the serum concentrations of triacylglycerols and phospholipids. It could thus be suggested that the decrease in serum lipids had caused a decrease in egg yolk lipids. It is not known how black cumin seeds lower the contents of triacylglycerols and phospholipids in serum, but it is tempting to speculate that a component of the seeds inhibits in the liver the flux of acetyl-CoA into the lipogenic pathway. Such an effect would also dampen cholesterol synthesis.

Akhtar *et al.* (2003) reported that inclusion of black cumin seeds in the diet to a level of 1.5% raised hen-day egg production from 59 to 77%. In contrast, in this study treatment with black cumin decreased the rate of egg production by up to 16%. The discrepancy may be explained by the fact that the lowering of egg-yolk cholesterol after feeding black cumin seeds was much greater in this study than in that reported earlier by Akhtar *et al.* (2003). After administration of a synthetic HMG-CoA reductase inhibitor to laying hens egg-yolk cholesterol was reduced by 30% and egg production by 20% without affecting egg weight (Elkin *et al.*, 1993). It is reasonable to suggest that cholesterol is needed by the hen for the maintenance of egg production. Possibly, there is a maximum reduction of the cholesterol content of egg yolk at which eggs are no longer produced. It would appear that the lower concentrations of serum cholesterol seen in this study were still sufficiently high to sustain egg formation albeit that total egg production had dropped. Unfortunately, the eggs were not weighed, but egg weight may not have been changed as was observed in the study of Elkin *et al.* (1993). In any event, there was no effect on the width and length of the eggs, indicating that egg shell formation was unaffected.

There was a significant increase in hen body weight after feeding the diets containing black cumin seeds. It may be concluded that black cumin seeds may not have toxic effects, at least not at the inclusion level fed in this study. The increase in body weight seen after feeding black cumin seeds may be explained by storage of feed energy not used for egg production. For the birds fed the highest level of black cumin seeds versus the control hens, the expected increase in body weight would be 119 g as based on the following calculations. The amount of metabolizable energy not used for egg production equals 5544 kJ. This value is based on a lesser egg production of 594 g/90 days while assuming that egg weight is 60 g, the energy value of an egg is 420 kJ and the efficiency of metabolizable energy conversion into egg is 75%. The extra energy requirement for maintenance due to the increase in body weight is estimated as 3167 kJ. This value is based on an increase in body weight by 106 g for an average period

Table 5: Effect of dietary black cumin seeds on serum lipid concentrations in laying hens

	Black cumin (g/kg diet)		
Serum lipid	0	10	30
Total lipids (mg/dl serum.)	1513±112	1236±71	1225±121
Cholesterol (mmol/l serum.)	4.06±0.39	3.44±0.38	3.12±0.44
Phospholipids (mmol/l serum)	5.15±0.57	4.56±0.70	3.61±0.55
Triacylglycerols (mmol/l serum)	7.92±0.90	6.42±0.61	6.98±0.12

Means ± SEM, n = 6 time points with average values for 10 hens each

^{ab}Values within a line with different superscript letter are significantly different (P<0.05)

Table 6: Effect of dietary black cumin seeds on egg-yolk lipid concentrations

	Black cumin (g/kg)		
Egg yolk lipid	0	10	30
Total lipids (mg/g egg yolk)			
Initial	327±11	309 ± 10	302±13
Final	303 ^{a}±10	246^{b}±11	200^{c}±13
Cholesterol (mg/g egg yolk)			
Initial	17±0.7	18±0.4	17±0.3
Final	20^{a}±0.5	13^{b}±0.2	11^{c}±0.3
Phospholipids (mg/g egg yolk)			
Initial	106±1	107±2	106±1
Final	89^{a}±2	68^{b}±3	79^{c}±13
Triacylglycerols (mg/g egg yolk)			
Initial	182±22	192±3	181±7
Final	173 ^{a}± 3	150^{b}±4	138^{c}±5

Means ± SEM, n = 10 eggs

^{abc}Values in the same line with different superscript letter are significantly different (P<0.01)

of 45 days and a energy requirement for maintenance of 380 kJ/kg metabolic weight. Because feed intake was identical for the control hens and their counterparts fed the highest amount of black cumin, the latter animals had an excess of 2377 kJ which is equivalent to 1782 kJ in the form of extra weight, when assuming a 75% efficiency of energy incorporation. When the energy content of the weight gain is 15 kJ/g, then 119 g would be gained. This value corresponds well with the measured increase in body weight of 106 g. In contrast to this study, Akhtar *et al.* (2003) found that the feeding of black cumin seeds lowered body weight of the hens, which was associated with an increase in egg production.

In conclusion, this study shows that black cumin seeds effectively lowered egg yolk lipids, confirming the data published by Akhtar *et al.* (2003). Further research may focus on the identification of the active principle in black cumin. Knowledge of the chemical structure of the active principle could be important for the development of new hypocholesterolemic drugs and for studies on the mechanisms controlling cholesterol metabolism.

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