Effects of Dietary Tamarind on Cholesterol Metabolism in Laying Hens

S. R. Chowdhury,* D. K. Sarker,† S. D. Chowdhury,† T. K. Smith,*,[1] P. K. Roy,‡ and M. A. Wahid†

*Department of Animal and Poultry Science, University of Guelph, Ontario, Canada, N1G 2W1;
†Department of Poultry Science, and ‡Department of Biochemistry,
Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ABSTRACT An experiment was conducted to evaluate the potential for dietary tamarind to alter serum and egg yolk cholesterol concentrations and overall performance in different layer strains. Thirty, 43-wk-old, Hisex Brown, ISA Brown, Lohmann Brown, Starcross Brown, Babcock B-300, and Starcross-579 strains (5 hens per strain) were fed diets supplemented with 0 (control), 2, 4, 6, or 8% oven-dried tamarind for 6 wk. Egg production, egg mass, and efficiency of feed utilization followed a quadratic response with a maximum when the diet contained 2% tamarind and a minimum when 8% tamarind was fed ($P < 0.05$). There were no differences ($P > 0.05$) among strains for egg production, egg weight, yolk weight, egg mass, feed consumption, or feed efficiency. Yolk weight increased linearly ($P < 0.05$) with increasing levels of dietary tamarind in wk 1, 2, and 3 as well as when averaged over 6 wk. Egg yolk cholesterol concentrations were not affected by dietary tamarind. Serum cholesterol concentrations, however, decreased quadratically with increasing levels of dietary tamarind ($P < 0.05$). It was concluded that 2% supplemental dietary tamarind could decrease serum cholesterol concentrations and increase layer performance.

(*Key words*: cholesterol, layer, tamarind)

2005 Poultry Science 84:56–60

INTRODUCTION

Eggs contain many essential nutrients, such as protein, calcium, phosphorus, retinol, α-tocopherol, folate, and other B vitamins. Egg yolk contains sterols, phospholipids, and triglycerides. The yolk from a large egg may contain 213 mg of cholesterol (USDA, 1991). Over 95% of the yolk cholesterol is associated with triglyceride-rich lipoproteins (Graffin, 1992). To avoid elevations in blood cholesterol and reduce the risk of coronary heart disease, consumption of no more than 300 mg of cholesterol daily and limited consumption of eggs has been recommended (Weggemans et al., 2001). The poultry industry has continued to seek to reduce egg cholesterol concentrations so that an egg with reduced cholesterol will be available to those consumers who need to lower their dietary cholesterol intake. Egg yolk cholesterol concentrations have been shown to vary depending on genetics of the laying hen (Chowdhury et al., 2002). Genetic selection of hens for lower egg cholesterol has resulted in a slight reduction in egg cholesterol concentration. This is also, however, associated with a decline in egg production (Hargis, 1988). The use of nutritional strategies to reduce egg cholesterol concentrations is an attractive alternative.

Spices exhibit a wide range of physiological and pharmacological properties in addition to enhancing the taste and flavor of foods (Sambaiah and Srinivasan, 1991a). Spices have also been found to intensify salivary flow and gastric juice secretion and hence aid in digestion (Srinivasan and Sambaiah, 1991). Tamarind (*Tamarindus indica*), which is sour, is one of the common spices used in food preparation in India. Tamarind has been used since ancient times not only as a flavoring agent but also as a preservative and for its medicinal properties (Ayres et al., 1980). Tamarind has also been reported to act as an antiviral agent in the early stages of rubella virus infection (Mastromarino et al., 1997). Antibacterial activities of tamarind against *Bacillus subtilis* and *Escherichia coli*, as well as antiyeast activity of tamarind against *Saccharomyces cerevisiae* have also been reported (De et al., 1999).

To our knowledge, the effect of dietary tamarind on cholesterol metabolism in laying hens has not been investigated. The current study was conducted, therefore, to determine the efficacy of tamarind as a hypocholesterolemic agent in different strains of laying hens.

MATERIALS AND METHODS

Experimental Birds and Diets

Thirty pullets that were 43 wk old and from 6 different strains (5 birds per strain, 6 birds per diet) were used in

Received for publication June 4, 2004.
Accepted for publication September 22, 2004.
[1]To whom correspondence should be addressed: tsmith@uoguelph.ca.

TABLE 1. Composition of experimental diets (%)

	Dietary tamarind (%)				
Ingredient	0	2	4	6	8
Wheat	55.00	54.00	53.00	54.00	52.00
Rice polish	19.00	18.00	17.00	14.00	14.00
Soybean seed, heat processed	10.50	10.50	10.50	10.50	10.50
Fish meal	4.00	4.00	4.00	4.00	4.00
Sesame meal	4.00	4.00	4.00	4.00	4.00
Tamarind[1]	0.00	2.00	4.00	6.00	8.00
Oyster shell	5.50	5.50	5.50	5.50	5.50
Bone meal	1.50	1.50	1.50	1.50	1.50
Common salt	0.25	0.25	0.25	0.25	0.25
Vitamin-mineral mixture[2]	0.25	0.25	0.25	0.25	0.25
Calculated values					
ME (kcal/kg)	2,903	2,895	2,885	2,877	2,870
Lysine	0.74	0.74	0.70	0.73	0.73
Methionine	0.35	0.36	0.35	0.34	0.34
Calcium	2.83	2.78	3.00	2.94	2.96
Total phosphorus	0.73	0.75	0.76	0.76	0.75
Analyzed values					
Dry matter	89.8	89.2	88.8	88.3	87.8
Crude protein	16.46	16.51	16.40	16.27	16.22
Cholesterol (μg/g)	84	83	86	80	82

[1]Analyzed tamarind values: dry matter, 66%; crude protein, 8.92%; crude fat, 0.16%; crude fiber, 8.47%; soluble carbohydrate, 77.14%; ash, 5.3%. Calculated ME value was 2,830 kcal/kg.

[2]Provided per kilogram of diet: vitamin A (retinyl palmitate), 12,000 IU; cholecalciferol, 2,500 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K_3, 4.0 mg; thiamin, 1.5 mg; riboflavin, 5.0 mg; pantothenic acid, 10.0 mg; niacin, 30 mg; pyridoxine, 4.0 mg; choline chloride, 250 mg; folic acid, 0.5 mg; biotin, 220 μg; vitamin B_{12}, 12 μg; butylated hydroxytoluene, 250 mg; manganese, 48 mg; zinc, 40 mg; iron, 24 mg; copper, 16 mg; iodine, 0.6 mg; selenium, 0.12 mg; DL-methionine, 100 mg.

the current study. The strains included Lohmann Brown, Babcock B-300, ISA Brown, Hisex Brown, Starcross-579, and Starcross Brown.[2] Hens were caged individually and provided with 14 h of light daily. All the birds were fed isoenergetic and isonitrogenous mash diets for 6 wk; the diets were formulated to meet or exceed the nutrient requirements of laying hens (National Research Council, 1994). Diets were supplemented with 0 (control), 2, 4, 6, or 8% oven-dried (6 h at 70°C), ripe, and seedless tamarind in crushed form. Compositions of the experimental diets are shown in Table 1. Feed and water were provided ad libitum.

Experimental Parameters Measured

Birds were randomly assigned to diets. Body weights, feed consumption, and feed efficiency were measured weekly during the 6-wk experiment. Daily egg production was recorded. Egg weights were determined weekly, and egg mass was calculated. Eggs were collected weekly for cholesterol analysis. Yolk weights were recorded individually, and yolk was extracted and analyzed for cholesterol concentration.

Blood Collection

Blood was collected from each bird weekly from the wing vein using sterilized syringes and needles. Serum was isolated 4 to 6 h after blood collection. Serum samples were maintained at −5°C for up to 4 d until cholesterol analysis.

[2]Bangladesh Agricultural University Poultry Farm, Mymensingh, Bangladesh.

Lipid Extraction from Diet and Yolk

Fifteen milliliters of chloroform:methanol (2:1 vol/vol) was blended with 1 g of yolk using a vortex mixer and allowed to extract for 12 h. Diet lipid was extracted by the same procedure using a 5-g sample with 40 mL of chloroform:methanol.

Cholesterol Analysis

Serum, extracted yolk, and diet samples were analyzed for cholesterol according to the colorimetric method of Abell et al. (1952).

Analysis of Dietary Nutrients

Diets were analyzed for dry matter and CP according to the methods of the AOAC (1980).

Statistical Analyses

Data were analyzed by ANOVA using the GLM procedure of SAS software for a factorial arrangement (2-factor analysis, diet and strain) (Kuehl, 1994; SAS Institute, 2000). Orthogonal polynomial contrasts were used to determine the nature of the response to diets. Differences among strains, when significant, were also ordered using Tukey's test (Kuehl, 1994). Statements of statistical significance were based on $P < 0.05$.

TABLE 2. Effects of dietary tamarind and genetic strain on performance of laying hens[1]

Diet and strain	Egg production (%)	Egg weight (g)	Egg mass[2] (g/d/hen)	Feed intake (g/d/hen)	Feed efficiency[3]
0% tamarind	75	59	44	115	2.60
2% tamarind	80	59	47	113	2.43
4% tamarind	76	58	44	115	2.63
6% tamarind	75	60	45	115	2.58
8% tamarind	62	59	37	114	3.15
Linear	0.01	NS[4]	0.007	NS	0.002
Quadratic	0.02	NS	0.01	NS	0.006
Pooled SD	9	3	4	1	0.26
Strain[5]					
Lohmann Brown	71	61	43	114	2.69
Babcock B-300	79	57	45	114	2.58
ISA Brown	69	60	42	114	2.77
Hisex Brown	71	59	42	115	2.83
Starcross-579	76	59	44	115	2.60
Starcross Brown	76	59	44	114	2.59

[1]Values are least square means as averaged over 6 wk; n = 6 for diet, n = 5 for strain.

[2](Egg production × egg weight)/100.

[3]Feed intake/egg mass (g/g).

[4]$P > 0.05$.

[5]There was no significant effect of strain on laying hen performance.

RESULTS AND DISCUSSION

It was observed that cholesterol concentrations in all diets were very low and similar to that found in the control diet (Table 1).

Layer Performance

Egg production, egg mass, and feed efficiency as averaged over 6 wk responded quadratically to increasing levels of dietary tamarind ($P < 0.05$). Egg weights and feed consumption, however, were not affected by diet (Table 2). Feeding lower levels of dietary tamarind increased egg production and egg mass. The efficiency of feed use also increased when birds were fed lower levels of dietary tamarind due to increased egg production. It has been shown that the feeding of tamarind can intensify salivary flow and gastric juice secretion and thereby enhance digestion (Srinivasan and Sambaiah, 1991). Tamarind might also improve gut health by decreasing the population of pathogenic microorganisms (De et al., 1999). Feeding lower levels of dietary tamarind increased layer performance compared with controls, whereas the feeding of higher levels decreased layer performance. The decreased layer performance when birds were fed higher levels of dietary tamarind might be due to the metabolic burden of excretion of tamarind metabolites, as no lesions were observed. Chowdhury and Smith (2001) reported similar results when laying hens were fed high levels of dietary putrescine.

In the current study there were no ($P > 0.05$) strain effects on egg production, egg weight, egg mass, feed consumption, or feed efficiency. Chowdhury et al. (2002), however, reported significantly higher egg production in

TABLE 3. Effects of dietary tamarind and genetic strain on egg yolk weight (g)[1]

Diet and strain	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1–6
0% tamarind	13.7	13.7	13.9	14.2	14.1	14.3	14.0
2% tamarind	13.7	13.9	14.1	14.4	14.4	14.3	14.1
4% tamarind	14.6	14.7	14.8	14.5	14.3	14.0	14.5
6% tamarind	14.7	14.8	14.8	14.5	14.3	14.0	14.5
8% tamarind	14.6	14.6	15.1	14.8	14.5	14.5	14.7
Linear	0.007	0.001	0.007	NS	NS	NS	0.01
Quadratic	NS[2]	NS	NS	NS	NS	NS	NS
Pooled SD	0.8	0.6	0.8	0.7	0.5	0.6	0.5
Strain[3]							
Lohmann Brown	14.5	14.5	15.0	14.8	14.6	14.5	14.7
Babcock B-300	13.6	13.9	14.2	14.4	14.3	14.5	14.2
ISA Brown	14.8	14.7	14.7	14.6	14.4	14.2	14.6
Hisex Brown	14.4	14.5	14.7	14.6	14.3	14.0	14.4
Starcross-579	13.9	14.3	14.1	14.2	14.1	14.0	14.1
Starcross Brown	14.3	14.0	14.3	14.3	14.2	14.0	14.2

[1]Values are least square means; n = 6 for diet, n = 5 for strain.

[2]$P > 0.05$.

[3]There was no significant effect of strain on egg yolk weight.

TABLE 4. Effects of dietary tamarind and genetic strain on egg yolk cholesterol concentrations (mg/g)[1]

Diet and strain	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1–6
0% tamarind	14.9	14.7	14.8	14.8	14.9	14.8	14.8
2% tamarind	14.8	14.9	14.8	14.9	14.8	14.8	14.8
4% tamarind	14.9	14.6	14.9	15.0	14.7	14.9	14.8
6% tamarind	14.7	14.8	14.7	14.9	14.6	14.6	14.7
8% tamarind	14.8	14.7	14.5	14.7	15.0	14.9	14.7
Linear	NS[2]	NS	NS	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS	NS
Pooled SD	0.2	0.3	0.3	0.2	0.4	0.3	0.1
Strain							
Lohmann Brown	15.1[a]	14.9[a]	14.9[a]	15.0[a]	14.9[a]	14.9[a]	14.9[a]
Babcock B-300	15.0[a]	14.9[a]	14.9[a]	15.0[a]	15.0[a]	15.0[a]	14.9[a]
ISA Brown	14.4[b]	14.3[b]	14.3[b]	14.5[a]	14.5[a]	14.6[a]	14.3[b]
Hisex Brown	14.8[ab]	14.8[ab]	14.8[ab]	15.0[a]	14.9[a]	14.5[a]	14.8[a]
Starcross-579	14.6[ab]	14.5[ab]	14.5[ab]	14.6[a]	14.7[a]	14.8[a]	14.6[ab]
Starcross Brown	15.1[a]	15.0[a]	15.0[a]	15.1[a]	14.8[a]	15.1[a]	15.0[a]

[a,b]Means with the same superscript within a column are not significantly different.

[1]Values are least square means; n = 6 for diet, n= 5 for strain.

[2]$P > 0.05$.

the Babcock B-300 strain compared with other strains. It is likely that the discrepancy was due to reduced egg production at the end of the production cycle and high individual variability in the current study.

Egg Yolk Weight

Egg yolk weights increased linearly with increasing levels of dietary tamarind in wk 1, 2, and 3 as well as overall (Table 3). Increased egg yolk weights might have been due to increased nutrient use arising from feeding of dietary tamarind. Increased egg yolk weight, however, did not result in changes in egg weight. There were no significant effects of strain on egg yolk weight, in agreement with results published by Chowdhury et al. (2002).

Egg Yolk Cholesterol Concentrations

The feeding of tamarind did not result in any differences in yolk cholesterol concentrations (Table 4). Yolk cholesterol concentrations in control eggs were comparable with those reported by Jiang et al. (1991), who found 14.6 mg of cholesterol/g of yolk in eggs laid by White Leghorn hens. There were significant differences among strains in yolk cholesterol concentrations in wk 1, 2, and 3, as well as averaged over 6 wk. Strain variation in egg yolk cholesterol concentrations might be due to inherent genetic differences or the interaction between diet and strain. Han and Lee (1992) observed significantly lower yolk cholesterol concentrations in hens laying brown eggs.

Serum Cholesterol

Serum cholesterol concentrations responded quadratically ($P < 0.01$) with increasing levels of dietary tamarind (Table 5). Lower levels of dietary tamarind might have reduced serum cholesterol concentrations by increasing the conversion of cholesterol to bile acids. It has been shown that feeding of 2.5% tamarind stimulated the rate of bile flow and increased secretion of bile acids in rats

TABLE 5. Effects of dietary tamarind and genetic strain on serum cholesterol concentrations in laying hens (mg/100 mL)[1]

Diet	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1–6
0% tamarind	209	210	210	211	211	210	210
2% tamarind	191	189	188	187	184	182	187
4% tamarind	193	190	188	186	184	182	187
6% tamarind	190	188	186	184	183	181	185
8% tamarind	188	187	185	183	182	179	184
Linear	0.003	0.001	0.001	0.001	0.001	0.001	0.001
Quadratic	0.01	0.006	0.002	0.008	0.003	0.001	0.001
Pooled SD	8	8	7	7	7	7	7
Strain[2]							
Lohmann Brown	195	194	193	191	191	189	192
Babcock B-300	191	190	189	188	187	184	188
ISA Brown	193	192	189	187	187	185	189
Hisex Brown	198	196	195	192	190	189	193
Starcross-579	193	193	191	191	189	187	191
Starcross Brown	194	193	192	190	189	185	190

[1]Values are least square means; n = 6 for diet, n= 5 for strain.

[2]There was no significant effect of strain on serum cholesterol concentrations in laying hens.

(Sambaiah and Srinivasan, 1991b). No changes in serum cholesterol concentrations were observed, however, when rats were fed 2.5% tamarind for 1 mo (Sambaiah and Srinivasan, 1991a). It was likely that the inclusion level of dietary tamarind or the duration of the experiment were not sufficient to show a significant hypocholesterolemic effect in rats.

The reduction of serum cholesterol when tamarind was fed to the laying hens might also be due to a reduction in synthetic enzyme activity. Qureshi et al. (1983) reported a dose-dependent inhibition of hepatic β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase, and fatty acid synthetase when chickens were fed polar fractions of garlic powder equivalent to 1, 2, 4, 6, and 8% fresh garlic paste. The feeding of 2, 4, 6, or 8% oven-dried and crushed tamarind decreased serum cholesterol concentrations 12 to 14%. Chowdhury et al. (2002) reported that feeding of 2, 4, 6, or 8% sun-dried garlic paste reduced serum cholesterol concentrations by 15, 28, 33, and 43%, respectively. The serum cholesterol concentrations of laying hens fed the control diet in the current study (209 to 211 mg/100 mL) were slightly lower than those reported by Chowdhury et al. (2002) (230 to 243 mg/100 mL). There were no differences among strains in serum cholesterol concentrations in the current study in contrast to the results of Chowdhury et al. (2002). The contradictory results might be due to interaction between dietary garlic and strains of laying hens in studies reported by Chowdhury et al. (2002).

The physiologically active components in tamarind have not yet been determined. Further studies are needed to chemically and pharmacologically characterize the components. Organoleptic studies are also needed to determine the effects of tamarind on egg color and flavor. We concluded that 2% supplemental dietary tamarind could decrease serum cholesterol concentrations and increase the performance of laying hens.

ACKNOWLEDGMENTS

Financial support for this experiment was provided by Bangladesh Agricultural University, Mymensingh, Bangladesh. Statistical advice of Ian McMillan is gratefully acknowledged.

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