Efficacy of neem leaf meal as a hypocholesterolemic dietary additive in laying pullets

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Abstract

An experiment was carried out with laying pullets to investigate the effects of feeding neem (*Azadirachta indica*) leaf meal (NLM) at different dietary concentrations on cholesterol metabolism and productivity of laying chickens. Forty, 26-week old laying pullets belonging to Shaver 579 strain was fed NLM for 12 weeks. Five dietary treatments, each of four replications, containing either 0, 5, 10, 15 or 20g NLM per kg were compared. The birds were reared in a pyramid-type laying cage. Blood and eggs were collected three times maintaining four weeks interval for the determination of cholesterol concentration respectively in serum and egg yolk. Eggs from birds laid after 6 weeks of feeding NLM were used for the determination of internal and external characteristics of eggs. Body weight gain, egg weight, total egg mass, FCR, survivability, and internal and external egg quality characteristics were studied. Use of NLM at 10 and 15 g/kg showed negative quadratic effect ($P<0.01$) on total lipids and yolk cholesterol after 4 weeks and 8 weeks of supplementation. Similar result was obtained for serum cholesterol ($P<0.05$) after 8 weeks of supplementation. Analysis of performance data showed no significant difference from control group except a linear decrease in feed consumption ($P<0.01$). A linear increase ($P<0.05$) in albumen index and a positive quadratic effect ($P<0.01$) on Haugh unit were also found. Based on the result, it may be concluded that NLM could be considered as a hypocholesterolemic dietary feed additive with dietary inclusion levels between 10 and 15 g/kg.

Key words: Neem, layer, cholesterol, additive, pullet

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Introduction

The egg fat is often criticized for its cholesterol content. Daily consumption of no more than 300mg of cholesterol has been recommended to avoid elevation in blood cholesterol and reduce the risk of coronary heart disease (Weggemans et al. 2001). An egg contains about 200-250mg cholesterol and the perception to eggs by the public as a major source of dietary cholesterol is seen as a significant factor contributing to the overall decline in its consumption (Yaffee et al. 1991). Poultry nutritionists are trying to reduce cholesterol in eggs by various ways. This has involved a number of different approaches, including genetic selection and nutritional or pharmacological manipulation. It has been found that a reduction of yolk cholesterol by 34% was found when laying hens were fed with pharmacological amounts of copper (Pesti and Bakalli, 1998). It has been indicated that feeding hens with whole chia seed (*Salvia hispanica* L.) up to 28% of the diet resulted in a maximal reduction in yolk cholesterol (Ayerza and Coates, 2000). Reduction of yolk cholesterol by 15, 28, 32 and 43% was possible when hens were fed garlic paste at 2, 4, 6 or 8 g/kg, respectively (Chowdhury et al. 2002). The production of "Omega Eggs" was possible by feeding hens a flaxseed-based diet, contained 180mg cholesterol (Lewis et al. 2000). Neem, a large evergreen fast growing perennial tree, is native to Bangladesh and inhabitant of Southeast Asia. Neem leaves have relieved so many different pains, fevers, infections and other complaints that it has been called the "village pharmacy". Generally, NLM contain (on dry matter basis) crude protein 185.1 to 206.8, crude fiber 174.2 to 245.7, ether extract 24 to 45, nitrogen free extract 406 to 502 and ash 65 to 72 g/kg respectively (Bais et al. 2002). A very limited research results are available on NLM as a hypocholesterolemic agent in laying hens. Interest to consider NLM as a hypocholes-terolemic agent arose from a broiler study where a significant reduction in abdominal fat was observed (Chowdhury et al. 2004). Therefore, an attempt was taken in this study to investigate the hypocholesterolemic effect of neem leaf meal and its effects on egg quality and laying performance.

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Materials and Methods

Collection, processing and storage of NLM

The fresh green neem leaves were harvested from neem trees. The leaves were cleaned, made free of stems and sun-dried on a polyethylene sheet and stored in a polyethylene bag until used for diet formulation.

Experimental birds and diets

Forty, 26-week old laying pullets of Shaver 579 strain were used to conduct the feeding trial. Chickens were almost similar with regard to body weight. The birds were reared in cages measuring 42×40×42 cm. Total duration of feeding trial was 12 weeks. Two birds were kept in each cage and considered as an experimental unit (replicate). Five dietary treatments, each of four replications, containing either 0, 5, 10, 15 or 20g NLM per kg feed were compared. Diets were identical in composition that met or exceeded the nutrient requirements of laying pullets and were prepared based on NRC feeding standards.

Measurement of experimental parameters

The birds were exposed to almost similar care and management in all dietary groups throughout the experimental period. Egg production and feed consumption were recorded daily, while body weight was recorded twice in a month, initially and at the end of trial and egg weight was recorded weekly. Feed conversion ratio, body weight gain and livability were calculated. Eggs were collected once in every 4 weeks' interval for cholesterol analysis. Eggs for the determination of internal and external quality characteristics were collected at 32 weeks of age and preserved at 4 to 8^0C until analyses were carried out.

Blood collection and separation of serum

Blood (3-4ml) sample was collected from each bird in every 4 weeks' interval from the jugular vein, serum were separated and stored at -12 to -20^0C until cholesterol analysis.

Chemical analysis of egg yolk and blood serum

About 3g of hard-cooked yolk sample was taken in a centrifuge tube and sonnicated with 45ml of chloroform: methanol (2:1, v/v) solvent mixture and was kept overnight for complete extraction of lipid. Total lipids in egg yolk were determined according to Solver et al. (1978). Cholesterol concentration in egg yolk was determined following *Liebermann-Burchard* method developed by Solver et al. (1978). Serum cholesterol was determined with the help of diagnostic kits (Cholesterol Liquicolor Complete Kit, HUMAN GMBH-GERMANY).

Statistical analysis

Data were analyzed by analysis of variance using the General Linear Model Procedure of SAS (SAS 1997). Orthogonal polynomial contrasts were used to determine the nature of the responses to increasing concentrations of NLM in the diets. Least Significant Difference (LSD) was calculated where significant variation in any trait was observed between different diet groups.

Results and Discussion

Cholesterol and related parameters

Yolk weight

The mean values of yolk weight were close to each other and did not differ significantly except at 38 weeks of age when a positive quadratic effect ($P<0.01$) was found. It was interesting to note that yolk weight slightly increased up to the level of NLM 10g/kg and then reduced at 38 weeks of age. Feeding garlic to Japanese quail of different layer strains as hypocholesterolaemic agents showed results similar to this study.

Total lipids in egg yolk

The highest and the lowest values were 30.2 g/100g yolk for the control and 23.7 g/100g yolk for 10 g/kg level at 34 weeks. The results showed a negative quadratic effects ($P<0.01$) in all weeks. The lipid content decreased up to the point of 10 g/kg at 30 and 34 weeks and then increased while decreased up to the point of 15 g/kg at 38 weeks and then increased. The results clearly indicate that lipid content in NLM fed layers may decline up to certain levels.

Serum cholesterol concentration

Although serum cholesterol concentrations showed a declining trend due to dietary inclusion of NLM, no statistical differences in data were apparent at 30 and 34 weeks of age. But serum cholesterol concentration showed a negative quadratic result ($P<0.05$) at 38 weeks of age

(Table 2). In this case, it was reduced by 26 and 31% at 10 and 15 g/kg levels respectively. It has been stated that a reduction of 30% serum cholesterol in human was possible by feeding neem (Chowdhury et al. 2004) which seemed to be closer to 10 and 15g/kg feeding levels as observed in this study. The result indicates that NLM has a hypocholesterolemic effects.

Egg yolk cholesterol

The effect of NLM on egg yolk as hypocholesterolemic ingredient is shown in Table 2. Significant negative quadratic effects (P<0.01) of NLM was observed at 30 and 38 weeks of age. At 34 weeks, similar trend in result was also noted although it was not statistically significant. The result obtained from yolk cholesterol showed that a reduction of 16% during 30 and 34 weeks and 18% during 38 weeks of age with 10 g/kg supplementation level was possible. It indicates that dietary supplementation of NLM up to 10 g/kg level had the depleting effect on egg yolk cholesterol.

Egg quality characteristics

The data on egg quality characteristics of laying pullets fed different dietary levels of NLM are shown in Table 3. Shell colour of different dietary groups including control group were deep brown. It was not affected by NLM. Mean values of shell quality characteristics were found to be similar including that of control group. Analysis of data on Haugh unit values showed statistically significant (P<0.01) results. Mean values of Haugh unit showed that there was a positive quadratic effect (P<0.01). Albumen index was found to be linearly increased (P<0.01) as the dietary concentration of NLM was increased. The data on internal egg quality characteristics suggest that both Haugh unit and albumen index improved due to supplementation of NLM in the diet. There was no effect on yolk color score.

Table 1. Yolk weight (g) and total lipids in yolk (g/100g yolk) of laying pullets fed different levels of dietary NLM (26-38 weeks)

Parameter	Age (week)	Dietary levels of NLM (g/kg)					Linear effect	Quadratic effect
		0	5	10	15	20		
Yolk weight (g/egg)	30	19.1	18.1	20.1	19.1	18.2	NS	NS
	34	18.0	18.6	18.7	18.3	19.1	NS	NS
	38	16.6^{b}	19.3^{ab}	20.0^{a}	19.2^{ab}	18.1^{ab}	NS	**
Total lipid in yolk (g/100g)	30	29.7^{a}	26.2^{b}	25.1^{b}	27.0^{b}	29.6^{a}	NS	**
	34	30.2^{a}	27.6^{bc}	23.7^{d}	25.7^{cd}	29.7^{ab}	NS	**
	38	30.1	27.5	26.4	25.5	29.3	NS	**

*Means with uncommon superscript differed significantly; ** P<0.01; NS, non-significant.*

Table 2. Serum cholesterol (mg/dl) and yolk cholesterol concentrations (mg/g yolk) of laying pullets fed different levels of dietary NLM (26-38 weeks)

Parameter	Age (week)	Dietary levels of NLM (g/kg)					Linear effect	Quadratic effect
		0	5	10	15	20		
Serum cholesterol (mg/dl)	30	238.0	187.0	172.0	163.0	217.0	NS	NS
	34	241.0	207.0	215.0	212.0	207.0	NS	NS
	38	285.0	230.0	210.0	196.0	241.0	NS	*
yolk cholesterol (mg/g yolk)	30	13.2^{a}	13.1^{a}	11.1^{b}	12.4^{a}	13.3^{a}	NS	**
	34	13.5^{a}	12.9^{ab}	11.4^{b}	11.6^{ab}	12.2^{ab}	NS	NS
	38	14.1^{a}	12.1^{b}	11.6^{b}	11.3^{b}	13.2^{ab}	NS	**

*Means with uncommon superscript differed significantly; ** P<0.01; *P<0.05; NS, non-significant*

Table 3. Egg quality characteristics of laying pullets fed diets containing NLM at different dietary levels (26 -38 weeks)

Variables	Dietary levels of NLM (g/kg)					Linear effect	Quadratic effect
	0	5	10	15	20		
Egg weight (g/egg)	61.0	62.5	62.0	57.5	61.5	NS	NS
Shell weight (g/egg)	6.2	6.3	6.5	5.7	6.4	NS	NS
Percent shell	10.2	10.2	10.5	10.0	10.0	NS	NS
Shell thickness (mm)	0.38	0.39	0.39	0.35	0.36	NS	NS
Shape index (%)	77.0	75.7	75.3	74.1	75.6	NS	NS
Albumen index	0.06	0.07	0.08	0.08	0.08	**	NS
Haugh Unit	66.3^{b}	75^{a}	77.8^{a}	76.5^{a}	76.8^{a}	**	**
Yolk index	0.40	0.38	0.38	0.39	0.40	NS	NS
Yolk colour score	6.5	7.5	7.5	6.5	7.5	NS	NS

*Means with uncommon superscript differed significantly; **, P<0.01; NS, non-significant*

Table 4. Productive performance of laying pullets fed diets containing NLM at different levels (26 -38 weeks)

Variables	Dietary levels of NLM (g/kg)					Linear effect	Quadratic effect
	0	5	10	15	20		
Feed consumption (g/bird/d)	117.0^{a}	115.0^{ab}	116.0^{abc}	112.0^{bc}	112.0^{c}	**	NS
Hen day egg production (%)	78	79	79	75	77	NS	NS
Egg weight (g/egg)	57.4	57.8	59.1	57.9	58.0	NS	NS
FCR	2.59	2.48	2.47	2.60	2.53	NS	NS
Body weight gain (g/day)	63.0	69.0	71.0	75.0	83.0	NS	NS

*Means with uncommon superscript differed significantly; ** P<0.01; NS, non-significant*

Laying performance

Supplementation of NLM in the diet linearly decreased feed consumption (P<0.01). The result showed that addition of NLM in the diet above 10 g/kg significantly reduced feed consumption as compared to control group (Table 4). Reduced feed consumption of broilers at a dietary level of 20 g/kg NLM was reported previously (Chowdhury et. al. 2004). The mean values of egg production and egg weight were almost similar to that of control group. These results agreed with the results of feeding garlic reported previously (Ayerza and Coates 2000). Garlic oil based diet did not affect egg weight significantly (P<0.05) and use of sun-dried garlic paste in six layer strains could not alter egg weight. In this study, NLM up to 20 g/kg levels although could not affect FCR in spite of a significant negative linear effect on feed consumption. The lower egg production at 15 and 20 g/kg levels with lower feed consumption probably contributed to this result. Feeding sun-dried garlic paste could not significantly affect FCR among the strains (Ayerza and Coates 2000). In spite of little increases in body weight at 15 and 20 g/kg level, no significant effect of NLM was apparent in this study.

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