Journal of Science and Technology 12(June 2014): 12-17

EFFECT OF DIETARY MULBERRY LEAF MEAL ON EGG QUALITY OF LAYING HENS

M. Kamruzzaman[1*], M.A. Khatun[1], M.S. Islam[2], M.Z. Rahman[1] and T. Yeasmin[1]

[1]Department of Dairy and Poultry science, Hajee Mohammad Danesh Science and Technology University
[2]Department of Anatomy and Histology, Hajee Mohammad Danesh Science and Technology University

Received 28 November 2013, revised 30 May June 2014, accepted 26 June 2014

ABSTRACT

The study was performed to determine the effects of various dietary levels of mulberry (*Morus alba* L.) leaf meal on production performance, egg qualities and egg-yolk cholesterol. In this study, forty eight 30-wk-old laying hens (Hi-sex brown) were divided into 4 dietary groups each with 4 replications (3 birds/replication) and offered manually prepared diets supplemented with 0, 3, 6 and 9% mulberry leaf meal for 8 weeks. Eggs were collected and weighed daily. Laying performance, egg quality and feed conversion ratio were evaluated. Results showed that the feed intake, egg production, egg weight, egg mass, feed conversion ratio, live weight and egg qualities were not significant ($P<0.05$) among the treatment groups. However, the egg yolk cholesterol concentration was significantly decreased ($P<0.05$) in accordance with increasing level of mulberry leaf meal in diet. Egg-yolk cholesterol decreased at 9.4, 12.5 and 14.8% with 3, 6 and 9% dietary supplementation of mulberry leaf meal respectively. It was observed that the supplementation of mulberry leaf meal up to the investigation level of (9%) potentiality reduced egg-yolk cholesterol without showing any adverse effect on egg production performance and egg quality.

Key words: Egg quality, egg-yolk cholesterol laying performance, mulberry leaf meal

INTRODUCTION

Poultry production is one of the most important sectors of livestock that provides cheapest animal protein (egg and meat) for human consumption within the shortest period of time. Poultry production has greatly flourished during last three decades in Bangladesh. However, the acute dearth of fluctuating feed supply and their price are the major constraints to poultry production in developing countries like Bangladesh. The feed cost usually constitutes the major proportion which ranges between 60-75% of the total cost of poultry production (Ojewola *et al.* 2005). The prices of conventional protein source feed ingredients such as groundnut cake; fish meal and soybean meal are always high and cannot permit profit maximization in poultry ventures. In view of this, current research interest in the poultry industry is aimed to find alternatives to those elusive feed ingredients. Mulberry leaf (*Morus alba)* may be such an alternative source of dietary protein for commercial livestock and poultry operations. It is a tree fodder which grows well in the tropics and subtropics. It is reported to have excellent nutritional value as forage. It is grown extensively for its leaves which are used for raising silkworms in the sericulture industry. Mulberry leaf meal is rich in protein (15-35%), minerals (Ca: 2.42-4.71%; P: 0.23-0.97%) and metabolizable energy (1130-2240 kcalkg^{-1}) with absence of or negligible anti-nutritional factors (Sarita *et al.* 2006). Mulberry leaf contains carotene which can be converted with varying efficiency by animals to vitamin A as well as xanthophylls which may have potentiality in pigmentation of egg yolk (Sarita *et al.* 2006).

Cholesterol is biosynthesized in the liver of laying hens and secreted into the plasma in the form of very low-density lipoproteins (VLDL) which transfer to the ovary and form high cholesterol containing yolk. Egg-yolk cholesterol has been shown to vary with species of bird, breed or strain as well as age of fowl. Egg-yolk cholesterol contents can be altered by (i) genetic selection such as upward direction method or selection of hens that produce low-cholesterol eggs and (ii) diet alteration. Mulberry leaf also contains phytosterols (plant sterols) which are structurally similar to cholesterol that act in the intestine to lower cholesterol absorption and helps in reduction of cholesterol in the blood vessels (Ray, 2003). So, mulberry leaf diets may inhibit the synthesis of cholesterol and fatty acids in the liver. Thus, the

**Corresponding author: Dr. Md. Kamruzzaman, Lecturer, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Cell phone: +8801774731456, Email: mitoodvm@gmail.com*

mulberry leaf could be supplemented in laying hen diet. However, there is limited research works regarding the effect of mulberry leaf meal with optimum inclusion level on egg quality and cholesterol content. Therefore, the present study investigated the effect of dietary mulberry leaf meal in laying hen performance, egg quality as well as cholesterol content in egg.

MATERIALS AND METHODS

This study was conducted at the Poultry farm and the laboratory of Poultry Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. A total 48 Hi-sex brown laying hens (age 30 weeks) were assigned into four dietary treatment groups with four replications of three (3) birds in each. The experimental birds were housed in cages. The cages and the poultry house were disinfected and fumigated properly before placing the birds. Mulberry leaves were collected from the local area of Dinajpur district. The leaves were initially cut into small pieces and then sun-dried for about fifteen (15) days. The sun-dried mulberry leaves were milled into a powder. The diets were formulated to as per recommendation of the National Research Council (NRC, 1994) to satisfy the nutrients requirement of the laying hens. Diets were supplied with 0 (control), 3, 6 and 9% sun-dried mulberry leaf meal and fed for 8 weeks. Water was provided *ad libitum.* The chemical composition of the experimental diets is shown in the Table 1. During the experimental period, eggs were collected and weighed daily. Data on feed intake were collected weekly. Initial and final live weights of birds were taken. The eggs used in the experiment were collected per hen on day zero (0) and after 15 days interval up to two months. Egg production recorded daily but external and internal quality characteristics of eggs were determined bi-weekly. Egg weight, shape index, shell dry weight, shell thickness, albumin index, fresh albumin weight, yolk index, fresh yolk weight and Haugh unit were measured. For quality determination, egg weight was recorded by an electric weighing balance. The length and width of eggs were measured by a slide calipers. Then the eggs were carefully broken down on a glass plate (40×20 cm) to determine the internal egg qualities. Feed conversion ratio (FCR) was calculated as gram feed consumption per day per hen divided by egg mass per day per hen. Egg mass was calculated by multiplying egg weight by egg production. Haugh unit was determined according to Nesheim *et al.* (1979) by using the formula. Cholesterol content of extracted yolk was determined by spectrophotometric method (Libermann-Burchard reaction) as described by (Kenny 1952).

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1980). The significance differences between the treatment means were calculated by the Duncan's Multiple Range Test (Duncan, 1955). All analyses were performed by MSTAT-C Program.

RESULTS AND DISCUSSION

Laying performances: The result regarding laying performance were shown in Table 2.

Egg production: The egg production was not differed significantly (P>0.05) among the layer groups fed the diets supplemented with different levels of mulberry leaf meal. The result indicates that the feeding of mulberry leaf meal up to 9% in the diet of laying hen has no detrimental effect on egg production. Feeding of mulberry leaf meal up to 6% levels showed slightly higher egg production whereas the production was slightly decreased when the birds received 9% mulberry leaf meal in the diet. These results are closed to the previous report of Lokaewmanee *et al.* (2009), however slightly differed from the observations of Ravindran *et al.* (1986), who found decreased egg production with the increased level of the mulberry leaf meal. Similarly, egg production of White Leghorn birds was not different from the control groups by feeding up to 9% mulberry leaf meal (Suda T, 1999).

Egg weight: The egg weight in different dietary treatments during experimental periods did not differ significantly (P>0.05). The results indicate that inclusion of mulberry leaf meal up to 9% in the diet of laying hens has no inimical effect on egg size. However, feeding of mulberry leaf meal with higher levels showed a tendency to reduce egg weight. The results are consistent with the report of Tateno *et al.* (1999) and Sudo *et al*. (2000). Both the studies found no significant difference in egg size after the birds exposed to 15% mulberry leaf meal in the diet.

Egg mass output: The results of the present study showed that the egg mass output (gm/hen/day) with the inclusion of 3-6% mulberry leaf meal was improved but slightly decreased with 9% mulberry leaf meal, although the differences were not significant(P>0.05). The results are agreement with the report of Tateno *et al*. (1999) and Sudo *et al.* (2000).

Live weight: During the experimental period live weight in different dietary treatments was almost similar and the differences were not significant (P>0.05). These results indicate that inclusion up to 9% mulberry leaf meal had no adverse effect on live weight. However, the live weight slightly increased in the dietary treatment with 6% mulberry leaf meal in comparison to control. This is in agreement with the results of Machii (2000) who observed no adverse effect of mulberry leaf meal on body weight

when mulberry leaves were given as part of the diet to domestic fowl.

Feed intake: Feed intake of laying hens in different dietary treatments during experimental period was almost statistically similar and the differences were not significant (P>0.05). The result clearly showed that mulberry leaf meal up to 9% dietary level had no bad effect on feed consumption. Similar results have been observed by Lokaewmanee *et al.* (2009), who found that there was no adverse effect in feed intake compared to control. But, Ravindran *et al.* (1986), Limcangco-Lopez (1989), Udedibie and Opara (1998), Odunsi (2003) and Akande *et al.* (2007) reported a reduction trend in feed intake with increased dietary leaf meals in the diets for broilers and laying hens. A decrease in feed intake for increased levels of mulberry leaf may be due to bulkiness and unpalatable taste which may affect the appetite of the birds.

Feed conversion ratio: Feed conversion ratio in different dietary treatments at 3, 6 and 9% level was almost similar and the differences were not significant (P>0.05). The results indicate that there was no detrimental effect on feed conversion ratio after feeding up to 9% of mulberry leaf meal. This is in agreement with the results of Machii (2000) who also observed no adverse effect of mulberry leaf meal on feed conversion ratio (FCR) when mulberry leaves were given as part of the diet to domestic fowl.

External and internal egg quality: It was observed that the shape index, shell thickness, albumin weight, albumin index, yolk weight, yolk index and Haugh unit (HU) of the eggs laid by hens fed different diets were almost similar during the experimental period (Table 3). These results indicate that feeding of mulberry leaf meal up to 9% had no adverse effect on external and internal qualities of eggs. However, egg shell weight (g) decreased slightly after supplementation of 3-9% mulberry leaf meal. But egg shell thickness slightly improved at the level of 6% mulberry leaf meal. Albumin weight decreased in the dietary treatments 3 and 6% but a little bit increased in dietary treatment 9% from those of control groups. Albumin index was improved slightly after inclusion of mulberry leaf meal in comparison to control. Moreover, yolk weight and yolk index were slightly decreased due to the inclusion level of mulberry leaf meal 3-9% but yolk index slightly improved at 6% level of mulberry leaf meal. Haugh unit was decreased in the laying hens group fed diet supplemented with 6% mulberry leaf meal but improved in the group fed diet supplemented with 9% of mulberry leaf meal. Similar results have been obtained by Tateno *et al.* (1999) and Sudo *et al.* (2000) who did not found any significant differences in the external and internal qualities of eggs up to 9% of mulberry leaf meal.

Egg-yolk cholesterol: This result showed that egg-yolk cholesterol was decreased significantly by supplementation of mulberry leaf meal in layer-ration (P<0.05). It is evident from Table-3 that a tendency of reduced egg-yolk cholesterol was observed in the dietary treatments with inclusion of 3-9% mulberry leaf meal. However, the highest level of cholesterol was 11.6 mg g^{-1} at 3% level and lowest level was 10.9 mg g^{-1} of egg-yolk at 9% of mulberry leaf meal whereas cholesterol of "control egg" ranged from as low as 10 mg g^{-1} of yolk to as high as 18 mg g^{-1} of yolk (USDA, 2008). During the experimental periods egg-yolk cholesterol was reduced to 0.8 mg/gm, 1.2 mg g^{-1} and1.5 mg g^{-1} after dietary supplementation of 3-9% of mulberry leaf meal as compared to the control. Thus, the result of the study clearly showed that mulberry leaf meal at 3, 6 and 9% dietary level had beneficial effect in reduction of egg yolk cholesterol. The similar results obtained from (Machii 1990) who found reduced egg-yolk cholesterol at 2% level of mulberry leaf meal. Liver is the organ that regulates the deposition of lipids and phospholipids in egg-yolk (Bell and Freeman 1971). Since liver and serum cholesterol decreased by supplementation of mulberry leaf meal which may lead in decreasing egg-yolk cholesterol. Thus, the reduction of egg-yolk cholesterol by dietary mulberry leaf meal supplementation may be due to a lesser deposition of cholesterol by liver in egg-yolk during yolk synthesis. Mulberry leaves contain phytosterol that is responsible for lower absorption of cholesterol from the intestine resulting lower deposition of cholesterol in egg-yolk. As a result, cholesterol of egg-yolk was reduced.

Table 1. Chemical composition of experimental diets

Feed ingredients	Dietary level of mulberry Leaf Meal (MLM)			
	T_1(Kg) (0%)	T_2(Kg) (3 %)	T_3(Kg) (6 %)	T_4(Kg) (9 %)
Maize	53	53	51	51
Soybean meal	22.6	21.1	20.1	20.1
Rice polish	11.5	10	10	9
Meat & bone meal	4	4	4	2
Oyster shell	7.8	7.8	7.8	7.8
DCP	0.75	0.75	0.78	0.75
Mulberry leaves	0	3	6	9
Salt	0.35	0.35	0.35	0.35
Vitamin-mineral premix*	*	*	*	*
Calculated composition:				
ME (Kcal/Kg)	2727.9	2742.3	2693.9	2698.5
CP (%)	17.77	17.53	17.06	16.69
CF (%)	3.28	3.52	3.05	3.20
Ca (%)	3.51	3.45	3.6	3.49
P (%)	0.45	0.50	0.70	0.46
Lysine (%)	0.94	0.96	0.90	0.85
Methionine (%)	0.28	0.32	0.34	0.35

*Added vitamin-mineral premix (Rena-Layer; Renata Animal Health Ltd.) @ 250 g per 100 kg which contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin k_3: 800 mg; vitamin B_1: 600 mg; vitamin B_2: 2 mg; vitamin B_3: 12 mg; vitamin B_5: 3.2 mg; vitamin B_6: 1.8 mg; vitamin B_9: 2 mg; vitamin B_{12}: 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL – Methionine: 20 mg; L- lysine:12 mg.

Table 2. Effect of mulberry leaf meal (*Morus alba*) on laying performance

Parameters	Mulberry Leaf Meal (MLM) (%)				Level of significance
	T_1 0% (Control)	T_2 3 % MLM	T_3 6 % MLM	T_4 9 % MLM	
Live weight (gm)	1745 ± 23.4	1730 ± 18.3	1754 ± 15.8	1742 ± 20.1	NS
Egg production (%)	88.48 ± 0.70	88.53± 0.67	88.78 ± 0.59	88.32 ± 0.45	NS
Feed intake (gm/hen/d)	112.8 ± 2.45	112.3 ± 2.08	111.8 ± 2.03	112.2 ± 2.31	NS
Egg weight (gm/egg)	63.40 ± 0.53	63.33 ± 0.51	62.84 ± 0.44	63.36 ± 0.41	NS
Egg mass (gm/hen/d)	56.84 ± 1.72	56.92 ± 2.10	56.86 ± 1.56	56.31 ± 1.60	NS
Feed conversion ratio (gm feed /gm egg)	1.98 ± 0.07	1.97 ± 0.05	1.95 ± 0.04	1.96 ± 0.08	NS

Values are expressed as mean ± standard error of means.
NS: Not significant (P >0.05). Means represent four replicates, three birds per replicate.
MLM= Mulberry Leaf Meal.

Table 3. Effect of mulberry leaf meal (*Morus alba*) on quality characteristics of egg

Parameters	Mulberry Leaf Meal (MLM) (%)				Level of significance
	T_1 0% (Control)	T_2 3 % MLM	T_3 6 % MLM	T_4 9 % MLM	
Egg Shell weight (gm/egg)	6.56 ± 0.09	6.41 ± 0.12	6.53 ± 0.09	6.50 ± 0.11	NS
Shape index (%)	80.04 ± 0.67	80.54 ± 0.86	81.59 ± 0.64	79.93 ± 0.83	NS
Shell thickness (mm)	0.40 ± 0.02	0.40 ± 0.02	0.41 ± 0.00	0.40 ± 0.01	NS
Albumin weight (gm/egg)	37.06 ± 0.77	36.87 ± 0.67	36.87 ± 0.70	37.86 ± 0.67	NS
Albumin index (%)	8.35 ± 0.28	8.40 ± 0.32	8.55 ± 0.38	8.40 ± 0.30	NS
Yolk weight (gm/egg)	17.24 ± 0.09	17.09 ± 0.08	17.12 ± 0.1	17.15 ± 0.11	NS
Yolk index (%)	42.7 ± 0.42	42.5 ± 0.36	42.9 ± 0.39	41.3 ± 0.35	NS
Yolk Cholesterol (mg/gm)	12.8 ± 0.28^a	11.6 ± 0.29^b	11.2 ± 0.32^b	10.9 ± 0.28^c	*
Haugh unit (%)	89.07 ± 2.33	88.56 ± 1.92	88.85 ± 2.12	89.19 ± 2.30	NS

Values are expressed as mean ± standard error of means.
NS: Not significant (P >0.05). Means represent four replicates, three birds per replicate.

CONCLUSION

Based on the results of present study it may be concluded that mulberry leaf is a good source of protein and it has significant effect on the reduction of egg yolk cholesterol of laying hens without affecting the bird's feed intake, body weight and egg quality characteristics. The results of the study may suggest that supplementation of mulberry leaf meal (*Morus alba* L.) up to the investigation level of 9% in diets has high potential as commercial applications for production of low-cholesterol containing eggs. Therefore, mulberry leaf meal may be used along with the other conventional feed ingredients. However, further study is to be needed to understand the active principle(s) of cholesterol lowering and other beneficial effects of mulberry leaf meal observed in this study prior to the practical use of it as unconventional feed for poultry.

REFERENCES

Akande TO, Adeyeri M.K, Longe OG, Odunsi AA. 2007. Response of laying chickens to graded levels of Tephrosia bracteolate leaf meal fed with soybean meal or full fat soybean meal. Livestock Research for Rural Development. 19:108.

Bell DJ and Freeman BM. 1971. Physiology and Biochemistry of Domestic Fowl. Vol. 1, 2. Academic Press London, UK.

Duncan DB. 1955. Multiple Ranges and Multiple F test. Biometric. 11: 5-42.

Kenny AP. 1952. The determination of cholesterol by the Liebermann-Burchard reaction. Biochem. J. 52: 611-619.

Limcangco-Lopez PD. 1989. The use of shrubs and tree fodders by non-ruminants. pp 61-73 in Proc. Workshop Shrubs and tree fodders for farm animals, Denpasar, Indonesia.

Lokaewmanee K, Mompanuon S, Khumpeerawat P, Yamauchi K. 2009. Effects of dietary mulberry leaves (*Morus alba* L.) on egg yolk color, Journal of poultry science. 46:112-115.

Machii H. 1990. On gamma-aminobutyric acid contained in mulberry leaves. Journal of Sericulture Science (Japan). 59: 381-389.

Machii H. 2000. Evaluation and utilization of mulberry for poultry production in Japan. In: Mulberry for Animal Production. FAO Animal Production & Health Roma, Italy. 147: 241-248.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC, USA.

Nesheim MC, Austic RE, Card IE. 1979. Poultry production. 12th ed. Lea Febiger, Philadelphia, PA, USA.

Odunsi AA. 2003. Assessment of Lablab purpureus leaf meal as a feed ingredient and yolk coloring agent on the diet of layers. International Journal of Poultry Science. 2:71-74.

Ojewola GS, Okoye FC and Ukoha OA. 2005. Comparative utilization of three animal protein sources by broiler chickens. Int. J. Poult. Sci. 4970: 462-467.

Ravindran V, Kornegay ET, Rajaguru ASB, Potter LM and Cherry JA. 1986. Cassava leaf meal as a replacement for coconut oil meal in broiler diets. Poultry Science. 65:1720-1727.

Ray Sahelian MD. 2003. Phytosterols supplements side effects and benefits, role in cholesterol metabolism and prostate health. Nutrition Research Division, Food Directorate, Health Products and Food Branch, Health Canada, Banting Research Centre, Ottawa, Ontario K1A 0K9, Canada.

Sarita S, Rashmi K, Anju T. and Rajesh Prakash. 2006. Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). International Journal of Food Science and Nutrition. 57(5/6): 305-313.

Singh B, Makkar and HPS. 2001. The potential of different levels of cocoa bean shell on the mulberry tree foliage as animal feed supplement haematological characteristics of mature cocks. in India. Indian Veterinary Research Institute, Proceedings of 11 Annual Conference of Animal Regional Station, Palamper 176061. Himachal Science Association; Ibadan, Sept. 4: 35-37.

SPSS. 2006. Statistical Package for Social Scientists. Version 14.0. SPSS Inc., Chicago, IL, USA.

Steel RDG. and Torrie JH. 1980. Principles and procedures of statistics. New York, McGraw-Hill. 137- 191: 236-372.

Suda T. 1999. Inhibitory effect of mulberry leaves on ammonium emission from poultry excrement. Abstracts of Gunma Agriculture-related Experiment Stations Meeting. 7-8.

Sudo M, Kuramoto H and Iso M. 2000 .Studies on functional poultry eggs. Effects of mulberry leaves on quantity & quality of eggs. Bull. Ibaraki Prefectural Poultry Experiment Station, 35 (in Japanese).

Tateno H, Yatabe T. and Iso M. 1999. Studies on foliage of unused resources. Effects of mulberry leaves on egg production as a poultry food. Bulletin of Ibaraki Prefectural Poultry Experiment Station. 33:15-20 (in Japanese)

Udedibie ABI and Opara CC. 1998. Responses of growing broilers and laying hens to the dietary inclusion of leaf meal from Alchornia cordifolia. Animal Feed Science Technology. 71:157-164.