EC NUTRITION
Research Article

Effects of Dietary Supplementation with Neem and Mulberry Leaf Powders on Production Performance, Egg Quality, and Egg Yolk Cholesterol in Laying Hens

Swarno Shekhor Roy[1], Sabbir Hossen Sabuz[2]*, Mst. Afroja Khatun[1] and Tahera Yeasmin[1]

[1]Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Bangladesh

[2]Department of Animal Science and Nutrition, Hajee Mohammad Danesh Science and Technology University, Bangladesh

***Corresponding Author:** Sabbir Hossen Sabuz, Department of Animal Science and Nutrition, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Rangpur, Bangladesh.

Received: July 17, 2026; **Published:** August 30, 2026

Abstract

Objective: This study evaluated the dietary effect of a fixed level of neem leaf powder (NLP) with graded levels of mulberry leaf powder (MLP) on production performance, egg quality characteristics and egg yolk cholesterol in commercial laying hens.

Methods: A total of 180 laying hens aged 32 weeks were randomly allocated into five treatments in a completely randomized design (CRD). Each treatment consisted of 36 birds with three replicates of 12 birds each. Hens were fed a basal diet supplemented with 0 (control diet), 2.5%, 5.0%, 7.5% and 10% MLP. In addition, all treatment groups except the control received 1.0% NLP in the diet. The feeding trial was conducted for eight weeks. Production performance including hen-day egg production, body weight, egg weight, as well as internal and external egg quality traits and yolk cholesterol were evaluated.

Results: The results showed that dietary supplementation with NLP and MLP had no significant effect ($P > 0.05$) on production performance and most egg quality characteristics compared with the control, although overall numerical improvements were observed. Albumen diameter was the only egg quality trait significantly affected by the dietary treatments ($P < 0.05$). However, egg yolk cholesterol was significantly decreased ($P < 0.05$) with the inclusion of 1% neem leaf powder (NLP) together with increasing levels of mulberry leaf powder (MLP). The greatest reduction was recorded in hens fed the diet with 1.0% NLP and 7.5% MLP.

Conclusion: Supplementation of laying hen diets with 1.0% NLP in combination with MLP effectively reduced egg yolk cholesterol without adversely affecting laying performance or egg quality. Although the greatest cholesterol-lowering effect was achieved with 7.5% MLP, dietary inclusion of MLP up to 10.0% was well tolerated, indicating its potential as a functional feed ingredient for producing low-cholesterol eggs.

Keywords: *Phytogenic Feed Additive; Neem Leaf Powder; Mulberry Leaf Powder; Egg Yolk Cholesterol; Laying Hens*

Introduction

Laying hens play a significant role in the global poultry industry providing one of the most affordable and widely consumed sources of high-quality animal protein through eggs. Together with chicken meat, eggs are consumed by approximately 45% of the world's population, highlighting their substantial contribution to food security and human nutrition [28,36]. Eggs are regarded as nutrient-dense, complete natural foods because of their high nutritional profile, containing superior quality proteins, lipids, vitamins, minerals, other bioactive nutrients required for human health and maintenance of a balanced diet [19,37].

The exceptional nutritional value of eggs is largely attributed to their balanced and unique chemical composition. A typical egg contains approximately 74% water, 13% protein, 11% lipids, and smaller amounts of carbohydrates, vitamins, minerals, and carotenoids. Structurally, it consists of approximately 9 - 12% shell, 60% albumen, and 30 - 33% yolk. The yolk serves as the principal lipid-rich component, with its lipid fraction consisting predominantly of triacylglycerols (63.3%) followed by phospholipids (29.7%), and cholesterol (5.2%).

Despite their high biological value, palatability, and cost effectiveness, eggs have long been the subject of consumer concern because of their cholesterol content, which is almost exclusively localized in the yolk. This misconception regarding dietary cholesterol has contributed to "cholesterol phobia", leading many health-conscious consumers, particularly in Western countries, to limit their egg consumption [7].

These growing concerns have stimulated efforts to develop "designer eggs," to reduce health-negative components such as cholesterol and triglycerides while enriching eggs with health-positive nutrients, including omega-3 fatty acids, antioxidants, vitamins, and minerals [7]. These eggs may be produced by laying hen diets modification through the incorporation of specific additives that play a vital role in improving both egg productivity and quality.

A significant body of research has explored nutritional strategies that improve laying hens' productivity, health, and egg quality. Among the various approaches adopted for the designer eggs production, eggs can also be transformed into functional foods through dietary manipulation using herbs and medicinal plants that have functional and therapeutic properties.

Dietary manipulation of poultry diets can alter the cholesterol content of eggs. Previous attempts to lower egg yolk cholesterol have centered mostly on genetic selection, pharmacological intervention or alteration of the laying hen diets with feed additives that convey positive egg traits. However, genetic selection had limited cholesterol reducing effect [4], and the use of pharmaceutical agents may result in undesirable drug residues in eggs [20]. In this scene, use of phytogenic feed additives to reduce egg cholesterol could be an attractive alternative.

Among the numerous medicinal plants evaluated as poultry feed additives, mulberry (*Morus alba*) has emerged as a promising candidate because of its rich phytochemical profile and nutritional value. Mulberry belongs to the Moraceae family [40]. Mulberry leaf powder has been considered a promising alternative feed ingredient for poultry with a high nutritional value [31,34], owing to its rich content of protein (15 - 35%), minerals (2,42 - 4,71%) and metabolizable energy (1.130 - 2.240 kcal/kg) [30]. Previous studies have demonstrated that dietary supplementation with mulberry leaf powder improves laying performance, feed efficiency, antioxidant status [8,18] and egg quality characteristics [21] while reducing serum and yolk cholesterol concentrations [9].

Similarly, Neem (*Azadirachta indica*), a tropical evergreen tree of the family Meliaceae, has attracted considerable interest as a natural phytogenic feed additive because of its nutritional composition and diverse pharmacological properties. Neem leaves are rich in bioactive compounds, including azadirachtin, nimbin, nimbidin, quercetin, limonoids, carotenoids, and other polyphenolic constituents, which exert antioxidant, hypolipidemic, antimicrobial, hypoglycemic, anti-inflammatory, hepatoprotective, and immunomodulatory functions [1,27,33].

In addition to these functional phytochemicals, neem leaves contain appreciable amounts of crude protein, ether extract, minerals, and other nutrients, making them a valuable feed resource for poultry [7]. Previous studies have demonstrated that dietary inclusion of neem leaf products as a feed ingredient can improve growth performance, feed efficiency, immune status, hematological and serum biochemical indices, and lipid metabolism while reducing cholesterol concentrations in poultry [2]. In laying hens, supplementation with neem leaf powder at levels up to 1% of the diet has been reported to maintain production performance and egg quality without adversely affecting serum biochemical parameters, suggesting its suitability as a safe phytogenic feed additive [11].

Although the beneficial effects of mulberry and neem have been investigated individually in some studies, limited information is available regarding their combined dietary supplementation in commercial laying hens. Furthermore, the potential of this combination to reduce egg yolk cholesterol while maintaining production performance and egg quality has not been adequately investigated.

Therefore, the present study was designed to evaluate the effects of supplementing laying hen diets with a fixed level of neem leaf powder (NLP) and different dietary inclusion levels of mulberry leaf powder (MLP) on production performance, egg quality characteristics, and egg yolk cholesterol concentration. It was hypothesized that this dietary supplementation would reduce egg yolk cholesterol without adversely affecting production performance or egg quality.

Materials and Methods

Preparation of phytogenic feed additives

Mulberry leaves, collected from Dinajpur district of Bangladesh, were chopped into small pieces and then sun dried under the shade. The dried leaves were ground in a laboratory blender. The fresh green neem leaves were harvested from the premises of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. The leaves were cleaned and then sun-dried on a polyethylene sheet. Dirt was separated by handpicking. The dried neem leaves were ground into a fine powder using the same laboratory blender. Both neem leaf powder (NLP) and mulberry leaf powder (MLP) were stored in separate airtight polyethylene bags and preserved in the feed storage room under suitable conditions until used. The required dose of NLP and MLP were thoroughly mixed with the commercial basal diet before feeding.

Experimental design and treatments layout

A Completely Randomized Design (CRD) was employed for the study. A total of 180 laying hens aged 32 weeks were used in the experiment. The experimental birds were randomly allocated into five dietary treatments, with each treatment consisting of 36 birds. Each treatment was further divided into three replicates of 12 birds each. The duration of the experiment was eight weeks. Neem leaf powder was supplemented in each group except control group. Increasing dietary inclusion levels of mulberry leaf powder were provided in each group as 0, 2.5%, 5.0%, 7.5% and 10%. The layout of the experiment is given in table 1.

Replication	Treatment					
	T_0	T_1	T_2	T_3	T_4	
1	12	12	12	12	12	60
2	12	12	12	12	12	60
3	12	12	12	12	12	60
Total	36	36	36	36	36	180

Table 1: *Experimental layout showing distribution of birds and treatments.*

Here T_0-control group (Only basal diet), T_1-basal diet+1% NLP+2.5% MLP, T_2-basal diet+1% NLP+5.0% MLP, T_3-basal diet+1% NLP+7.5% MLP, T_4-basal diet+1% NLP+10.0% MLP.

Experimental diets and management of birds

All the birds were fed a basal diet throughout the experiment. The ingredient and chemical compositions of the basal diet are presented in table 2. The diet was formulated according to the recommendation of the National Research Council [22] to meet the nutrients requirement of the laying hens. The experimental diets were prepared by supplementing the basal diet with neem leaf powder (NLP) and mulberry leaf powder (MLP) according to the study layout and were offered for a period of eight weeks. Clean drinking water was provided *ad libitum*.

Feed ingredients	**Dietary level of mulberry and neem leaf powder**				
	T_0 (Kg)	**T_1 (Kg)**	**T_2 (Kg)**	**T_3 (Kg)**	**T_4 (Kg)**
Maize	55.5	53.5	52	50	47
Soybean meal	22	20	19	20	19
Rice polish	7	7	7	6.5	6.5
Meat and bone meal	7	7	7	6.5	6.5
Lime stone	8	8	8	8	8
DCP	0.75	0.75	0.78	0.75	0.75
Neem leaves	0	1	1	1	1
Mulberry leaves	0	2.5	5	7.5	10
Salt	0.5	0.5	0.5	0.5	0.5
Vitamin-mineral premix*					
Calculated composition:					
ME (Kcal/Kg)	2727.9	2752.10	2762	2774.35	2767.90
CP (%)	17.77	17.53	17.06	16.69	16.23
CF (%)	3.28	3.52	3.05	3.20	3.12
Ca (%)	3.51	3.45	3.6	3.49	3.10
P (%)	0.45	0.50	0.70	0.46	0.53
Lysine (%)	0.94	0.96	0.90	0.85	0.82
Methionine (%)	0.28	0.32	0.34	0.35	0.38

Table 2: *Chemical composition of experimental diets.*

**Added vitamin-mineral premix (Rena-Layer; Renata Animal Health Ltd.) @ 250g per 100 kg.*

Birds were housed in conventional wire cages under standard husbandry conditions. A lighting schedule of 16h light and 8h dark was maintained throughout the study. Adequate ventilation was ensured, and birds were reared under the prevailing environmental conditions of Bangladesh. All birds were vaccinated following standard vaccination schedule. Feed was offered twice daily while clean drinking water was provided *ad libitum*. Uniform management practices were maintained for all treatment groups to minimize environmental variation.

Data collection and parameters recorded

During the experimental period, eggs were collected and weighed daily. Initial and final live weights of birds were taken. The eggs used in the experiment were collected from each hen on day zero and at two-week intervals up to end of the trial. Egg production was recorded daily but external and internal quality characteristics of eggs were determined bi-weekly. Egg weight, albumin height, albumen diameter, fresh albumin weight, yolk height, yolk weight, 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 onto a glass plate (40×20 cm) to determine the internal egg qualities. Haugh unit was calculated using the formula described previously [23].

Determination of yolk cholesterol value

Egg yolk cholesterol concentration was determined at the end of the experiment. Twelve eggs were randomly collected from each treatment, with three eggs obtained from each replicate. Cholesterol was determined by the spectrophotometric Liebermann-Burchard method according to Kenny [16]. Cholesterol concentration was calculated following the procedure described in the reference method. The obtained values were expressed as the mean egg yolk cholesterol concentration for each treatment group and used for subsequent statistical analysis.

Statistical analysis

The collected data were analyzed using one-way analysis of variance (ANOVA) in SPSS (version 26.0). Each replicate cage was considered as the experimental unit for all production performance variables. For egg quality traits, measurements recorded from individual eggs were averaged within each replicate before statistical analysis. Data are presented as mean ± standard error of the mean (SEM). When a significant treatment effect was found, means were compared using Duncan's Multiple Range Test. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Productive performance

The effects of dietary supplementation with neem leaf powder and mulberry leaf powder at different inclusion levels on egg production, egg weight and body weight are presented in table 3. Dietary supplementation with NLP and MLP had no significant ($P > 0.05$) effect on these production parameters compared with the control group, although numerically higher values were observed in the supplemented groups. Similarly, dietary mulberry fiber showed no significant influence on the laying performance and final body weight of tested hens although it improves liver lipid metabolism and egg quality [13]. Laying hens fed a 2.5% combination of moringa and neem leaf meals showed increased egg production intensity [15]. Likewise, no significant differences in egg weight were recorded through supplementation of neem leaf meal [38]. A previous study has shown that laying rate is insignificant when mulberry leaf powder was supplemented below the level of 2% [18]. In addition, no significant differences ($P > 0.05$) were observed in growth performance of laying hens when diet contained 5, 10 and 15% neem leaf meal (NLM). The present findings demonstrated that dietary supplementation with neem leaf powder and mulberry leaf powder did not adversely affect laying performance or body weight of laying hens. Similar observations were reported by [5], who found no significant improvement in egg weight and final body weight with the addition of garlic powder in white leghorn hens.

Parameters	Treatments					Level of significance
	T_0	T_1	T_2	T_3	T_4	
Average weight of egg (g)	62.44 ± 0.26	63.21 ± 0.27	65.27 ± 0.28	64.23 ± 0.21	64.01 ± 0.27	NS
Egg production %	82.25 ± 0.58	85.77 ± 0.69	84.25 ± 0.64	83.50 ± 0.65	84.74 ± 0.52	NS

Initial body weight (kg/bird)	1.73 ± 0.43	1.73 ± 0.33	1.77 ± 0.38	1.75 ± 0.67	1.69 ± 0.24	NS
Final body weight (kg/bird)	1.90 ± 0.94	1.98 ± 0.80	1.96 ± 0.45	1.89 ± 0.65	1.91 ± 0.48	NS

Table 3: *Effect of neem leaf powder and mulberry leaf powder on performance of layer hens.*

All data are presented as mean values with their respective standard errors of the mean (SEM). NS denotes non-significant differences (P > 0.05).

In this experiment, the dietary treatments were as follows:

- *T_0: Basal diet without NLP and MLP.*
- *T_1: Diet supplemented with 1.0% NLP and 2.5% MLP.*
- *T_2: Diet supplemented with 1.0% NLP and 5.0% MLP.*
- *T_3: Diet supplemented with 1.0% NLP and 7.5% MLP.*
- *T_4: Diet supplemented with 1.0% NLP and 10.0% MLP.*

Egg quality traits

The present study showed that dietary neem leaf powder and mulberry leaf powder supplementation had no significant (P > 0.05) effects on most egg quality parameters except for albumen diameter (Table 4). No significant difference in egg weight, shell thickness, shell weight, albumin height, albumin weight, yolk weight, yolk height, and yolk diameter through supplementation of neem leaf meal [35]. 0.1%-0.3% neem leaf powder supplementation through feed can be unaffected egg composition parameters in commercial layers. Dietary supplementation of neem leaf powder did not significantly affect egg composition parameters in commercial layers [30]. that mulberry leaf powder in diets did not adversely affect egg quality or haemato-biochemical parameters [32]. Similar findings were reported in a separate study [17]. Dietary Mulberry fiber had no harmful effects on the laying performance or egg quality in laying hens [13]. Insignificant effect was exhibited on external and internal egg quality by mulberry leaf powder inclusion in ration [21]. Contrary to the present study, egg quality was improved by the addition of mulberry leaf to the diet [39] that may be due to increasing antioxidant capacity and lipid metabolism. Additionally, researchers have found that the addition of more than 10% mulberry leaf powder negatively affected egg quality [3].

Parameters	Treatments					Level of significance
	T_0	T_1	T_2	T_3	T_4	
Egg length (mm)	57.51 ± 1.34	59.31 ± 0.63	62.13 ± 2.54	59.50 ± 1.18	58.27 ± 1.31	NS
Egg width (mm)	45.87 ± 0.98	44.30 ± 0.22	44.73 ± 0.18	44.42 ± 0.93	43.48 ± 0.42	NS
Egg shell weight (g)	6.67 ± 0.19	7.06 ± 0.04	6.51 ± 0.16	6.54 ± 0.04	6.71 ± 0.26	NS
Albumen weight (g)	38.64 ± 0.52	39.09 ± 0.61	39.78 ± 0.48	39.07 ± 0.89	35.08 ± 0.75	NS
Albumen height (mm)	6.79 ± 0.28	8.26 ± 0.39	6.98 ± 0.36	7.70 ± 0.42	6.10 ± 0.37	NS
Albumen diameter (mm)	91.47 ± 2.14^a	89.82 ± 1.84^b	92.83 ± 1.09^a	92.28 ± 1.01^a	91.83 ± 2.07^a	*
Yolk weight (g)	20.83 ± 0.46	15.46 ± 0.60	17.87 ± 0.74	18.01 ± 0.65	16.06 ± 0.88	NS
Yolk height (mm)	17.12 ± 0.66	15.46 ± 0.63	16.68 ± 0.15	17.19 ± 0.50	16.55 ± 0.45	NS
Yolk diameter (mm)	37.43 ± 0.49	37.60 ± 0.36	39.55 ± 0.93	40.95 ± 0.80	40.60 ± 0.52	NS

Haugh unit (%)	81.37 ± 5.02	89.98 ± 1.48	81.82 ± 4.77	86.5 ± 3.18	81.10 ± 4.18	NS
Yolk cholesterol (mg/g)	13.38 ± 0.48^{c}	11.52 ± 0.51^{a}	12.25 ± 0.21^{b}	11.29 ± 0.34^{a}	12.10 ± 0.38^{b}	*

Table 4: *Egg quality parameters and yolk cholesterol of layer chickens fed on diets with neem and mulberry leaf powders as phytogenic feed additives.*

All data are presented as mean values with their respective standard errors of the mean (SEM). NS denotes non-significant differences (P > 0.05). Mean values in the same row with different superscripts (a, b, c) are significantly different at the level of 5% (P < 0.05). A single asterisk (*) indicates statistical significance at the 5% level.

In this experiment, the dietary treatments were as follows:

- *T_0: Basal diet without NLP and MLP.*
- *T_1: Diet supplemented with 1.0% NLP and 2.5% MLP.*
- *T_2: Diet supplemented with 1.0% NLP and 5.0% MLP.*
- *T_3: Diet supplemented with 1.0% NLP and 7.5% MLP.*
- *T_4: Diet supplemented with 1.0% NLP and 10.0% MLP.*

Egg yolk cholesterol

The effect of dietary supplementation of NLP and MLP on egg yolk cholesterol are presented in table 4. It was found in this study that adding NLP and MLP to the diet had a statistically significant (P < 0.05) effect in reducing the egg yolk cholesterol level. Yolk cholesterol was comparatively lower in the groups fed with a diet mixed with 1% NLP and different dietary inclusion levels of MLP (2.5%, 5%, 7.5% and 10% respectively in T_0, T_1, T_2 and T_3 groups). Hen receiving diet containing 1.0% NLP in combination with 7.5% MLP showed the lowest egg yolk cholesterol concentration among all treatment groups. The decrease in yolk cholesterol is thought to originate from the mulberry leaf having antioxidant property in laying hens [17]. Researchers have found that different levels (0.4, 0.8, and 1.2%) of mulberry leaf extract significantly reduced the egg cholesterol levels [39]. Yolk cholesterol level decreased significantly (P < 0.05) by the addition of 2%, 4% and 6% neem leaf meal in the diet of the birds [24]. Neem leaf meal in laying hen diet exhibited significant egg yolk cholesterol depleting effect [10,12]. This cholesterol reducing effect may be associated with it's active compounds like flavonoids and phenols having capability to downregulate the expression of genes involved in fatty acid and cholesterol biosynthesis, and upregulate the expression of fatty acid oxidation-related genes in the liver [9]. These active ingredients may also interfere with cholesterol metabolism and improve lipid utilization leading to low cholesterol egg production [10]. Although dietary supplementation with 10% MLP was well tolerated and did not adversely affect laying performance or egg quality, it failed to provide further reductions in egg yolk cholesterol compared with the 7.5% MLP diet [25,39]. These findings are consistent with the results of the present study.

These results suggest that the decrease in the cholesterol level of the yolk in the present study is because of the mulberry and neem leaves those have hypocholesterolemic effect that may be attributed to the presence of various bioactive compounds. The present findings suggest a plateau response in egg yolk cholesterol reduction, as increasing the inclusion level of mulberry leaf powder from 7.5% to 10% did not result in any further significant decrease in yolk cholesterol. The absence of additional cholesterol reduction at higher inclusion level may reflect diminished bioavailability of phytochemicals due to increased dietary fiber and anti-nutritional factors. These findings suggest that 7.5% MLP supplementation with 1.0% NLP resulted in the greatest reduction in egg yolk cholesterol under the present study conditions. This reduction may be associated with the bioactive phytochemicals present in neem and mulberry leaves, particularly flavonoids and phenolic compounds, which are known to modulate lipid metabolism. However, as the present study did not investigate the underlying molecular mechanisms, further studies are required to confirm the biological pathways involved.

Conclusion

The present study demonstrated that dietary supplementation with 1.0% neem leaf powder (NLP) in combination with varying levels of mulberry leaf powder (MLP) significantly reduced egg yolk cholesterol without adversely affecting production performance or most egg quality traits in commercial laying hens. The dietary treatments were well tolerated throughout the experimental period. The greatest reduction in egg yolk cholesterol was observed in hens fed the diet containing 1.0% NLP and 7.5% MLP. Increasing the MLP level to 10% did not result in any further reduction in egg yolk cholesterol, suggesting a plateau response. Overall, supplementation of neem leaf powder and mulberry leaf powder in laying hen diets may offer a promising natural feeding strategy for producing low-cholesterol eggs while maintaining productive performance and egg quality. Further studies investigating the underlying molecular mechanisms, nutrient digestibility, antioxidant status, and long-term economic feasibility are warranted to support the practical application of these phytogenic feed additives in laying hen nutrition.

Study Limitations

The present study has some limitations. Firstly, all supplemented groups received a fixed level of neem leaf powder (1.0%) with varying levels of mulberry leaf powder, the independent effects of these phytogenic additives could not be distinguished. In addition, the relatively short experimental period and lack of molecular, antioxidant, and nutrient utilization pathways limit a comprehensive understanding of the underlying mechanisms. Further investigations using factorial designs and long-term evaluations are recommended to explore the individual and interactive effects of neem and mulberry leaf powders in laying hen nutrition.

Conflict of Interest

The authors declared there is no potential conflict of interest.

Bibliography

1. Abbas Ghulam., *et al*. "*Azadirachta indica*: the medicinal properties of the global problems-solving tree". Biodiversity and biomedicine. Academic Press (2020): 305-316.

2. Akintomide AA., *et al*. "Haematology and serum biochemistry of cockerels fed diets containing neem leaf meal". *Applied Tropical Agriculture* 23.1 (2018): 12-16.

3. Al-kirshi Riyadh., *et al*. "Utilization of mulberry leaf meal (*Morus alba*) as protein supplement in diets for laying hens". *Italian Journal of Animal Science* 9.3 (2010): e51.

4. Ansah GA., *et al*. "Selection for low yolk cholesterol in Leghorn-type chickens". *Poultry Science* 64.1 (1985): 1-5.

5. Asrat Meseret., *et al*. "Effects of supplementation of different levels of garlic (*Allium sativum*) on egg production, egg quality and hatchability of White Leghorn chicken". *Livestock Research for Rural Development* 30.3 (2018): 37.

6. Bais B., *et al*. "Nutritive value of sares and neem leaves in Marwari goats". *Indian Journal of Animal Nutrition* 19.3 (2002): 266-268.

7. Bonsu FRK., *et al*. "Medicinal response of broiler chickens to diets containing neem (*Azadirachta indica*) leaf meal, haematology and meat sensory analysis". *World Applied Sciences Journal* 19.6 (2012): 800-805.

8. Chen Dandan., *et al*. "Effects of mulberry leaf flavonoid and resveratrol on methane emission and nutrient digestion in sheep". *Animal Nutrition* 1.4 (2015): 362-367.

9. Chen Fang., *et al*. "Dietary *Morus alba* L. leaf supplementation improves hepatic lipid accumulation of laying hens via downregulating CircACACA". *Poultry Science* 102.11 (2023): 103042.

10. Dey B., *et al*. "Efficacy of neem leaf meal as a hypocholesterolemic dietary additive in laying pullets". *Bangladesh Journal of Animal Science* 40.1-2 (2011): 13-17.

11. Gayathri SL., *et al*. "Effect of dietary supplementation of *Azadirachta indica* leaf meal on production performance, egg quality and serum biochemical profile of Vanaraja laying hens". *Indian Journal of Poultry Science* 55.3 (2020): 183-188.

12. Ghasi S., *et al*. "Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats". *Journal of Ethnopharmacology* 69.1 (2000): 21-25.

13. Hu Hong., *et al*. "Mulberry branch fiber improved lipid metabolism and egg yolk fatty acid composition of laying hens via the enterohepatic axis". *Microbiome* 12.1 (2024): 73.

14. Huang Zhenwu., *et al*. "Dietary mulberry-leaf flavonoids improve the eggshell quality of aged breeder hens". *Theriogenology* 179 (2022): 177-186.

15. Igugo RU., *et al*. "Effects of substitution of antibiotics with medicinal plant leaf meals on egg-laying performance and egg qualities". SSRN 5086609 (2022).

16. Kenny AP. "The determination of cholesterol by the Liebermann-Burchard reaction". *Biochemical Journal* 52.4 (1952): 611-619.

17. Kılınç Gözde. "The effects of black mulberry (*Morus nigra* L.) leaf powder on performance, egg quality, egg antioxidant capacity, and yolk cholesterol levels in laying hens". *Journal of Anatolian Environmental and Animal Sciences* 8.4 (2023): 714-719.

18. Lin WC., *et al*. "Effects of mulberry leaves on production performance and the potential modulation of antioxidative status in laying hens". *Poultry Science* 96.5 (2017): 1191-1203.

19. Mason Pamela. "The importance of eggs in an environmentally sustainable diet". *Nutrition Bulletin* 48.3 (2023): 400-410.

20. Mathur Shalini and Rameshwar Singh. "Antibiotic resistance in food lactic acid bacteria-a review". *International Journal of Food Microbiology* 105.3 (2005): 281-295.

21. Munțiu-Rusu M-I., *et al*. "The effects of dietary mulberry leaf powder in laying hens' diets on internal and external egg quality parameters and yolk color". (2022): 129-137.

22. National Research Council. "Nutrient requirements of poultry". National Academy of Sciences Press, Washington DC, USA (1994).

23. Nesheim MC., *et al*. "Poultry Production. 12th edition". Lea & Febiger. Philadelphia (1979).

24. Olabode AD., *et al*. "Influence of neem (*Azadirachta indica* A. Juss) leaf meal on the serum biochemistry and yolk cholesterol of laying birds (a case study at Ishiagu, Ivo local government area of Ebonyi State)". *International Journal of Agriculture and Biosciences* 3.4 (2014): 177-180.

25. Olteanu Margareta., *et al*. "Effect of dietary mulberry leaves on performance parameters and nutrient digestibility of laying hens". *Indian Journal of Animal Sciences* 82.8 (2012): 914-917.

26. Opara Maxwell. "Physiological response of laying birds to Neem (*Azadirachta indica*) leaf meal-based diets: body weight organ characteristics and haematology". *Online Journal of Health and Allied Sciences* 5.2 (2006).

27. Osho BI., *et al*. "Nutrient composition and antioxidant properties of neem (*Azadirachta indica*) and sweet potato leaves as alternative resource in poultry production". *Applied Tropical Agriculture* 26.1 (2021): 181-189.

28. Pal Mahendra and Judit Molnár. "The role of eggs as an important source of nutrition in human health". *International Journal of Food Science and Agriculture* 5.1 (2021).

29. Patel SN., *et al*. "Feeding strategies for the production of designer eggs and meat". *Veterinary Today* 2.7 (2024): 378-382.

30. RP Patil., *et al*. "Effect of neem (*Azadirachta indica*) leaf powder on egg composition parameters of commercial layers". *Indian Journal of Animal Production and Management* 30.3-4 (2017).

31. Saddul D., *et al*. "The potential of mulberry (*Morus alba*) as a fodder crop: The effect of plant maturity on yield, persistence and nutrient composition of plant fractions". *Asian-Australasian Journal of Animal Sciences* 17.12 (2004): 1657-1662.

32. Siddiqui Rukkiya., *et al*. "Effect of replacing rice polish with mulberry (*Morus alba*) leaf powder in diet containing phytase enzyme on performance and haemato-biochemical parameters of commercial laying hens". *Indian Journal of Veterinary Sciences and Biotechnology* 18.1 (2022).

33. Singh MK., *et al*. "Neem leaf meal supplementation for profitable poultry production: A review". *Indian Journal of Poultry Science* 52.3 (2017): 239-245.

34. Siti N Wayan., *et al*. "Effect of *Moringa oleifera* leaf powder in diets on feed digestibility and external egg quality characteristics in laying hens". *International Journal of Fauna and Biological Studies* 6.4 (2019): 113-118.

35. Venkatesh KR., *et al*. "Use of mulberry leaves as supplementary food in cow and goat to improve milk production". *International Journal of Applied Research* 1.13 (2015): 81-84.

36. Wadhwa D., *et al*. "Production of heart-healthy eggs through dietary manipulations of hens". *Himachal Journal of Agricultural Research* 43.1 (2017): 1-9.

37. Walker Sam and JI Baum. "Eggs as an affordable source of nutrients for adults and children living in food-insecure environments". *Nutrition Reviews* 80.2 (2022): 178-186.

38. Yigrem Minichle., *et al*. "Effect of neem (*Azadirachta indica*), girawa (*Vernonia amygdalina*) leaves meal and garlic (*Allium sativum*) on performance and egg qualities of layer chickens". *Journal of Applied Poultry Research* 33.4 (2024): 100469.

39. Zhang Bo., *et al*. "Positive effects of Mulberry leaf extract on egg quality, lipid metabolism, serum biochemistry, and antioxidant indices of laying hens". *Frontiers in Veterinary Science* 9 (2022): 1005643.

40. Zhang Hongxia., *et al*. "Effects of mulberry fruit (*Morus alba* L.) consumption on health outcomes: A mini-review". *Antioxidants* 7.5 (2018): 69.

Volume 21 Issue 6 August 2026