Effect of dietary supplementation of *Moringa oleifera* leaf meal on egg quality, composition and anti-stress activity of Vanaraja laying hens

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ABSTRACT

An experiment was conducted to study the effect of dietary supplementation of *Moringa oleifera* leaf meal (MLM) on egg quality, composition and anti-stress activity of Vanaraja laying hens. One hundred twenty, Vanaraja laying hens of 24 weeks of age were randomly divided into four groups of 30 each with three replicates of 10 in each group. Four experimental diets were fed to birds in which T₁ (control-antibiotic – oxytetracycline powder @ 50 g/ quintal) and in rest of the 3 treatment groups T₂, T₃ and T₄, the birds were fed with MLM at levels of 0.25, 0.5 and 1.0%, respectively. The egg quality parameters studied at 36 weeks of age did not show any significant difference between the treatment groups except for the yolk index. The activities of serum enzymes like alanine transaminase and aspartate transaminase were also not differed significantly, except for alkaline phosphatase. The heterophil / lymphocyte ratio showed significant difference (P<0.05) between the treatment groups of MLM, where in the highest mean value was showed by T₄ (1% MLM). No significant influence of dietary MLM was noticed on the nutrient composition and amino acid profile of eggs. From the above findings, it is concluded that MLM up to 1% level in the diet of Vanaraja laying hens is beneficial in terms of egg quality, composition and anti-stress activity.

Key words: Anti-stress activity, Egg quality, Moringa leaf meal, Vanaraja laying hens

In spite of rapid growth, the poultry industry suffered many setbacks in recent times due to rising cost of feed, emergence of new or re-emerging existing diseases, fluctuating market price of egg and broilers, etc. which need to be addressed to make the poultry sector as a sustainable enterprise (Chatterjee and Rajkumar 2015). Unavailability of poultry products and low purchasing ability of the rural people devoid them of access to the highly nutritious products like egg and meat, thereby, resulting in malnutrition. Backyard poultry farming can be used as a tool for alleviating rural poverty, malnutrition and provision of employment in rural areas (Sharma and Chatterjee 2009).

*Moringa oleifera*, commonly called as Moringa is available cheaply throughout the rural and semi urban areas of India and it can be used as an alternative feed resource in poultry ration (Agbede 2003). Moringa Leaf Meal (MLM) contains several bioactive compounds and essential nutrients, that made MLM as an ideal candidate not only as feed ingredient but also as phytogenic feed additive (Joshi and Mehta 2010) to promote layer performance and to enrich the egg yolk with carotenoids, flavonoids, and selenium (Melesse et al. 2011). Researchers showed that, this Moringa tree have had several medicinal properties such as hypocholesterolemic properties (Olugbemi et al. 2009), anti-inflammatory activities (Ezeamuzie et al. 1996), hepatoprotective (Pari and Kumar 2002), antihypertensive (Faizi et al. 1998), strong coagulative and antimicrobial properties (Fahey et al. 2001) anti-tumor activities (Murakami et al. 1998) and antioxidant activities (Moyo et al. 2012). The usage of antibiotic growth promoters in the animal feed have been banned due to the development of antibiotic resistance (Cogliani et al. 2011), motivating the search for alternative growth promoters, such as phytogenic feed additives (Windisch et al. 2008). The MLM can be used as a promising antibiotic replacer. The present study focuses on the effect of supplementation of MLM of Vanaraja laying hens on egg quality, composition and anti-stress activity.

MATERIALS AND METHODS

Stock, diets and husbandry: Vanaraja laying hens (120) of 24 weeks of age were selected at random and divided into four groups of 30 each with three replicates of 10 in each group in a complete randomized design. Experiment was conducted for a period of 12 weeks. The birds were placed in 12 pens having floor space of 18 sq. ft. each. Rice husk was used as litter material. MLM was prepared by sun drying, followed by grinding the leaves. In control group T₁, the birds were fed with antibiotic – oxytetracycline (OTC) @ 50 g/ quintal and in rest of the 3 treatment groups...
The birds were fed with MLM at levels of 0.25, 0.5 and 1.0%, respectively (Table 1). A measured quantity of feed was given on daily basis. The birds were given restricted amount of 125 g of feed per bird for first six weeks, followed by 130 g of feed for next six weeks. Standard management practices were followed and clean drinking water was made available ad lib throughout the experiment.

Table 1. Ingredient and nutrient composition of experimental diets (% air dry basis)

| Ingredient               | T1  | T2  | T3  | T4  |
|--------------------------|-----|-----|-----|-----|
| Yellow Maize             | 58.46 | 58.26 | 58.01 | 57.51 |
| Soybean meal             | 20.7 | 20.7 | 20.7 | 20.7 |
| De-oiled rice bran       | 10.2 | 10.2 | 10.2 | 10.2 |
| Shell grit               | 8.7  | 8.7  | 8.7  | 8.7  |
| Dicalcium phosphate      | 1.15 | 1.152 | 1.152 | 1.15 |
| DL-methionine            | 0.08 | 0.08 | 0.08 | 0.08 |
| Common salt              | 0.4  | 0.4  | 0.4  | 0.4  |
| Vitamin B complex        | 0.02 | 0.02 | 0.02 | 0.02 |
| Vitamin ABDK             | 0.02 | 0.02 | 0.02 | 0.02 |
| Mineral mixture*         | 0.12 | 0.12 | 0.12 | 0.12 |
| Choline                  | 0.05 | 0.05 | 0.05 | 0.05 |
| Toxin Binder             | 0.05 | 0.05 | 0.05 | 0.05 |
| Antibiotics              | 0.05 | –    | –    | –    |
| Moringa leaf meal        | –    | 0.25 | 0.5  | 1    |
| TOTAL                    | 100  | 100  | 100  | 100  |

Nutrient composition (Calculated value)

| Ingredient          | T1  | T2  | T3  | T4  |
|---------------------|-----|-----|-----|-----|
| ME (kcal/kg)        | 2601 | 2608 | 2615 | 2622 |
| CP (%)              | 15.99 | 15.96 | 16.02 | 16.08 |
| Lysine (%)          | 0.79 | 0.79 | 0.80 | 0.80 |
| Methionine (%)      | 0.31 | 0.33 | 0.34 | 0.34 |
| Calcium (%)         | 3.23 | 3.24 | 3.25 | 3.24 |
| Phosphorous (%)     | 0.35 | 0.35 | 0.35 | 0.35 |

*Trace Min CB (Venky’s India Private Limited, Pune).

Composition: Each 1 kg Trace Min CB contains Manganese: 90 g, Zinc: 80 g, Iron: 90 g, Copper: 15 g, Iodine: 2 g, Selenium: 300 mg.

Egg quality: Twelve eggs were randomly chosen in each treatment from the eggs laid during the last five consecutive days of experimental period to measure egg-shell weight, shell thickness and Haugh unit (HU). The cleaned egg-shells were dried for 24 h, weighed and expressed as % of whole egg. The shell thickness was measured at three different locations (middle, broad and narrow ends) using a micrometer gauge (Mitutoyo Code, 7027, Japan).

Serum bio-chemical parameters: On 36th week of experimental feeding, around 3 ml of blood was collected from brachial vein from 9 birds in each dietary treatment. Subsequently serum was separated and the activities of enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were estimated in the serum by auto-analyzer using diagnostic kits (Coral Clinical Systems, Goa, India). Also, at the time of blood collection, blood smear was prepared, fixed and further stained using Wrights Giemsa staining procedure to measure Heterophils (H) and Lymphocytes (L).

Nutrient composition of eggs: For analyzing the egg composition, 6 eggs (two eggs per replicate) were randomly chosen from each dietary treatment from the eggs laid during the last three consecutive days of the experiment. The protein and fat content of the eggs were analyzed by following the method of AOAC (1995). The amino acid analysis of eggs was done at EVONIK, Mumbai, India.

Statistical Analysis: The data obtained from the experiment were statistically analyzed according to Snedecor and Cochran (1994). The data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range (DMR) Test (Duncan 1955) to test the difference between treatments means, wherever necessary.

RESULTS AND DISCUSSION

The egg quality parameters (albumen %, Yolk %, egg shell weight, shell thickness, Haugh unit and shape index) did not show any significant difference (P>0.05) between

Table 2. Effect of dietary supplementation of Moringa leaf meal on the egg quality parameters of Vanaraja laying hens at 36 weeks of age

| Parameter           | Concentration in the diet | SEM | P value |
|---------------------|---------------------------|-----|---------|
|                     | 50 g/ quintal OTC (T1)    |     |         |
|                     | 0.25% MLM (T2)            |     |         |
|                     | 0.5% MLM (T3)             |     |         |
|                     | 1% MLM (T4)               |     |         |
| Egg weight (g)      | 51.46 | 52.37 | 51.53 | 51.59 | 0.211 | 0.392 |
| Albumen, %          | 53.35 | 53.49 | 52.99 | 55.83 | 0.452 | 0.103 |
| Egg yolk, %         | 36.56 | 36.66 | 37.25 | 34.71 | 0.445 | 0.206 |
| Shell, %            | 10.07 | 9.83  | 9.76  | 9.46  | 0.118 | 0.334 |
| Yolk color score    | 6.91  | 6.75  | 7.08  | 6.83  | 0.074 | 0.444 |
| Shell thickness     | 0.345 | 0.352 | 0.352 | 0.351 | 0.001 | 0.204 |
| Albumen index       | 13.26 | 14.36 | 13.63 | 13.71 | 0.668 | 0.634 |
| Yolk index          | 43.64 | 44.63 | 46.34 | 45.74 | 0.307 | 0.010 |
| Haugh unit          | 69.50 | 68.38 | 70.60 | 67.98 | 1.022 | 0.812 |
| Shape index         | 79.79 | 79.91 | 84.05 | 81.01 | 1.189 | 0.274 |

*Means with different superscript in a row differs significantly (P<0.05); **SEM, Standard Error of Mean; OTC, Oxytetracycline; MLM, Moringa leaf meal.
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Table 3. Effect of dietary supplementation of Moringa leaf meal on the nutrient composition of eggs in Vanaraja laying hens

| Parameter   | Concentration in the diet | SEM | P value |
|-------------|---------------------------|-----|---------|
|             | 50 g/ quintal OTC (T1)    |     |         |
| Dry matter  | 93.85                      | 1.08| 0.648   |
| Protein     | 46.54                      | 0.74| 0.574   |
| Ether extract| 45.13                      | 0.58| 0.624   |
|             | 0.25% MLM (T2)            |     |         |
| Dry matter  | 93.25                      | 1.08| 0.648   |
| Protein     | 46.68                      | 0.74| 0.574   |
| Ether extract| 45.29                      | 0.58| 0.624   |
|             | 0.5% MLM (T3)             |     |         |
| Dry matter  | 93.75                      | 1.08| 0.648   |
| Protein     | 46.95                      | 0.74| 0.574   |
| Ether extract| 45.57                      | 0.58| 0.624   |
|             | 1% MLM (T4)               |     |         |
| Dry matter  | 94.68                      | 1.08| 0.648   |
| Protein     | 48.08                      | 0.74| 0.574   |
| Ether extract| 46.21                      | 0.58| 0.624   |

SEM, Standard Error of Mean; OTC, Oxytetracycline; MLM, Moringa leaf meal.

Table 4. Effect of dietary supplementation of Moringa leaf meal on the amino acid profile of eggs in Vanaraja laying hens

| Parameter     | Concentration in the diet | SEM | P value |
|---------------|---------------------------|-----|---------|
|               | 50 g/ quintal OTC (T1)    |     |         |
| Methionine    | 1.43                      | 0.024| 0.674   |
| Cystine       | 1.01                      | 0.027| 0.724   |
| Methionine +Cystine | 2.45               | 0.032| 0.548   |
| Lysine        | 3.05                      | 0.035| 0.045   |
| Threonine     | 2.07                      | 0.024| 0.748   |
| Arginine      | 2.78                      | 0.012| 0.642   |
| Isoleucine    | 2.45                      | 0.011| 0.746   |
| Leucine       | 3.91                      | 0.042| 0.456   |
| Valine        | 2.93                      | 0.026| 0.548   |
| Histidine     | 1.13                      | 0.009| 0.486   |
| Phenyl alanine| 2.31                      | 0.21 | 0.724   |
| Glycine       | 1.50                      | 0.008| 0.842   |
| Serine        | 3.23                      | 0.014| 0.546   |
| Proline       | 1.72                      | 0.022| 0.684   |
| Alanine       | 2.54                      | 0.024| 0.482   |
| Aspartic acid | 4.58                      | 0.032| 0.548   |
| Glutamic acid | 5.79                      | 0.035| 0.672   |

SEM, Standard Error of Mean; OTC, Oxytetracycline; MLM, Moringa leaf meal.

the treatment groups, except for the parameter yolk index (Table 2). Similar to the findings of the present study, Ebenebe et al. (2013) did not observe any difference on egg weight, shell thickness and shape index due to dietary inclusion of MLM up to 7.5% in the diet of brown laying hens. The findings of the present study on Albumin index and Haugh unit corroborated with the findings of Wei et al. (2016). Swain et al. (2017) also observed non significance difference on egg quality parameters (albumen %, yolk %, shell %, shape index and shell thickness due to dietary inclusion of MLM in the diet of Vanaraja laying hens. From this study, it is therefore suggested that MLM can be supplemented up to 1% in the diet of Vanaraja laying hens without affecting the egg quality parameters.

The dietary supplementation of MLM had no influence on nutrient composition of eggs of Vanaraja laying hens during the entire period of study (Table 3). Similarly, the amino acid composition of egg protein was also not influenced due to dietary supplementation of MLM in the diet of Vanaraja laying hens (Table 4). There is no available information in literature on the influence of MLM on egg nutrient composition in general and amino acid profile in particular. However, the results of the present study suggested that dietary inclusion of MLM up to 1% level did not affect the egg nutrient composition and amino acid profile.

The activities of serum enzymes such as AST and ALT were not influenced due to dietary supplementation of MLM up to 1% in the diet of Vanaraja laying hens (Table 5). Wei et al. (2016) reported higher activity of AST in the serum of laying hens due to MLM supplementation at 15% in the diet. The above researcher, however, did not observe any variation in AST activity at a lower level of supplementation, a similar finding observed in the present study. Ahmad et al. (2017) reported lower ALT activity in layers by dietary inclusion of MPM at 0.15% in the diet. But in the present study, no such variation could be observed. This could be attributed to the compositional variation of MLM and MPM. Alkaline phosphatase activity in the serum is an indicator of stress. In the present study, dietary supplementation of MLM at 1% significantly reduced the ALP activity in the serum as compared to 0.25 or 0.5%, and control. This above finding suggested that MLM supplementation can reduce stress in laying hens.

The heterophil to lymphocyte ratio, another indicator of stress increased linearly with levels of MLM.
supplementation (Fig. 1). This finding further suggested that dietary supplementation of MLM can reduce the level of stress in laying hens. This could be attributed to the hepatoprotective, antioxidant and antimicrobial properties of MLM (Ramadan, 2017).

Dietary supplementation of MLM up to 1% level in the diet of Vanaraja laying hens did not affect the production performance (egg production, egg weight and feed conversion) in the present study (result not presented). Further, MLM up to 1% level in the diet had not significantly affected the egg quality and composition. On the other hand, MLM at 1% level increased the anti-stress activity as evidenced by lowering ALP activity and improving H:L ratio. From the overall findings, it can be concluded that MLM can be supplemented up to 1% level in the diet of Vanaraja laying hens.

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REFERENCES

Agbede J O. 2003. Equi-protein replacement of fishmeal with Leucaena leaf protein concentrate: an assessment of performance characteristics and muscle development in the chicken. International Journal of Poultry Science 2(6): 421–29.

Ahmad S A, Khaliqie A, Pasha T N, Mehmood S, Hussain K, Shaheen M S, Naeem M, Shafiq M and Ahmad S. 2017. Effect of Moringa oleifera (Lam.) pods as feed additive on egg antioxidants, chemical composition and performance of commercial layers. South African Journal of Animal Sciences 47: 864–74.

AOAC. 1995. Official Methods of Analysis, 13th edn. Association of Official Analytical Chemists, Washington DC, USA.

Chatterjee R N and Rajkumar U. 2015. An overview of poultry production in India. Indian Journal of Animal Health 54(2): 89–108

Cogliani C, Goossens H and Greko C. 2011. Restricting antimicrobials in food animals. Lessons from Europe Microbiology 6: 274–9.

Duncan D B. 1955. Multiple range and multiple F test. Biometrics 11: 1–42.

Ebenene C I, Anigbogu C C, Anizoba M A and Ufele A N. 2013. Effect of various levels of Moringa leaf meal on the egg Quality of Isa Brown breed of layers. Advances in Life Science and Technology 14: 45–49.

Ezeamuzie I C, Ambedaderomo A W, Shode F O and Ekwebelem S C. 1996. Antiinflammatory effects of Moringa oleifera root extract. International Journal of Pharmacognosy 34: 207–12.

Fahey J W, Zakmann, A T and Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Corrigendum Phytochemistry 59: 200–37.

Faiyi S, Siddiqui B S, Saleem R, Ahab K, Shaheen F and Gilani A H. 1998. Hypotensive constituents from the pods of Moringa oleifera. Planta Medica 64: 225–8.

Joshi P and Mehta D. 2010. Effect of dehydration on the nutritive value of drumstick leaves. Journal of Metabolism Systems Biology 1: 5–9.

Melesse A, Tiruneh W and Negasse T. 2011. Effects of feeding Moringa stenopetala on nutrient intake and growth performance of Rhode Island Red Chicken under tropical climate. Tropical and Subtropical Agriculture 14: 485–92.

Moyo B, Oyedemi S, Masika P J and Mucheje V. 2012. Polyphenolic content and antioxidant properties of Moringa oleifera leaf extracts and enzimatic of liver from goats supplemented with Moringa oleifera leaves/sunflower seed cake. Meat Science 91: 441–7.

Murakami A, Kitazono Y, Jiwajinda S, Koshimizu K and Ohigashi H. 1998. Niaziminin, a thio carbamate from the leaves of Moringa oleifera, holds a strict structural requirement for inhibition of tumorpromoter-induced Epstein–Barr virus activation. Planta Medica 64: 319–23.

Olugbemi T S, Mutayoba S K and Lekule F P. 2009. Moringa oleifera leaf meal as a hypocholesterimic agent in laying hen diet. Livestock Research for Rural Development 22(4): 201–05.

Pari L and Kumar N A. 2002. Hepatoprotective activity of Moringa oleifera on antitubercular drug-induced liver damage.
in rats. Journal of Medicinal Food 5 (3): 171–177.
Rajkumar U, Rama Rao S V and Sharma R P. 2010. Backyard poultry farming-changing the face of rural and tribal livelihoods. Indian Farming 59: 20–24
Ramadan S G A. 2017. Impact of supplementation of Moringa oleifera in diet of broiler chicks on their behaviour, welfare, performance and immune responses Alexandria Journal of Veterinary Sciences 55(1): 50–59.
Sharma R P and Chatterjee R N. 2009. Backyard poultry farming and rural food security. Indian Farming 59: 36–37.
Snedecor G W and Cochran W G. 1994. Statistical methods. Oxford and IBH Publishing Company, New Delhi.
Swain B K, Naik P K, Chakurkar E B and Singh N P 2017. Effect of supplementation of Moringa oleifera leaf meal on the performance of Vanaraja laying hens, Indian Journal of Animal Sciences 87(3): 353–55.
Wei L J, Wang H J, Zhang S G, and Qi G H. 2016. Evaluation of Moringa oleifera leaf in laying hens: Effects on laying performance, egg quality, plasmabiochemistry and organ histopathological indices. Italian Journal of Animal Science 15(4): 658–65.
Windisch W, Schedle K, Plitzner C and Kroismayr A. 2008. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science 86: 140–48.