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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 T_1 (control-antibiotic – oxytetracycline powder @ 50 g/ quintal) and in rest of the 3 treatment groups T_2, T_3 and T_4, 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 T_4 (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 (Ezeamuzle *et al.* 1996), hepatoprotectiveness (Pari and Kumar 2002), antihypertensiveness (Faizi *et al.* 1998), strong coagulative and antimicrobial properties (Fahey *et al.* 2001) anti-tumor acitivities (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 T_1, the birds were fed with antibiotic – oxytetracycline (OTC) @ 50 g/ quintal and in rest of the 3 treatment groups

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T_2, T_3 and T_4, 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	Parts per quintal			
	T_1	T_2	T_3	T_4
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
Di-calcium 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)				
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 (T_1)	0.25% MLM (T_2)	0.5% MLM (T_3)	1% MLM (T_4)		
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	15.63	13.71	0.668	0.634
Yolk index	43.64^{c}	44.63 ^{bc}	46.34 ^{a}	45.47 ^{ab}	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

^{a,b}Means with different superscript in a row differs significantly (P<0.05); SEM, Standard Error of Mean; OTC, Oxytetracycline; MLM, Moringa leaf meal.

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 (T_1)	0.25% MLM (T_2)	0.5% MLM (T_3)	1% MLM (T_4)		
Dry matter	93.85	93.25	93.75	94.68	1.08	0.648
Protein	46.54	46.68	46.95	48.08	0.74	0.574
Ether extract	45.13	45.29	45.57	46.21	0.58	0.624

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 phospatase 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

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

Parameter	Concentration in the diet				SEM	P value
	50 g/ quintal OTC (T_1)	0.25% MLM (T_2)	0.5% MLM (T_3)	1% MLM (T_4)		
Methionine	1.43	1.42	1.44	1.54	0.024	0.674
Cystine	1.01	1.03	1.05	1.16	0.027	0.724
Methionine +Cystine	2.45	2.47	2.49	2.69	0.032	0.548
Lysine	3.05	3.05	3.05	3.19	0.035	0.045
Threonine	2.07	2.06	2.05	2.16	0.024	0.748
Arginine	2.78	2.78	2.78	2.89	0.012	0.642
Isoleucine	2.45	2.45	2.45	2.55	0.011	0.746
Leucine	3.91	3.88	3.89	4.07	0.042	0.456
Valine	2.93	2.91	2.90	3.07	0.026	0.548
Histidine	1.13	1.12	1.14	1.19	0.009	0.486
Phenyl alanine	2.31	2.31	2.31	2.45	0.21	0.724
Glycine	1.50	1.49	1.48	1.57	0.008	0.842
Serine	3.23	3.22	3.23	3.34	0.014	0.546
Proline	1.72	1.73	1.74	1.81	0.022	0.684
Alanine	2.54	2.54	2.54	2.67	0.024	0.482
Aspartic acid	4.58	4.54	4.51	4.79	0.032	0.548
Glutamic acid	5.79	5.78	5.80	5.88	0.035	0.672

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

Table 5. Effect of dietary supplementation of Moringa leaf meal on the different serum enzyme activities of Vanaraja laying hens

Parameter	Concentration in the diet				SEM	P value
	50 g/ quintal OTC (T_1)	0.25% MLM (T_2)	0.5% MLM (T_3)	1% MLM (T_4)		
AST (µ/l)	28.87	37.37	35.73	40.14	2.04	0.260
ALT (µ/l)	18.77	18.27	21.22	18.55	0.460	0.069
ALP (µ/l)	198.53[a]	193.41[a]	189.467[a]	159.165[b]	21.93	0.001

[a,b]Means with different superscript in a row differs significantly (P<0.05) ; SEM, Standard Error of Mean; OTC, Oxytetracycline; MLM, Moringa leaf meal.

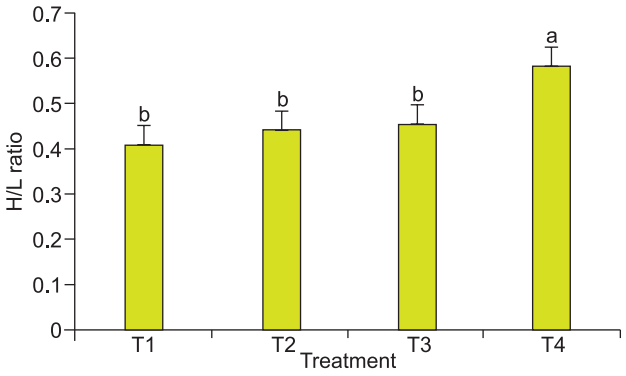

Fig. 1. Effect of dietary supplementation of Moringa leaf meal on heterophil to lymphocyte ratio of Vanaraja laying hens.

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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