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Effect of Supplemental DL-Methionine to Low Protein Diet on Production Performance, Egg Quality and Serum Biochemical Indices of Vanaraja Laying Hens

A. K. Panda, B. Sahoo and Anil Kumar
ICAR-Central Institute for Women in Agriculture, Bhubaneswar, 751003, India
*Correspondence: akpanda59@gmail.com

ABSTRACT

The present study was conducted to evaluate the effect of supplemental DL-Methionine(DLM) to low protein diet on production performance, egg quality and serum biochemical indices of Vanaraja laying hens. Ninety six Vanaraja laying hens (28 wks) were randomly distributed into four dietary treatment groups with three replicates of 8 birds in each pen. A standard diet containing 2600kcal/kg ME, 15% CP, 0.7% lysine and 0.28% methionine was formulated which served as control. Subsequently, three experimental diets were formulated to contain 0.32, 0.36 and 0.40% DLM in the diets. A measured quantity of each diet was fed to one of the treatment groups for a period of 8 weeks to study their performance. Dietary supplementation of DLM had no influence on body weight gain, egg production and feed efficiency of Vanaraja laying hens. However, egg weight and egg mass output increased significantly due to supplemental DLM at 0.04% (0.32% DLM diet) with no further improvements thereafter. None of the egg quality parameters (albumen, yolk and egg shell%; albumen index, yolk index, shape index and eggshell thickness) except the Haugh unit was influenced due to the levels of supplemental DLM in the diet of Vanaraja laying hens. Increasing the levels of DLM to 0.32% in the diet significantly improved the Haugh unit compared to the control group. Dietary supplementation of DLM at 0.04% significantly increased the serum protein and calcium concentration of laying hens. From the findings of the present study, it is concluded that 0.04% supplemental DLM to the diet containing 2600 kcal/kg ME, 15% CP, 0.7% lysine and 0.28% methionine is adequate in eliciting optimum performance in Vanaraja laying hens.

KEY WORDS: DL-methionine, Egg quality, Production performance, Serum biochemical indices, Vanaraja laying hens

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INTRODUCTION

The importance of small scale poultry farming in rural areas has been recognized globally to alleviate poverty, hunger and malnutrition in developing countries like India (Panda et al., 2020a; Rajkumar et al., 2021). Vanaraja is a dual-purpose chicken variety developed by the ICAR-Directorate of Poultry Research, Hyderabad, India, aimed at rural communities where it can be reared in backyard on natural, scavenged food with minimal supplementation. The birds can lay up to 110-120 eggs per year under backyard system of rearing. However, many farmers are rearing these birds under intensive system of production due to high market price of egg produced from Vanaraja (Panda et al., 2020 b). In intensive system of poultry farming, feed costs around 60-70% of the total cost of production. Availability of good quality feed resources is one of the major constraints in the intensive poultry production. This has compelled the researchers to find alternate feed resources and/ or formulating precise feeding practices to meet the requirement of poultry.

Amongst the nutrients required by the bird, protein is one of the most important nutrients, which has a major influence on growth, feed utilization and production performance (NRC, 1994). The requirements of protein and the critical amino acids predominantly depend upon the genetic makeup, growth and production potential of the bird (Witten

et al., 2004; Panda et al., 2007). Dietary requirements of protein are actually the requirements for amino acids in the dietary protein. Supplementation of DL-methionine (DLM) in the diet is the best probable way to decrease the nitrogen excretion in the excreta as chickens can effectively utilize around 40% of the dietary protein (Panda et al., 2011). To our knowledge there is no available information in the literature on the influence of DLM on production performance of Vanaraja laying hens in the intensive system of production. Therefore, the present study was conducted to evaluate the effect of supplementation of DL-Methionine on production performance and serum biochemical indices of Vanaraja laying hens.

MATERIALS AND METHOD

Stock, diet and husbandry

A total of 96, Vanaraja laying hens were randomly distributed into four dietary treatment groups with three replicates of 8 birds in each pen. These birds were reared in deep litter system having floor space of 20 sq ft in each pen. A lighting regime of 16h was provided and all laying hens were kept under uniform management conditions throughout the experimental period (28-36 wk). The experiment was conducted following the guidelines of Institute Animal Ethics Committee.

A standard diet containing 2600kcal/kg ME, 15% CP, 0.7% lysine and 0.28% methionine was formulated which served as control (T1) (Table 1).

Table 1. Ingredient and nutrient composition of experimental diets (% as such basis)

Ingredients	Parts per quintal			
	T1	T2	T3	T4
Maize	57.46	57.46	57.46	57.46
Soyabean meal	19.82	19.82	19.82	19.82
Deoiled rice bran	11.78	11.74	11.70	11.66
Stone grit	8.98	8.98	8.98	8.98
Dicalcium phosphate	1.15	1.15	1.15	1.15
DL - methionine	0.10	0.14	0.18	0.22
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
Coccidiostat	0.05	0.05	0.05	0.05
TOTAL	100	100	100	100
Nutrient composition (Calculated value)				
ME(kcal/kg)	2604	2604	2604	2604
CP (%)	15.0	15.0	15.0	15.0
Lysine (%)	0.71	0.71	0.71	0.71
Methionine (%)	0.28	0.32	0.36	0.40
Calcium (%)	3.20	3.20	3.20	3.20
Phosphorous (%)	0.34	0.34	0.34	0.34

*Trace Min CB (Venky's India Private Limited, Pune).
Composition: Each 1 kg Trace Min CB contains Manganese: 90g, Zinc: 80 g, Iron: 90.0g, Copper: 15.0g, Iodine: 2.0g, Selenium: 300mg

Subsequently, three experimental diets were formulated by supplementing dietary DL-methionine at 0.04, 0.08 and 0.12% to obtain 0.32 (T2), 0.36 (T3) and 0.40% (T4) DLM in the diets. The levels of calcium and non phytate phosphorus were constant in all the four diets. A measured quantity of each diet was fed to one of the treatment groups for a period of 8 weeks to study their performance.

Response Criteria

Egg production, feed efficiency and egg weight

Daily egg production was recorded on pen basis and percent hen day egg production was calculated. Measured quantity of feed was offered each day and the feed residue was recorded at 28d intervals and feed intake was calculated as g/bird/day. Feed efficiency was calculated as the quantity of feed consumed per unit of egg mass produced. All the eggs laid during the last three consecutive days of every 28 days period were collected and egg weight was recorded.

Egg quality

Twelve eggs were randomly chosen in each treatment from the eggs laid during the last three consecutive days of each 28-day period to measure the albumen weight, yolk weight eggshell weight, shell thickness and Haugh unit. 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

At the end of experimental feeding (40^{th} wk), around 3ml of blood was collected from brachial vein from 2 birds from each pen (total 6 birds) of the dietary treatment. Subsequently serum was separated and the concentrations of total protein, , calcium, phosphorous, cholesterol, triglycerides, creatinine and uric acid were estimated in the serum by auto-analyzer using diagnostic kits (Coral Clinical Systems, Goa, India)

Statistical analysis

Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor and Cochran, 1989) and the means of treatments were compared by Duncan multiple range test (Duncan, 1955). Significance was considered at $P \leq 0.05$ level.

RESULTS AND DISCUSSION

The effect of dietary supplementation of DL methionine (DLM) on body weight changes of Vanaraja laying hens during 28-36 weeks (wks) of age is presented in Table 2.

Table 2. Effect of supplementation DL methionine on body weight gain of Vanaraja laying hens

Parameters	% DL methionine				SEM	P value
	0.28 (T1)	0.32 (T2)	0.36(T3)	0.40 (T4)		
Initial body weight (g, 28 wk)	2204	2236	2238	2194	9.45	0.748
Body weight (g, 36 wk)	2328	2364	2320	2334	8.20	0.624
Body weight gain (g, 28-36 wks)	124	128	82	140	16.40	0.312

SEM- Standard Error of Mean

At the beginning of the experiment, the body weights varied between 2194 and 2238g and were comparable among all the dietary groups. The laying hens of all the dietary groups gained body weight during the experimental period which varied from 106 to 133g, however, the supplemental DLM had no influence on body weight gain of Vanaraja laying hens. Concomitant to the findings of the present study, Sohail et al. (2003) did not find any influence of dietary DLM supplementation on body weight gain of laying hens. In another study, El-Maksoud et al. (2011) also did not find any beneficial effect on body weight changes due to supplemental DLM to either low protein diet containing 12% CP or higher level of CP (14 or 16%).

The effect of DL methionine (DLM) supplementation in the diets on production performance of Vanaraja laying hens during 28-36 wks is given in Table 3.

Table 3. Effect of supplementation DL methionine on production performance of Vanaraja laying hens

Parameters	% DL methionine				SEM	P value
	0.28 (T1)	0.32 (T2)	0.36 (T3)	0.40 (T4)		
Hen housed egg production (%)	64.4	63.9	64.0	64.1	1.02	0.86
Egg weight (g)	52.1[a]	52.7[b]	52.5[b]	52.7[b]	0.08	0.05
Egg mass (g/day)	33.4[a]	33.7[ab]	33.8[b]	33.8[b]	0.02	0.56
Feed consumed/day (g)	135	134	134	135	-	-
Feed conversion ratio (g feed/g egg)	2.24	2.23	2.24	2.22	0.05	0.68

[a, b] Means with different superscript in a row differs significantly ; SEM- Standard Error of Mean

The hen housed egg production (HHEP) % varied between 63.9 to 64.1% and was comparable among the dietary groups. The egg weight (EW) was lowest in the control group containing 0.28% DLM in the diet. Increasing the level of dietary DLM from 0.28 to 0.32% significantly increased the EW. However, no further improved in EW could be observed by increasing the dietary DLM levels to either 0.36 or 0.40%. The similar trend was observed in egg mass (g/day), except in T2 group (0.32% DLM) where it was intermediate. Similar to the findings of the present study, Leeson and Caston (1996) reported higher egg weight and egg mass in diet containing higher levels of supplemental DLM compared to the lower level of DLM in diet containing 16.8% CP. Similarly, Gumpha et al. (2019) reported significant influence of higher levels of DLM (0.33 or 0.35%) on egg weight and egg mass in Vanaraja laying hens (35-46 wks) compared to diet containing 0.29% DLM, a similar finding observed in this study. The feed consumed per bird per day was similar across the dietary groups. The feed conversion ratio as measured by g feed required to produce g egg was significantly non-different because of dietary levels of DLM during the experimental period of eight wks (28-36 wks).

Protein and amino acids, especially methionine has a large influence on egg size (Panda et al., 2006). Methionine is the first limiting amino acid in maize-soybean based diets for laying hens. In the present study, egg weight as well as egg mass was improved significantly due to increase in the levels of DLM from 0.28 to 0.32%. As no significant increase in any of the production parameters were observed due to higher levels of DLM beyond 0.32%, it is implied that 0.32% DLM in 15% CP diet was adequate for optimizing the production performance in Vanaraja laying hens during 28 to 36 wks of age

The effect of DL methionine (DLM) supplementation on egg quality parameters in Vanaraja laying hens measured at 36 wks is presented in Table 4.

Table 4. Effect of supplementation DL methionine on egg quality parameters of Vanaraja laying hens

Parameters	% DL methionine				SEM	P value
	0.28 (T1)	0.32 (T2)	0.36 (T3)	0.40 (T4)		
Albumen (%)	58.1	58.2	58.5	58.3	0.25	0.71
Yolk (%)	32.9	32.9	32.5	32.9	0.22	0.82
Eggshell (%)	8.92	8.84	8.94	8.82	0.04	0.91
Albumen Index	6.84	7.02	7.18	7.24	0.28	0.34
Yolk Index	34.1	34.1	34.0	33.9	0.29	0.55
Shape Index	72.5	72.8	72.6	72.6	0.58	0.85
Haugh Unit	71.2[a]	75.2[b]	75.5[b]	75.3[b]	2.16	0.04
Eggshell thickness (mm)	0.36	0.36	0.36	0.37	0.003	0.92

[a, b] Means with different superscript in a row differs significantly SEM- Standard Error of Mean

None of the egg quality parameters (albumen, yolk and eggshell%; albumen index, yolk index, shape index and eggshell thickness) except the Haugh unit was influenced due to the levels of supplemental DLM in the diet of Vanaraja laying hens. Similar to the findings of the present study, Mohapatra et al. (2018) reported no influence of supplemental amino acids (lysine and methionine) on egg quality parameters like albumen %, yolk %, eggshell %, albumen index, yolk index, shape index and eggshell thickness in Vanaraja laying hens. The Haugh unit of the egg was 71.22 in the control diet containing 0.28% DLM. Increasing the levels of DLM to 0.32% in the diet significantly improved the Haugh unit (75.24) compared to the control group. No further increase in EW could be observed by further increasing the supplemental DLM levels from 0.32% to either 0.36 or 0.40%. The Haugh unit of the egg in the dietary group containing 0.32, 0.36 and 0.40% DLM was comparable. The Haugh unit is a measure of egg protein quality and the findings of the study suggested that diet containing 16% CP with 0.32% DLM is adequate to optimize the egg protein quality (Haugh unit) in Vanaraja laying hens. Novak et al. (2006) also suggested that the essential amino acids contents, especially lysine and DLM content of the diet in laying hen is important in maintaining optimum egg protein quality.

The influence of dietary supplementation of DL methionine (DLM) on serum biochemical parameters of Vanaraja laying hens measured at 36 wks of age is given in Table 5.

Table 5. Effect of Effect of supplementation DL methionine on different serum biochemical parameters of Vanaraja laying hens

Parameters	% DL methionine				SEM	P value
	0.28 (T1)	0.32 (T2)	0.36 (T3)	0.40 (T4)		
Total protein (g/dl)	5.72[a]	6.24[b]	6.18[b]	6.30[b]	0.10	0.05
Creatinine (mg/dl)	1.60	1.65	1.67	1.69	0.03	0.46
Uric acid (mg/dl)	5.54	5.29	5.52	5.48	0.12	0.35
Calcium (mg/dl)	11.8[a]	12.9[b]	12.8[b]	12.7[b]	0.11	0.043
Phosphorus (mg/dl)	5.73	5.64	5.16	5.72	0.25	0.67
Triglyceride (mg/dl)	274	269	284	288	9.78	0.48
Cholesterol (mg/dl)	184	180	178	189	3.24	0.65

[a, b] Means with different superscript in a row differs significantly SEM- Standard Error of Mean

Lowest total protein (5.72 g/dl) concentration in the serum was observed in the laying hens fed the control diet containing 0.28% DLM. Increasing the dietary DLM level to 0.32% in the diet significantly increased the concentration of total protein (6.24 g/dl) in the serum of laying hens. Further increased in the levels of dietary DLM to either 0.36 or 0.40% did not have any additional advantage on the total protein concentration in the serum. Alagaswany et al. (2011) also reported higher total protein concentration in serum of hens fed diet containing higher levels of EAA in diet containing 16% CP. The serum, creatinine and uric acid concentrations in the serum were not influenced due to the levels of dietary DLM in the present study. The calcium (Ca) in the serum was lowest in the control diet containing 0.28% DLM. Increasing the dietary DLM level to 0.32% and beyond (0.36 or 0.40%) significantly elevated the Ca levels in serum. This is in line with the findings of Gumpha et al. (2019) who reported higher serum Ca concentration in Vanaraja laying hens due to supplemental essential amino acid in the diet, which might have triggered calcium binding protein synthesis leading to higher calcium absorption from the intestine. The concentrations of phosphorus (P), triglycerides, and total cholesterol in serum of Vanaraja laying hens were not influenced by the dietary levels of DLM. Ding et al.(2016) also did not find significant effect of dietary amino acid levels on the serum concentration albumin, triglycerides and cholesterol in laying hens.

CONCLUSION

In the present study, dietary supplementation of DLM at 0.04% to the diet containing 0.28% DLM significantly improved egg weight and egg mass, Haugh unit, serum protein and calcium concentration. No further improvement in any of these parameters could be observed due to higher levels of DLM supplementation (0.08 or 0.12%). Thus it is concluded that diet containing 2600kcal/kg ME, 15% CP, 0.7% lysine and 0.32% DL-methionine is adequate in eliciting optimum performance in Vanaraja laying hend during 28 to 36 weeks of age.

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