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Effect of methionine and lysine supplementation to low protein diet and its influence on production performance, egg quality and egg composition of Vanaraja laying hens

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Abstract
The effect of Methionine and lysine supplementation to low protein diet and its influence on production performance, egg quality and egg composition parameters were evaluated in Vanaraja laying hens. One hundred twenty (n=120), Vanaraja laying hens of thirty weeks of age were selected and randomly distributed into four dietary treatments with three replicates in each group having 10 birds in each replicate pen. Four diets were formulated with 2600 kcal/kg of metabolizable energy (ME) and 13% crude protein (CP). The dietary treatments were: T1- diet contains 0.65% lysine and 0.28% methionine; T2- diet contains 0.70% lysine and 0. Highest and lowest HHEP was observed in the group containing lowest level of supplemental EAAs (0.65% Lys and 0.28% Met) and highest level of supplemental EAAs (0.80% Lys and 0.40% Met) respectively. Enhancing the Lysine and Methionine supplementation to 0.70% and 0.32% respectively, increased the egg weight significantly ($P<0.05$). Significantly ($P<0.05$), higher egg mass (g/day) was observed in the dietary group containing lowest essential amino acid (EAAs) compared to highest EAAs. Feed conversion ratio was highest in the dietary group containing 0.65% Lys and 0.28% Met (T1). The egg quality parameters had no effect of supplemental EAAs to low CP diet. It was concluded that diet containing 0.65% lysine and 0.28% methionine (T1) is adequate for optimum production performance and egg quality in Vanaraja laying henss.

Keywords: Methionine, lysine, low protein diet, Vanaraja

1. Introduction
Poultry keeping for commercial production of egg and meat is one of India's most innovative industries. The popularity of poultry meat is on the rise during the last three decades and is presently accounting for more than half of the total meat consumed in the country [1]. However, the commercial poultry activity is concentrated in urban and semi-urban areas only and the rural poultry sector scenario remains unchanged. Chicken population in rural areas increased marginally from 63 million to 75 million during the last 40 years. Adopting poultry farming in rural areas utilizing chicken varieties which demand low inputs in terms of nutrition and management and perform better is a potential tool to increase the availability of poultry products and economic status of the rural people in India.

In the 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 as the area expansion for cultivation is limiting. This has compelled the researchers to find alternate feed resources and/ or formulating low nutrient density based balance diet to meet the requirement of poultry.

Among the nutrients needed by the bird, protein is one of the most important nutrient, which has a major influence on growth, feed utilization and production performance [2]. Protein is an important component of chicken egg (6.5 to 7 g protein /egg). Thus, dietary protein has a major influence on production performance of the bird [3, 4].

The most important nutrient in the poultry feed is protein which is the costliest among all the nutrients. Farmers growing birds under intensive or rural farming are uncapable of bearing such high feed cost. In fact, feeding high protein diet to the poultry leads to nitrogen excretion which further can only utilize approximately 40% of the dietary protein, it seems logical to decrease the level of protein in the diet [5]. So it is justified to reduce the dietary CP and balance it by supplementation of essential amino acids (EAA).

Lysine (Lys) and methionine (Met) are the two most important to use the sub-optimals of dietary CP and provision of balanced amino acids by synthetic amino acid supplements [8, 9]. So, researchers have been successful by supplementation of lysine and methionine to a low protein diet in the other poultry species and breeds without affecting their production performance.

Many chicken varieties have been developed across the country for rural poultry farming and one such promising variety is 'Vanaraja'. Vanaraja is a dual- up to 110-120 eggs per year under backyard system of rearing [10]. However, many farmers are rearing these birds under an intensive system of production due to the high market price of egg produced from Vanaraja birds.

To our knowledge, no information is available in the literature on supplementation of essential amino acids to low protein diet in Vanaraja laying hens. Therefore, the present experiment was conducted to effect of methionine and lysine supplementation to low protein diet and its influence on production performance, egg quality and egg composition of Vanaraja laying hens.

2. Materials and Methods

2.1 Experimental site

This research was carried out at the poultry unit of the ICAR-Central Institute for Women in Agriculture, Baramunda, Bhubaneswar, and Odisha, India. Located at latitude 20.284911 and longitude 85.786717 with average annual rainfall of 1436.1mm and humidity varies from 48 to 85%.

2.2 Experimental design

One hundred twenty (*n=120*), Vanaraja laying hens of thirty weeks of age were selected and randomly distributed into four dietary treatments with three replicates in each group having 10 birds in each replicate pen. Four diets were formulated with 2600 kcal/kg of metabolizable energy (ME) and 13% crude protein (and 0.36% methionine and T4– diet contains 0.80% lysine and 0.40% methionine. The calcium (Ca) and non phytate phosphorus (NPP) was constant in all the diets.

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

Ingredient	Parts per quintal			
	Diet 1	Diet 2	Diet 3	Diet 4
Maize	59.46	59.46	59.46	59.46
Soyabean meal	13.20	13.20	13.20	13.20
Deoiled rice bran	16.00	15.89	15.79	15.68
Shell grit	9.70	9.70	9.70	9.70
Dicalcium phosphate	0.90	0.90	0.90	0.90
Common salt	0.40	0.40	0.40	0.40
Vitamin premix	0.015	0.015	0.015	0.015
B-complex	0.015	0.015	0.015	0.015
Trace mineral	0.120	0.120	0.120	0.120
Toxin binder	0.100	0.100	0.100	0.100
Antibiotics	0.050	0.050	0.050	0.050
Lysine	0.00	0.064	0.128	0.192
Methionine	0.04	0.08	0.12	0.16
Total	100	100	100	100
Nutrient composition (Calculated value)				
Metabolizable Energy (kcal/kg)	2602	2601	2601	2601
Crude Protein (%)	13.04	13.03	13.02	13.01
Lysine (%)	0.65	0.70	0.75	0.80
Methionine (%)	0.28	0.32	0.36	0.40
Calcium (%)	3.20	3.20	3.20	3.20
Nonphytate phosphorous (%)	0.30	0.30	0.30	0.30

2.3 Production performance

Body weight (BW) was recorded at the beginning of the experiment and subsequently at four weeks interval. The average feed consumption was recorded as gram/ hen per day. Feed conversion ratio (FCR) was calculated as weeks period were collected to measure the egg weight. The egg mass per day was calculated as follows

$$\text{Egg mass per day} = \frac{\text{Number of egg produced} \times \text{average egg weight}}{\text{Number of birds} \times \text{duration (days)}}$$

2.4 Egg quality and composition

For analyzing the egg quality and composition nine eggs (three eggs per replicate) were randomly chosen from each dietary treatment from the eggs laid on 38th week. The protein and fat content of the eggs were analyzed by following the method of AOAC [11] official method of analysis. Egg quality parameters were analyzed as follows

2.4.1 Characteristics of unopened egg

Egg weight: Egg weight was recorded by an electronic balance.

2.4.2 Characteristics of opened eggs

The eggs were broken and the contents were poured over a levelled plate placed on a table and the following characteristics were measured and/or calculated.

Shell weight

After pouring out the contents of the eggs completely on the plate, the egg shells were kept overnight in a hot air over for drying and the weights were recorded by an electronic balance next day.

Shell percent

The shell percent was calculated as follows

$$\text{Shell \%} = \frac{\text{Weight of the dried shell}}{\text{Weight of the egg}} \times 100$$

Shell thickness

Three pieces of the shell, one each from the narrow end, broad end and middle were

albumen by using a speedometer at three different points and the mean value was calculated.

Haugh unit (HU)

The Haugh unit was calculated according to Haugh [12] by using the following formula.

$$HU = 100 \log (H + 7.57 – 1.7 W^{0.37})$$

Where H = Average height of albumen (mm)

W = Average weight of egg (g)

Albumen weight

The albumen weight was calculated by carefully separating the albumen from the yolk and weighing in a previously weighed Petri dish, using the formula.

Albumen weight = (Weight of Petri dish + Albumen) – Weight of Petri dish.

Albumen percent: The albumen percent was calculated by using the formula.

$$\text{Albumen \%} = \frac{\text{Weight of albumen}}{\text{Weight of egg}} \times 100$$

Yolk weight

The yolk was weighed (after separating the albumen completely) in a previously weighed Petri dish and calculation was made using the formula.

Yolk weight = (Weight of Petri dish + Yolk) – Weight of Petri dish.

Yolk percent

The yolk percent was calculated as follows

$$\text{Yolk \%} = \frac{\text{Weight of the yolk}}{\text{Weight of the egg}} \times 100$$

Nutrient Composition of egg

For analyzing nutrient composition of egg, three eggs from each treatment were randomly chosen from each dietary treatment. The edible portion was removed, oven dried, milled into flour and kept in laboratory freezer for pending analysis. The protein and India Pvt. Ltd, Munbai, India by HPLC method described by Spackman *et al*. [13].

Statistical analysis

The data obtained from the experiment were statistically analyzed according to Snedecor and Cochran [14]. The data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range (DMR) Test [15] to test the difference between treatments means, wherever necessary.

3. Result

The effect of supplementation of EAAs to low CP diet on production performance of the Vanaraja laying hens in the whole experimental period (30-42 wks.) is given in the Table no. 2. The HHEP significantly (P<0.05) differed across the dietary treatments during the whole period of study. Highest and lowest HHEP was observed in the group containing lowest level of supplemental EAAs (0.65% Lys and 0.28% Met) and highest level of supplemental EAAs (0.80% Lys and 0.40% Met) egg weight was observed in diet containing 0.75% Lys and 0.36% Met compared to either 0.70% Lys and 0.32% Met and 0.80% Lys and 0.40% Met. The egg mass per day was comparable among the dietary treatment containing 0.65% to 0.70% Lys and 0.28 to 0.32% Met. Significantly (P<0.05), higher egg mass (g/day) was observed in the in the dietary group containing 0.65% Lys and 0.28% Met (T1). However, further increase in Lys and Met levels from 0.65% to 0.70% and 0.28% to 0.32%, respectively resulted in significantly (P<0.05) improved FCR with no further improvement. The lowest FCR was observed in the dietary groups containing highest level of supplemental EAAs.

Table 2: Effect of supplementation of essential amino acids to low protein diet on overall production performance of Vanaraja laying hens (30-42 weeks)

Parameters	T 1	T2	T3	T4	SEM	P value
Hen housed egg Production (%)	65.47^{c}	59.08^{ab}	62.06^{bc}	57.77^{a}	0.774	0.001
Egg weight (g)	52.21^{a}	53.96^{b}	52.48^{a}	53.68^{b}	0.152	0.000
Egg mass (g/day)	34.28^{b}	31.40^{b}	32.35^{ab}	30.60^{a}	0.418	0.008
Feed consumption/day (g)	140.9	140.9	140.9	140.9	-	-
Feed conversion ratio	2.427^{b}	2.230^{a}	2.294^{ab}	2.192^{a}	0.030	0.030

^{ab} Means with different superscript in a row differs significantly (P<0.05)

The egg quality parameters like albumen, yolk and eggshell percent (Table 3) had no effect of supplemental EAAs to low CP diet. The result revealed that albumen index, yolk index and shape index were not significantly (P<0.05) altered by supplementation of EAAs to low CP diet. Similarly, other egg quality parameters like Haugh unit and eggshell thickness did not vary due to variation in the supplementation of EAAs to low CP diet.

Table 3: Effect of supplementation of essential amino acids to low protein diet on egg quality parameters of Vanaraja laying hens (at the end of 38 weeks of age)

Parameters	T_1	T_2	T_3	T_4	SEM	P valu
Albumen (%)	58.87	58.92	59.55	59.73	0.28	0.64
Yolk (%)	32.03	31.75	31.59	30.76	0.30	0.51
Eggshell (%)	9.097	9.327	8.85	9.50	0.08	0.06
Albumen Index	3.89	4.024	4.88	3.49	0.23	0.18
Yolk Index	38.08	38.56	37.16	36.69	0.41	0.38
Shape Index	73.31	71.17	73.76	73.56	0.45	0.15
Haugh Unit	79.54	79.61	81.44	78.92	2.11	0.47
Eggshell thickness (mm)	0.35	0.364	0.34	0.36	0.00	0.49

SEM- Standard Error of Mean

The effect of supplementation EEAs to low protein diets on egg composition of Vanaraja laying hens at 42 wks of age is presented in Table 4. There was no significant effect of dietary protein content on either protein or fat content of the egg.

Table 4: Nutrient composition of egg (on as such basis)

Parameters	T1	T2	T3	T4
Proximate Composition				
Dry matter	94.68	94.57	94.29	94.97
Protein	49.23	51.63	51.42	50.85
Ether extract	45.45	42.94	42.87	44.12
Amino acid profile of egg				
Methionine	1.354	1.385	1.545	1.394
Cystine	0.751	0.858	0.865	0.802
Methionine+cystine	2.105	2.243	2.410	2.196
Lysine	3.021	3.035	3.292	3.044
Threonine	2.126	2.169	2.315	2.176
Arginine	2.586	2.623	2.750	2.593
Isoleucine	2.363	2.435	2.567	2.404
Leucine	3.812	3.903	4.117	3.876
Valine	2.907	3.005	3.187	2.973
Histidine	0.909	0.934	0.992	0.916
Phenyl alanine	2.363	2.438	2.618	2.415
Glycine	1.472	1.522	1.625	1.499
Serine	3.096	3.249	3.452	3.193
Proline	1.764	1.769	1.879	1.747
Alanine	2.627	2.657	2.848	2.632
Aspartic acid	4.619	4.731	5.032	4.690

4. Discussion

The overall HHEP also varied significantly due to variation in EEAs content of the diet. In the present study, the birds fed diet with the lowest EEAs content (0.65% Lys and 0.28% Met) elicited significantly higher HHEP. In a similar study, Gumpha [16] reported optimum the lowest level of 0.65% Lys and 0.28% Met is adequate for optimum egg production in Vanaraja laying hens. In a study with Matrouh laying hens, El Maksoud *et al.* [17] reported similar egg production (56.26%) due to supplemental AAs (0.74% Lys & 0.30% Met) to low CP (12%) diet compared to higher level of CP (14 to 16%) diet.

the overall egg weight was significantly higher in the dietary group containing the highest level of EAAs (0.80% Lys & 0.40% Met) was due to lower HHEP as egg production and egg weight is [20] reported higher egg weight and egg mass in diet containing lower level of CP (14.4%) compared to higher level of CP (16.8%) with equal levels of Lys and Met in the diet.

The feed consumed per day was similar across the dietary treatments during the entire period of study. The laying hens were offered a measured quantity of feed everyday based on their body weight, activity as well as production. No feed residues were left over the next day of feeding during the whole study. This could be the reason that the feed consumed everyday was 0.70% Lys the first and second limiting AAs in maize-soybean based diets for laying hens. Methionine is excretion by 30 to 35%.

None of the egg quality parameters (albumen %, yolk %, eggshell %, Haugh 0.61% Lys and 0.31% Met. In contrast, Novak *et al.* [24] reported no variation in eggshell % due to Lys and Met intake which was similar to the finding of the present study. Wu *et al.* [27] also reported no significant effect of dietary protein (14-16%) on Haugh unit, egg specific gravity and egg shell thickness. Similarly, Salah uddin *et al.* [28] reported comparable egg shell thickness and eggshell percentage in the diets containing 13 to 22% CP, confirming the findings of the present study.

So it was concluded that diet containing 0.65% lysine and 0.28% methionine (T1) is adequate for optimum production performance and egg quality in Vanaraja laying hens.

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