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Manju G Preedaa
Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

P Selvaraj
Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

P Visha
Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

MR Purushothaman
Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

N Murali
Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

Corresponding Author:
Manju G Preedaa
Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

Effect of dietary tryptophan supplementation on egg quality parameters

Manju G Preedaa, P Selvaraj, P Visha, MR Purushothaman and N Murali

Abstract
The study was conducted to determine the effect of dietary supplemental tryptophan on the external and internal egg quality parameters. A total of 350 White Leghorn layers of 18 weeks were allocated to seven experimental groups, each of which included 5 replicates and each replicate had 10 birds reared upto 45 weeks of age. Experimental diets consisted of two protein diets along with 5 supplemental levels of tryptophan. The basal diet consisted of normal protein (CP-17%) with 0.165% tryptophan and low protein diet with 0.153% tryptophan. Tryptophan was supplemented at 0.015 and 0.035% in normal protein diet to obtain 0.18% and 0.20% digestible tryptophan and at 0.012, 0.027 and 0.047% in low protein diets (CP- 16.23%) to obtain 0.165%, 0.18% and 0.20% digestible tryptophan respectively. Egg quality characteristics were assessed in six eggs per treatment collected randomly at fortnightly intervals. The results of the study indicate that external and internal egg quality parameters were not influenced either by the level of protein or tryptophan. However, shell thickness was higher in birds supplemented with 0.18% and 0.20% tryptophan of both normal and low protein diets, respectively during 31-37 weeks of age and overall experimental period. There was no significant alteration in egg cholesterol throughout the experimental period except at 29-35 weeks of age, wherein egg cholesterol was lower in treatments supplemented with tryptophan at 0.18% and 0.20% in both normal and low protein diets, respectively when compared to other diets.

Keywords: Layers, tryptophan, shell thickness, egg cholesterol

Introduction

Amino acids play an important role as component of protein and are essential for maintenance, production and other metabolic processes in poultry. Tryptophan is considered to be the third limiting amino acid in diets based on corn and soybean meal for laying hens. However, few studies have been conducted to determine the requirement of digestible tryptophan and its ideal ratio for laying hens.

Protein level or amino acids proportion in poultry feed make up a significant portion of feed cost and are major determinants of batch productivity, thus they directly interfere with the profitability of the poultry enterprise. Recent research is focused to maintain layer's performance and also to maximize profitability by lowering protein content of diets marginally with supplementation of synthetic amino acids

Among the essential amino acids that commonly constitute proteins, tryptophan is found in the lowest proportion in plasma or blood. It has relatively low tissue storage and the overall tryptophan concentration in the body was the lowest among all amino acids [1]. Tryptophan plays a significant role in low protein laying hen diets. Synthetic L-tryptophan is 99.3% digestible [2].

Because of serotonin's role in neurotransmission, dietary tryptophan content has been linked to alterations in bird's behavioral conditions like hysteria alleviation, reduced cannibalism and feather pecking [3, 4] Tryptophan reduced aggressive behaviour in birds and also modulated stress response including social and environmental adaptability, which led to more efficient egg production [5] Egg cholesterol content had been observed as dynamic and fluctuating factor and the level was reduced significantly by supplementing niacin.

Materials and methods

Three hundred and fifty Single Comb White Leghorn layers of 16 weeks were procured from commercial breeding farm (Namakkal) and the experiment was conducted from March to October 2016.

All the birds were reared under standard management conditions throughout the experimental period. Birds were vaccinated against Ranikhet disease (RDVF1) and Infectious Bronchitis (IB).
The experimental layer diets were formulated according to the breeder's specification (Venkateshwara Hatcheries Private Limited). In commercial formulation, the levels of essential amino acids were fixed in relationship with lysine, however in our experiment the essential amino acids were fixed based on digestible tryptophan.

The experimental groups and their diets are as follows

Treatment	Diets	No. of birds	No. of replicates	No. of birds / replicates
Diet I (T1)	Layer diet with 17% CP and 0.165% digestible tryptophan	50	5	10
Diet II (T2)	Layer diet with 16.23% CP and 0.153% digestible tryptophan	50	5	10
Diet III (T3)	Diet II+ 0.012% digestible tryptophan supplementation	50	5	10
Diet IV (T4)	Diet I+ 0.015% digestible tryptophan supplementation	50	5	10
Diet V (T5)	Diet I+ 0.035% digestible tryptophan supplementation	50	5	10
Diet VI (T5)	Diet II+ 0.027% digestible tryptophan supplementation	50	5	10
Diet VII (T7)	Diet II+ 0.047% digestible tryptophan supplementation	50	5	10

The ingredients of the diets are presented in the Table 1.

Table 1: Ingredients composition (%) of pre-layer and layer diet fed different levels of tryptophan and crude protein

Ingredients (%)	Pre-layer diet		Layer diet	
	Diet I (normal protein)	Diet II (low protein)	Diet I (normal protein)	Diet II (low protein)
Maize	50.8	52.5	49.6	52.2
Deoiled rice bran	15.0	15.0	15.0	15.0
Sunflower oil cake	8.9	10.0	5.1	4.3
Soyabean meal	15.6	12.4	16.5	14.7
Fish meal	3.0	3.0	3.1	3.2
Di calcium phosphate	0.7	0.7	0.56	0.58
Calcite	4.0	4.0	4.0	4.0
Shell grit	1.8	2.0	5.7	5.7
DL-Methionine (g/100kg)	89	96	141	165
Lysine (g/100kg)	8	87	0	34
L-Threonine (g/100kg)	0	0	34	62
SodaBicarb (g/100kg)	33	27	55	61

Additives and supplements (per 100 kg)
Vitamin premix ([1]Hyblend) - 100 g, trace mineral ([2]Ultra TM) - 100 g, toxin binder - 160 g, Vitamin B-complex ([3]Meriplex) - 100 g, liver stimulant (hepatocare) - 100 g, choline chloride (60%) - 150 g, oxytetracycline (10%) - 100 g, phytase - 20 g, NSP enzyme - 50 g. [1]Hyblend – nutritional value per gram-vitamin A - 82500 IU, vitamin B2 - 50 mg, vitamin D3 - 12000 IU, menaphthone sodium bisulphate and vitamin K (stabilized) - 10 mg. [2]Ultra TM - Each 5kg contains manganese - 270 g, zinc - 260 g, iron - 100 g, iodine - 10 g, copper - 10 g, cobalt - 5 g, selenium - 1.5 g [3]Meriplex - each gram contains vitamin B_1 - 8 mg, vitamin B_6 - 16 mg, vitamin B_{12} - 80 mcg, vitamin E_{50} - 80 mg, niacin - 120 mg, folic acid - 8 mg, calcium D pantothenate - 80 mg, calcium - 86 mg.

External egg quality parameters
Length and width of the egg was measured with electronic digital vernier caliper (Mitutoyo, Japan) with 0.05 mm accuracy. Shape Index (SI) was calculated using the formula: Shape Index = [egg width / egg length] × 100. Specific gravity was calculated using the formula: Specific gravity = Egg weight (g) / Volume of water dispersed (ml).
The shell weight was obtained by carefully placing the opened part in the shell along with intact shell membranes weighed on the electronic balance. Egg shell thickness (mm) was calculated according to [6].

Internal egg quality
The eggs were broken on a glass plate, laid evenly on the table, width of the thick albumen and yolk was measured using the vernier caliper with 0.05 mm accuracy. Height of the thick albumen and yolk was measured to 0.1 mm accuracy using Ames tripod micrometer.
Albumen index, yolk index, albumen weight and yolk weight were calculated according to Romanoff and Romanoff (1949). Albumen index = Maximum height of the thick albumen (mm) / Maximum width of the thick albumen (mm), Yolk index = [Yolk height (mm) / Yolk diameter (mm)], Albumen ratio% = [Albumen weight (g) / Egg weight (g)] x100 and Yolk ratio (%) = [Yolk weight (g) / Egg weight (g)] x100,
The yolk color of eggs was measured directly by using the Roche yolk color fan. The Haugh unit (HU) was obtained by the formula proposed by [7]. Haugh unit (HU) = 100 log ($H+7.57 – 1.7 W^{0.37}$), where W - Weight of egg (g) and H - Height of thick albumen (mm). Shell ratio was estimated from the expression shell ratio (%) = (Shell weight / Egg Weight) x 100.

Egg Cholesterol
Lipid extraction and cholesterol content of the egg yolk were determined by the method of [8, 9] respectively.

Results and discussion
External egg quality
The overall external egg qualities such as shape index, specific gravity, eggshell weight and eggshell thickness studied are presented in Table 2 and 3, respectively. The overall and biweekly observations of shape index, specific gravity and eggshell weight were not influenced by the level of protein or tryptophan. The observations of our study on shape index, specific gravity and eggshell weight are in agreement with results of [10, 11, 12, 13, 14, 15, 16].

The values of shape index, specific gravity and eggshell weight were in the range of 75.47 ± 0.84 to 78.78 ± 0.72, 1.13 ± 0.02 to 1.24 ± 0.01 and 11.55 to 12.65 (%), respectively. The above values were in normal range [17] suggesting that the eggs laid in current experiment were free from any deformation and were ideal in quality with proper calcification.

The shell thickness of eggs were significantly ($P<0.01$) higher in 0.18% tryptophan (T4 and T6) and 0.20% tryptophan (T5 and T7) of both normal and low protein diets, respectively, than eggs laid from birds fed normal protein with 0.165% tryptophan (T1) and low protein diets with 0.153% (T2) and 0.165% tryptophan (T3) during 31-37 of age and overall experimental period.

The increased shell thickness in present study might be due to increased utilization of calcium and phosphorus via niacin [18, 19] produced from supplemental tryptophan.

Table 2: Mean (± SE) of external egg quality parameters of White Leghorn layers fed different levels of tryptophan and crude protein during 23 to 45 weeks of age

Treatment	Shape index	Specific gravity (wt./vol.)	Shell weight (%)
T1	76.98 ± 0.22	1.185 ± 0.006	11.83 ± 0.03
T2	76.85 ± 0.23	1.182 ± 0.006	11.79 ± 0.03
T3	77.01 ± 0.20	1.184 ± 0.006	11.84 ± 0.05
T4	77.32 ± 0.24	1.192 ± 0.006	11.89 ± 0.05
T5	77.20 ± 0.23	1.195 ± 0.007	11.95 ± 0.05
T6	76.96 ± 0.19	1.190 ± 0.006	11.91 ± 0.04
T7	77.44 ± 0.23	1.191 ± 0.006	11.97 ± 0.04

Each value is a mean of six observations

Table 3: Mean (± SE) egg shell thickness (mm) of White Leghorn layers fed different levels of tryptophan and crude protein during 23 to 45 weeks of age

Treatment	Age in weeks											
	23	25	27	29	31	33	35	37	39	41	43	45	Overall
T1	0.35±0.01	0.35±0.01	0.35±0.01	0.38±0.01	0.38^a±0.01	0.38^a±0.01	0.38^a±0.01	0.38^a±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.37^a±0.01
T2	0.35±0.01	0.35±0.01	0.35±0.01	0.37±0.01	0.37^a±0.01	0.38^a±0.01	0.39^a±0.01	0.38^a±0.01	0.37±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.37±0.01
T3	0.35±0.01	0.35±0.01	0.35±0.01	0.38±0.01	0.38^a±0.01	0.39^a±0.01	0.38^a±0.01	0.38^a±0.01	0.37±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.37^a±0.01
T4	0.35±0.02	0.35±0.01	0.35±0.01	0.38±0.01	0.41^b±0.01	0.40^b±0.01	0.40^b±0.01	0.41^b±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38^b±0.01
T5	0.36±0.01	0.36±0.01	0.36±0.01	0.39±0.01	0.41^b±0.01	0.41^b±0.01	0.41^b±0.01	0.41^b±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38^b±0.01
T6	0.36±0.01	0.36±0.01	0.35±0.01	0.38±0.01	0.40^b±0.01	0.40^b±0.01	0.40^b±0.01	0.40^b±0.01	0.37±0.01	0.38±0.02=1	0.38±0.01	0.38±0.01	0.38^b±0.01
T7	0.36±0.01	0.36±0.01	0.36±0.01	0.39±0.01	0.40^b±0.01	0.40^b±0.01	0.40^b±0.01	0.40^b±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38±0.01	0.38^b±0.01

Each value is a mean of six observations

Internal egg quality

The internal egg quality parameters such as Haugh unit, albumen index, albumen weight (%), yolk weight (%), yolk index and yolk color were studied biweekly and the results are reported in Table 4. Tryptophan supplementation did not change the internal quality parameters.

The values of Haugh unit, albumen index, albumen weight (%), yolk weight (%), yolk index and yolk color were in the range of 83.30 ± 0.13 to 86.72 ± 0.59, 0.085 ± 0.02 to 0.089 ± 0.01, 58.47± 0.25 to 64.49 ± 0.43, 23.73 ± 0.76 to 29.30 ± 0.27, 0.361 ± 0.004 to 0.444 ± 0.005, 4.70 ± 0.30 to 5.90 ± 0.31, respectively.

A good quality egg generally has Haugh unit of 70 and above [20]. The overall Haugh unit of eggs recorded during different egg production cycles were in the above mentioned range (83 to 86). The albumen index in our study did not exceed the value of 0.1 which indicated the freshness of egg, whereas yolk index value was around 0.48 which indicated the spherical nature of the eggs and better integrity of yolk [21]

Table 4: Mean (± SE) of internal egg quality parameters of White Leghorn layers fed different levels of tryptophan and crude protein during 23 to 45 weeks of age

Treatment	Haugh unit	Albumen Index	Albumen weight (%)	Yolk weight (%)	Yolk Index	Yolk colour
T1	85.67 ± 0.13	0.086 ± 0.005	61.26 ± 0.28	26.90 ± 0.23	0.429 ± 0.002	5.55 ± 0.26
T2	85.51 ± 0.13	0.086 ± 0.006	61.19 ± 0.27	27.02 ± 0.23	0.426 ± 0.003	5.50 ± 0.21
T3	85.54 ± 0.15	0.086 ± 0.006	61.64 ± 0.26	26.52 ± 0.22	0.430 ± 0.003	5.50 ± 0.23
T4	85.65 ± 0.14	0.087 ± 0.005	61.45 ± 0.28	26.66 ± 0.24	0.431 ± 0.003	5.60 ± 0.18
T5	85.79 ± 0.16	0.088 ± 0.007	61.55 ± 0.23	26.50 ± 0.19	0.434 ± 0.002	5.50 ± 0.21
T6	85.57 ± 0.13	0.088 ± 0.005	61.20 ± 0.23	26.90 ± 0.19	0.432 ± 0.002	5.55 ± 0.20
T7	85.56 ± 0.16	0.088 ± 0.005	60.71 ± 0.23	27.31 ± 0.22	0.432 ± 0.002	5.45 ± 0.18

Each value is a mean of six observations

The present study revealed that supplementation of tryptophan at lower and higher levels did not alter the internal egg quality parameters. Also, these internal qualities were not influenced by crude protein levels. These findings are in agreement with [2, 10, 12, 15, 22, 23]. In contrary to our results, increased albumen and yolk (%) by tryptophan supplementation at 0.199% was observed by [10].

Egg Cholesterol

The egg yolk cholesterol during different periods from 20 to 43 weeks of age is presented in Table 5. There was no significant alteration in egg cholesterol throughout the experimental period except at 29-35 weeks of age. During this period egg cholesterol was lower in treatments supplemented with tryptophan at 0.18% and 0.20% in both normal protein (T4 and T5) and low protein (T6 and T7) diets, respectively, when compared to 0.165% tryptophan in normal (T1), low protein (T3) and low tryptophan 0.153% in low protein diets (T2).

The present findings are in agreement with [24] and the reduced egg cholesterol in the current study may be due to metabolic niacin produced from tryptophan. The mechanism behind this is that niacin reduces liver HMG-CoA reductase activity through its oxidative form ($NADP^+$) and decreases the cholesterol deposition in the egg yolk.

Table 5: Mean (± SE) egg cholesterol (mg/g of yolk) of White Leghorn layers fed different levels of tryptophan and crude protein during 23 to 45 weeks of age

Treatment	Age in weeks												
	23	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**39**	**41**	**43**	**45**	**Overall**
T1	17.09 ±0.53	17.22 ±0.26	17.26 ±0.23	17.72 ^{b}±0.49	17.78^{b} ±0.32	17.82^{b} ±0.32	17.34^{b} ±0.29	17.78 ±0.47	17.99 ±0.42	18.26 ±0.50	18.28 ±0.28	18.41 ±0.51	17.79^{b} ±0.12
T2	17.24 ± 0.41	17.31 ± 0.18	17.50 ± 0.31	17.77^{b} ± 0.66	17.88^{b} ± 0.29	17.83^{b} ± 0.35	17.68^{b} ± 0.23	17.96 ± 0.34	18.04 ± 0.59	18.35 ± 0.68	18.29 ± 0.20	18.50 ± 0.68	17.86^{b} ± 0.13
T3	17.22 ± 0.46	17.24 ± 0.54	17.39 ± 0.48	17.76^{b} ± 0.30	17.82^{b} ± 0.48	17.86^{b} ± 0.19	17.76^{b} ± 0.21	17.90 ± 0.10	17.86 ± 0.15	18.27 ± 0.28	18.15 ± 0.29	18.44 ± 0.42	17.81^{b} ± 0.10
T4	16.99 ± 0.42	16.95 ± 0.43	17.24 ± 0.27	16.46^{a} ± 0.23	16.52^{a}± 0.48	16.97^{a} ± 0.23	16.64^{a} ± 0.14	16.87 ± 0.17	17.28 ± 0.14	18.11 ± 0.37	17.98 ± 0.33	18.07 ± 0.55	17.19^{a} ± 0.11
T5	16.87 ± 0.55	16.91 ± 0.27	16.97 ± 0.48	16.21^{a} ± 0.43	16.44^{a} ± 0.33	16.81^{a} ± 0.18	16.60^{a} ± 0.08	16.87 ± 0.24	17.29 ± 0.15	18.08 ± 0.34	17.75 ± 0.33	18.03 ± 0.32	17.07^{a} ± 0.11
T6	17.03 ± 0.45	17.06± 0.47	17.18 ± 0.40	16.51^{a} ± 0.23	16.63^{a} ± 0.40	17.05^{a} ± 0.23	16.70^{a} ± 0.17	16.91 ± 0.34	17.25 ± 0.12	18.19± 0.29	17.95 ± 0.44	18.15 ± 0.43	17.22^{a} ± 0.11
T7	17.00 ± 0.40	17.02 ± 0.59	17.07 ± 0.25	16.45^{a} ± 0.26	16.60^{a} ± 0.37	17.02^{a} ± 0.21	16.54^{a} ± 0.12	16.88 ± 0.52	17.28 ± 0.14	18.20 ± 0.38	17.93 ± 0.21	18.18 ± 0.32	17.18^{a} ± 0.11

Means bearing different superscript within the column differ significantly (P<0.05)
Each value is a mean of six observations

Conclusion

In conclusion, currently protein sources in layer diets have been partially replaced by industrial amino acids such as tryptophan which leads to increased egg production. Also in our study supplemental tryptophan levels in layer diets has increased egg shell thickness and decreased egg cholesterol levels during peak production phase.

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