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Effect of L-threonine supplementation on egg production and quality parameters in white leghorn

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Abstract
The essential amino acids are one of the dietary factors affecting egg size, total solids, and internal egg quality characteristics. Threonine is an essential amino acid for birds as it is involved in a variety of intermediaries for protein metabolism. After methionine and lysine, threonine is considered the third-limiting amino acid in poultry. The availability of cost-effective synthetic threonine since 2002–2003 has made it possible to reduce the dietary CP requirements in animal diets, thus meeting precision nutrition demands. Our experiment aimed to determine the effect of L-Threonine on egg production and quality parameters in laying hens. The effect of dietary supplementation of L-Threonine at various levels (0.2%, 0.4%, 0.6%, and 0.8%) on production performance and egg quality traits for 12 weeks of administration in white leghorn birds resulted in improvements in production percentage, egg weight and egg mass, Haugh Unit, and Shape Index in white leghorn birds. In comparison to other treatments, the 0.4% threonine supplemented group produced the best results and could be recommended for laying birds.

Keywords: L-threonine, white leghorn, egg production

1. Introduction
Eggs are recognised for their great biological value and are used as a WHO reference protein for humans. Genetic and dietary advances have increased the production of laying hens. Despite these developments, additional research is still needed on a few nutritional issues (Figueiredo *et al*., 2012) [11]. Few authors disagree with the NRC's requirements since they are incompatible with the conditions of current productivity with various environmental conditions. Few studies have argued that these NRC/ICAR recommendations for amino acids are insufficient for the current strains of poultry. As a result, there is a need to update advice for laying hens in various production conditions.

The essential amino acids are one of the dietary factors affecting egg size, albumen and yolk characteristics, total solids, and internal egg quality (Figueiredo *et al*., 2012) [11]. Threonine is an essential amino acid since birds cannot synthesize it, and its catabolism results in a variety of intermediaries that are involved in protein metabolism. After methionine and lysine, threonine is considered as the third limiting amino acid in poultry (Kidd and Kerr, 1996) [14]. Since the availability of cost effective synthetic threonine in 2002–2003, it has become a possible tool to reduce the dietary CP requirements in animal diets, thus meeting precision nutrition demands (Ojano-Dirain and Waldroup, 2002) [15]. Many studies found that L-threonine supplementation improved egg production and egg quality parameters in a variety of rearing and feeding conditions (Azzam *et al*., 2011; 2017; Abdel-Wareth and Esmail, 2014) [2, 6, 1]. In laying hens fed a wheat-based diet, increasing levels of L-threonine supplementation improved productive performance (Hossaninejad *et al*., 2021) [12]. In contrast, Azzam and El-Gogary (2015) [5] reported that bodyweight gain, feed intake, and FCR were unaffected in hens by increasing the L-Threonine level in the diet. However, dietary threonine performs antioxidant function and plays an important role in maintaining intestinal barrier function by regulating intestinal protein synthesis and mucin production (Azzam *et al*., 2012, Bertrand *et al*., 2013) [3, 7]. However, threonine impact on egg production and its quality parameters are scant. Therefore, the current study sought to ascertain the effect of L-Threonine on egg production and quality parameters in laying hens.

2. Materials and Methods
2.1 Birds and housing: The birds and housing experiment was conducted at the Department of Instructional Livestock Farm Complex, Veterinary College Shivamogga, Karnataka. A total of three hundred and sixty (White leghorn) layer birds, aged 40 weeks, were randomly

allocated into 5 treatment groups, consisting of 6 replicates of 12 hens each. The birds were housed in cages consisting of four birds per cage. The experimental diet (Table 1) and water were fed *ad libitum* during the trial, and the photoperiod was 17 hours a day.

2.2 Experimental diet: Each group received the same basal diet formulated with corn, DORB, SBM, and crystalline amino acids for 12 weeks. Beginning with the 40th week, hens were fed an experimental diet for a period of 12 weeks.

2.3 Laying performance parameters: In the experiment, egg production percentage was measured as hen day egg production (HDEP %). A digital electronic scale was used to weigh individual eggs, and egg mass was calculated by multiplying the average egg weight with HDPE%. Abnormal eggs (broken eggs, soft shell eggs, and shell-less eggs) were discarded.

2.4 Egg quality parameters: Eggs were collected randomly every week (12 eggs per treatment) to measure egg quality parameters. Vernier caliphers were used to measure maximum width and length with the least count of 0.01 to calculate the egg shape index as described by Duman *et al*., 2016 [9]. By breaking the egg contents were poured, and shell thickness was measured in the middle part of the egg by removing shell membranes using the screw gauge. Albumin and yolk index are calculated by measuring the height and diameter of albumin and yolk, respectively. To assess internal quality, the Haugh unit was measured using the formula co-relating with albumin height.

$$HU = 100 * \log [(H+7.57) - (1.7*W^{0.37})]$$

Where HU= Haugh unit, H= Albumen height in mm, W= Egg weight in gm

Table 1: Proximate composition of basal diet

Constituents	Amount (%)*
Dry matter (%)	89.30
Ash (%)	11.50
Crude protein (%)	17.20
Ether extract (%)	3.5
Crude fibre (%)	8.1
Neutral detergent fibre (%)	10.5
Acid insoluble ash (%)	4.0
Salt (as NaCl) %	0.5
Acid detergent fibre (%)	3.50

*mean of observations

2.5 Statistical analysis
The statistical data analyses for the experiment were performed using SPSS Version 16.0 for Windows (SPSS Inc., Chicago, IL).

3. Results and Discussion
3.1 Production performance
Hen day egg production (%), egg weight and egg mass were calculated as indicators of production performance in layers and are indicated in Table 2. Egg production percentage was significantly higher in the T2 group compared to the control group (p<0.05). Further, significantly higher production percentages were found in the T1 and T3 groups when compared to the control group. The egg weight was significantly higher in the T2, T3 and T4 groups compared to the control and T1 groups (p<0.01), but there was no significant difference between the T2, T3 and T4 groups. The egg mass of all L-Threonine supplemented groups was significantly higher (p<0.01) than the control group. In the present experiment, overall production performance was higher in the group supplemented with 0.4% threonine. Similar results were reported by Faria *et al.* (2002) [10] with increased egg production, egg weight and egg mass on diets containing 0.53% threonine. Hossaninejad *et al.* (2021) [12] observed enhanced egg weight, hen-day egg production, and egg mass on supplementing 0.48% L-Threonine along with a wheat based diet in layers (Hy-line-W36). In comparison to hens fed a control diet, those provided with 2.0 g of threonine per kg of feed displayed improved egg production and egg mass with no effect on egg weight (Azzam *et al*., 2014) [44]. However, certain authors disagree that no additive effect of threonine on egg weight and egg production is observed in birds (Ishibashi *et al*., 1998; Azzam *et al*., 2011) [13, 2].
The aforementioned improved egg production and egg quality parameters with increased threonine levels might be due to improved gut morphology by increasing villus height and the villus height/crypt depth ratio in various regions of the intestine and thereby improving nutrient absorption (Xie *et al*., 2014) [18]. This could have enhanced protein synthesis, improved feed efficiency, and balanced the amino acids in the diet, which would have improved hen-day egg production, egg weight, and egg mass (Hossaninejad *et al*., 2021) [12].

3.2 Egg quality parameters
Egg quality parameters are indicated in Table 2. Quality parameters are the standards defining both internal and external quality of eggs. Shape index was significantly increased in T2 (p<0.01) compared to control followed by T1. Dietary threonine supplementation at various levels did not affect albumin index, yolk index and egg shell thickness. 0.4% Threonine supplemented group T2 showed Haugh unit significant relative to control group (p<0.01). Albumin index, yolk index and Haugh unit imply internal quality of egg. Similar results were reported Cardoso *et al*. (2014) [8] that feeding various levels of threonine in diet of white laying hens during laying period for 17 weeks unaffected egg internal quality parameters like albumin, yolk proportion and egg shell thickness. Feeding brown laying hens 0.4% level threonine for eight weeks didn't considerably have an effect on albumin height, eggshell thickness and eggshell percent however there has been significant improvement in Haugh unit (Abdel-Wareth and Esmail 2014) [1]. Contrasting results were also reported with no effect on Haugh unit on feeding various levels of threonine in layer diets of different age group (Valerio *et al*., 2000; Sa *et al*., 2007) [17, 16]

Table 2: Effect of L-Threonine on production performance and egg quality parameters in layers

Parameters	Dietary treatments					SEM	*p*-Value*
	Control	T1 (0.2%)	T2 (0.4%)	T3 (0.6%)	T4 (0.8%)		
Hen day egg production (%)	88.16^{a}	91.07^{b}	92.10^{c}	90.43^{b}	89.91^{ab}	0.65	0.05
Egg weight(g)	53.52^{a}	55.69^{b}	56.50^{c}	56.41^{c}	56.47^{c}	0.57	0.01
Egg mass(g/bird/day)	47.20^{a}	50.76^{b}	52.08^{b}	51.06^{b}	50.80^{b}	0.82	0.01
Albumin index	12.33	12.15	12.31	12.42	12.07	0.05	NS
Yolk index	44.62	44.49	45.17	44.49	44.65	0.13	NS
Haugh unit	89.89^{a}	90.91^{b}	91.07^{c}	91.05^{c}	91.02^{c}	0.23	0.01
Shape index	77.29^{bc}	77.71^{d}	78.11^{e}	76.91^{a}	77.28^{b}	0.20	0.01
Egg shell thickness(mm)	0.355	0.351	0.359	0.350	0.349	0.001	NS

*Values with different superscripts differ significantly (p<0.01 & p<0.05)

4. Conclusion

The effect of dietary supplementation of L-Threonine at various levels (0.2%, 0.4%, 0.6% and 0.8%) resulted in improvements in production percentage, egg weight and egg mass, HU and shape index in white leghorn. According to the present study, 0.4% L-Threonine supplementation was found to be optimal for egg production and quality parameters in white leghorn.

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