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Influence of dietary L-threonine on egg production, egg quality, immune functions, gut health and anti-oxidant status in laying hens

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ABSTRACT

This study was conducted on 1680 BV-300 layers, aged 40 weeks (1352±5.85g) divided into five groups (336 birds each) fed on various levels of dietary L-threonine in their diets: T_1 (100% threonine according to NRC recommendations), T_2 (100% threonine based on BV-300 requirements), T_3 (110% threonine of BV-300 requirements), T_4 (120% threonine of BV-300 requirements) and T_5 (130% threonine of BV-300 requirements), over a period of twenty weeks. Feed efficiency improved in T_4 and T_5 groups as compared to T_1 group. Egg mass was higher in T_3, T_4 and T_5 groups as compared to T_1 and T_2 groups. Albumin height, albumin index and Haugh Unit exhibited higher values in T_4 group than T_1 and T_2 groups. The total immunoglobulin level was elevated in T_3, T_4 and T_5 groups. The ND titre (HA-HI) was higher in T_5 group than T_1 and T_2 group. The proliferation response index of lymphocyte was higher in the T_5 group than the T_1 group. The phagocytic activity index of neutrophil was superior in T_5 group than T_1 and T_2 groups. Villus height was higher and villus surface area and the goblet cells number/villus were greater in T_4 and T_5 group than in T_2 group. The T_5 group exhibited a higher concentration of GSH-Px as compared to T_1 group. Additionally, T_5 group demonstrated a higher level of SOD activity than the other groups. The best relative economic efficiency was recorded in T_4 group. It can be concluded that dietary threonine level may be increased by 20% and 30% of BV-300 requirement for optimal performance and immune health, respectively in layers.

Keywords: Albumin index, Egg mass, Haugh unit, Threonine, Villus height

Dietary L-threonine (98.5%) (THR), the third limiting amino acid, is usually supplemented to the ration of layers to meet the amino acid balance. THR serves numerous functions in poultry, including the formation of uric acid, the re-synthesis of pancreatic enzymes, protein synthesis and protein turnover in the body, production of elastin, collagen and antibodies (Sá *et al.* 2007). It is vital for maintaining intestinal wall functions by regulating protein and mucin synthesis in the intestinal goblet cells (Montagne *et al.* 2000, Azzam *et al.* 2011). Threonine contributes around 30% of the amino acids of mucins glycoprotein. As a basic component of immunoglobulins, THR alone comprises 7 to 11% of overall amino acids (Sandberg *et al.* 2007). In the culture media, inclusion of 2mM- threonine can prevent apoptosis by stimulating growth of cells and enhancing lymphocytal production of antibody by synthesis of protein as well as signalling pathways at cellular level (Duval *et al.* 1991). Similarly, Newcastle disease infected poultry may experience increased antibody titres in the serum due to increased intake of dietary L- threonine (Jahanian, 2010).

High environmental temperatures and humidity induce heat stress in layer birds, thus diminishing not only feed consumption, production, weight and quality of eggs (Mashaly *et al.* 2004), but also disrupts the epithelium structure of the intestine (Burkholder *et al.* 2008), leading to a decrease in goblet cell numbers (Sandikci *et al.* 2004). Furthermore, the immune functions are adversely impacted as a result of this heat stress (Mahmoud and Yaseen, 2005). During any state of physiological stress, the activities of antioxidant enzymes serve as critical indicators of stress. The NRC recommendations for poultry have been predominantly assessed in a thermo-comfort zone; thus, in hot-humid conditions, the implications of elevated temperatures need to be brought into consideration when formulating a balanced diet for layers that includes the necessary amounts of amino acids. A higher dose of THR in the ration (higher than NRC recommendation) has earlier been advocated to attain optimal growth performance, immune-responses and resistance to diseases in birds (Debnath *et al.* 2019). In light of these considerations, the present trial was planned to explore the influence of dietary L-threonine on egg production, egg quality, intestinal

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health, immune responses and the economic status of laying hens.

MATERIALS AND METHODS

Experimental birds, housing, environmental condition and experimental duration: In a completely randomized design (CRD), one thousand six hundred eighty (1680) BV-300 laying hens, aged 40 weeks with a mean body weight of 1352±5.85g, were allocated in 420 metal wire cages (cage system), with four birds per cage. Each cage measured 60cm in width, 60cm in depth, and 40cm in height. Throughout the trial, a standard lighting schedule (16 hours light and 8 hours of darkness) was consistently maintained. The experiment was conducted from March to July, a period of hot and humid conditions. Temperature of the layer shed and its humidity were observed daily (at 0900 and 1500 hours). The average daily relative humidity in the shed was 75 ± 10%, while the average temperature was 34 ± 8°C. The birds were kept under standard hygienic conditions during the experiment. Immunization of the birds was performed at the 45th and 52nd weeks using the Lasota strain vaccine, in accordance with the standard vaccination schedule of the farm as per BV-300 guidelines. The experiment was conducted during the second phase of laying, spanning over a duration of twenty weeks (from 40 to 60 weeks).

Experimental design and diets: The present trial was conducted in agreement with the animal welfare and approved by the ethical committee of WBUAFS, Kolkata, India. The experimental layers were arranged in a completely randomized design (CRD), consisting of five (5) groups, each containing 336 birds. Each group was further divided into four (4) replicates, with each replicate consisting of eighty-four (84) birds (Table 1). All experimental layer birds were housed in cage system within a commercial layer house, maintained with a uniform basal mash ration under standard hygienic conditions throughout the trial period. Dietary L-threonine was administered using synthetic 98.5% feed quality L-threonine. Prier to the formulation of feed, amino acid analysis of individual feed ingredients was estimated by high performance liquid chromatography. All trial rations were analysed for the proximate as per AOAC, 1995. The basal feed formulation is detailed in Table 2. Feed was provided in iso-nitrogenous and iso-energetic mash form, along with fresh and clean

Table 2. Composition of basal diets and chemical analysis (Fulfilling 100% threonine level as per NRC 1994 without dietary L- threonine)

Ingredients	Period in weeks (40-60 wks)
Maize	57.0
SBM	3.6
GNC (SE)	17.0
Wheat Bran	10.3
DCP	1.0
Limestone (LSP)	5.0
Shell grit	5.0
Iodized Salt	0.2
L-Lysine HCl	0.13
DL-Methionine	0.15
Pre-mix[a]	0.62
Nutrient content DMB (%)	
CP[b]	17.52
EE[b]	3.19
TA[b]	9.43
AIA[b]	1.91
Crude Fibre[b]	7.44
Calcium[b]	4.05
Total Phosphorus[b]	0.87
Lysine[b]	0.80
Methionine[b]	0.40
Cystine[b]	0.352
Methionine and Cystine[b]	0.752
Arginine[b]	0.84
Tryptophan[b]	0.18
Threonine[b]	0.47
Calculated ME (Kcal/Kg)[c]	2560

a Premix provided the following per kilogram: Vitamin A, 7,000 IU, Vitamin D_3, 2,500 IU, Vitamin E, 36 mg, Vitamin K, 32 mg, Vitamin B_1, 2 mg, Vitamin B_2, 5.6 mg, Vitamin B_6, 4 mg, Vitamin B_{12}, 0.025 mg, Nicotinic acid, 38 mg, Folic acid, 1.1 mg, Calcium pantothenate, 10 mg, Biotin, 0.16 mg, Cu, 10 mg, Fe, 80 mg, Mn, 100 mg, Zn, 60 mg, I, 0.55 mg, Se, 0.12 mg.

Other premixes: Phytase 5000, Choline chloride, Toxin binder, Cocktail enzyme, Coccidiostat, Emulsifier, Antioxidant, Sodium bi-carbonate, Liver tonic and L-glutamic acid.

[b] analyzed values; [c] Calculated value

Table 1. Allotment of various experimental groups with L-threonine (%) and threonine: lysine in basal and trial rations

Experimental Group	Threonine Enhancement	% of threonine	Threonine/Lysine
T_1	Basal ration without dietary L- threonine, (100% Thr requirement of NRC, 1994)	0.47	0.59
T_2	Basal ration with dietary L- threonine (100% Thr requirement of BV-300 strain)	0.52	0.65
T_3	110% threonine r of BV-300 requirement	0.57	0.71
T_4	120% threonine of BV-300 requirement	0.62	0.78
T_5	130% threonine of BV-300 requirement	0.68	0.85

drinking water available *ad libitum* to all the birds throughout the trial period.

Egg production and egg quality parameters: Egg count was done on a daily basis and measured starting from the first egg of the second phase, with averages calculated for each replicate throughout the experiment. The hen-day egg production (HDEP) was assessed daily from the onset of the second phase of egg laying and was subsequently determined. Egg mass (EM) was computed using the formula EM = Egg number per hen per day × average egg weight (EW) (g). The cumulative feed conversion ratio (CFCR) values for each group were derived based on egg production and feed intake. CFCR was calculated by dividing the average feed consumption (kg) by the average EM (g) produced, which is the product of average EW (g) and egg production, expressed as kilograms of feed consumed per kilogram of egg output. At five-week intervals, fifty eggs from each group were randomly chosen for each storage duration (two days of eggs for each storage period) to evaluate various components of egg quality, including external factors {EW, egg shell index (SI), egg shell thickness}and internal quality metrics like albumin height (AH), albumin index (AI), yolk index (YI) and Haugh Unit (HU). SI, AI and YI were calculated using established formulas. HU was calculated as HU = 100 log ($H + 7.57 - 1.7\ W^{0.37}$). Here 'H' represents the height of thick albumin (mm) and 'W' denotes the egg weight (g).

Sample collection, sample analysis and measurements: Blood samples from the experimental layers (approximately 5mL) were obtained at the 60 weeks of age. For this, twenty layers (five layers per replicate) for each treatment were randomly selected. Blood was aseptically collected from the wing vein of each layer bird, utilizing EDTA for the assessment of total immunoglobulin levels. The total immunoglobulins in serum were determined using the zinc sulfate turbidity test. The Beta procedure of HA-HI test was conducted to measure the antibody titers against Ranikhet Disease virus. The *in vitro* neutrophil's phagocytic activity was also evaluated through the NBT Reductive Method to measure the phagocytosis of blood neutrophils. The serum samples were analyzed for the concentrations of super-oxide dismutase (SOD), glutathione-per-oxidase (GSH-Px), and catalase using a UV-spectrophotometer. The activity of SOD, GSH-Px and catalase was assessed by recording the changes of absorbance respectively at 480 nm, 412nm and 240nm, monitored by a UV-spectrophotometer.

After the completion of the trial, five hens from every dietary replicate (near to the average live weight of that replicate) were sacrificed. A five-centimetre segment from the midpoint of each jejunum (part of the small intestine) was excised for the assessment of villus height (VH), villus width (VW), crypt depth (CD) and goblet cell number. The jejunum was of particular significance as it serves as a primary site for absorption of nutrients in the birds (Horn *et al.* 2009). Measurements included VH (from the villus-crypt junction to the tip of the villus), CD (from the base of the crypt to the crypt-villus junction), villus width (VW), the VH: CD ratio, the VH: VW ratio, and the VSA is calculated as $[(\pi \times mh \times h) + (\pi \times mh/2)^2]$, where 'mh' represents the VW at the mid-VH and 'h' denotes VH (Law *et al.* 2007). VH, VW and goblet cell number were recorded from five villi per bird, ensuring that only complete, vertically oriented villi were included in the measurements. The goblet cells density was computed as the goblet cell number per unit of villus surface area (VSA) (mm^2).

Economic analysis: Economic analysis of layers was carried out by calculating the production cost (except feed cost, no other costs was considered) and the egg price. Net revenue = Egg price (Rs.)/hen/day- Feed cost (Rs.)/hen/day.

$$\text{Economic efficiency (EE)} = \frac{\text{Net return (Rs.)/hen/day}}{\text{Feed cost (Rs.)/hen/day}}$$

Statistical analysis: All data were subjected to one-way analysis of variance (ANOVA) as described by Snedecor and Cochran (1994) utilizing the SPSS (2002) (SPSS 21.0, Chicago, IL, USA). In instances where significant differences were identified (at $p<0.05$), Tukey's test was applied to compare the treatment means. The primary influence of threonine levels were assessed for both linear and quadratic response via polygonal contrasts. The linear as well as quadratic effects of the threonine levels were analyzed in relation to the NRC recommendation group and the BV-300 recommendation group. No statistical analysis was conducted for the cost assessment of various experimental diets.

RESULTS AND DISCUSSION

Feed consumption and CFCR: The feed consumption of the laying hens exhibited a linear increment. Feed efficiency improved ($p<0.01$) in the T_4 and T_5 group in comparison to the T_1 group (Table 3). Furthermore, the CFCR also demonstrated a linear improvement. The HDEP showed a linear increase, while EM per hen improved linearly as well as quadratically. The EM was higher ($p<0.001$) in the T_3, T_4 and T_5 group compared to the T_1 and T_2 group. Additionally, the average EM per hen also showed linear as well as quadratic increase in the T_3, T_4 and T_5 group.

The elevated concentrations of dietary L-threonine in the test diets enhanced the feed consumption of the laying hens, potentially attributable to an appetitive influence of threonine. This appetitive influence may have contributed to achieve an amino acid balance within the experimental diets. Increased levels of L-threonine could have triggered the regulatory mechanisms governing the appetite of the layers or change of amino acid composition in the plasma. Typically, CNS mechanisms are responsive to fluctuations in blood amino acid levels, which consequently lead to a reduction in feed consumption (Bertechini, 2012) although, such mechanisms were not verified during the current experiment.

An enhancement in the CFCR observed in the present experiment may be attributed to a higher intake of dietary L-threonine alongside methionine or lysine in the diets.

Furthermore, L-threonine exhibited a positive impact on productive performance, which is crucial for the transformation of digested feed into eggs. Additionally, the supplementation of threonine influenced VH, CD and goblet cell number (Zaefaria *et al.* 2008, Azzam *et al.* 2012), potentially leading to improved nutrient absorption and, consequently, enhanced feed efficiency. The current trial corroborated with the findings of Abdel–Wareth and Esmail (2014) as well as Azzam *et al.* (2016), who revealed that the FCR improved with higher levels of L-threonine in laying hens.

Egg laying performance and egg quality parameters: The EW, egg SI, egg shell thickness, and yolk index did not differ ($p>0.05$) amongst the different treatments applied. Conversely, AH, AI, and HU exhibited higher values ($p<0.05$) in the T_4 group as compared to the T_1 and T_2 group (Table 3) demonstrating a linear increase.

The dietary L-threonine might have resulted in an improved amino acid profile, thereby promoting more effective protein deposition in eggs. Moreover, threonine supplementation has been demonstrated to affect VH, CD, and the goblet cell number (Zaefaria *et al.* 2008, Azzam *et al.* 2012), which could enhance nutrient absorption and subsequently improve the feed conversion ratio (FCR), leading to an increase in both egg quantity and egg weight (EW), ultimately resulting in a significant rise in egg mass (EM). The current study was consistent with the findings of Abdel–Wareth and Esmail (2014), Azzam *et al.* (2016), who also reported a significant increase in EM. Furthermore, Azzam *et al.* (2016), Dong *et al.* (2017) and Fouad *et al.* (2017) indicated that raising the L-threonine levels contributed to increased EM and HDEP. In line with the present results, no effects were noted in eggshell quality (Azzam *et al.* 2011, Abdel–Wareth and Esmail, 2014, Hossaninejad *et al.* 2021). In our experiment, it was observed that increasing the L- threonine level led to an increased albumen height and albumin index. Therefore, the supplemented L-threonine group may be linked to the formation of thicker egg albumin with eggs storage quality. Consistent with the present trial, Azzam *et al.* (2014) also found that varying levels of dietary threonine resulted in improved albumin height in laying hens. The current findings clarified that hens with a higher level of dietary L- threonine could produce eggs of better quality and with a higher AI. The improved HU observed in the higher threonine supplemented groups of our trial may be attributed to the enhanced quality of the eggs. Higher quality eggs exhibited thicker whites with increased threonine supplementation. Consistent with the current study, Azzam *et al.* (2014), Abdel–Wareth and Esmail (2014) and Hossaninejad *et al.* (2021) noted that varying levels of dietary threonine led to improved interior quality of eggs, as reflected by HU in laying hens.

Immune responses: The total immunoglobulin level were elevated ($p<0.001$) in the T_3, T_4 and T_5 group when as compared to T_1 and T_2 group (Table 4). Furthermore, the mean total serum immunoglobulin level displayed a linear increment. In a similar manner, the ND titre (HA-HI) demonstrated a linear increase ($p<0.05$) in the T_5 group compared to T_1 and T_2 group. The proliferation response index of lymphocyte (LPR) was particularly linearly superior ($p<0.05$) in the T_5 group than in the T_1 group. The phagocytic activity index of neutrophil improved linearly as well as quadratically ($p<0.001$) in the T_5 group compared to T_1 and T_2 group.

L-threonine comprises approximately 7 to 11% of proteins present in immunoglobulin (Sandberg *et al.* 2007). An increase in dietary L-threonine may positively influence the effective production of immunoglobulins (Wang *et al.* 2006, Li *et al.* 2007, Debnath *et al.* 2019). The production of the most significant globulin, immunoglobulin, is affected by the elevated L-threonine levels in diets, either by directly influencing antibody production or by impacting viral replication, which subsequently may affect γ-globulin production (Bhargava *et al.* 1971). The current study corroborates with earlier findings in broilers (Debnath *et al.* 2019) as well as other researchers such as Rezeipour *et al.* (2012), Khan *et al.* (2018), who demonstrated that

Table 3. Influence of dietary L- threonine on feed consumption, FCR, laying performance and egg quality of BV-300 Laying Hens

Attributes	T_1	T_2	T_3	T_4	T_5	SEM	P value	Linear	Quadratic
Fedd Intake (g)/hen/day	117.86	117.87	118.38	118.01	118.19	0.13	0.07	0.05	0.23
FCR (kg feed/kg Egg mass)	2.13^{c}	2.12^{bc}	2.10^{abc}	2.07^{a}	2.09^{ab}	0.002	0.002	<0.001	0.33
Hen-day egg production (%)	91.30	91.82	92.75	93.63	92.98	0.70	0.19	0.04	0.38
Egg weight (g)/hen	60.71	60.75	60.79	61.00	60.87	0.39	0.96	0.47	0.90
Egg mass (g)/hen	55.42^{a}	55.78^{ab}	56.38^{bc}	57.11^{c}	56.59^{bc}	0.19	<0.001	<0.001	0.05
Shell index	75.24	75.55	75.90	78.02	76.61	1.12	0.43	0.15	0.69
Albumen height (mm)	5.75^{a}	5.80^{a}	5.85^{a}	6.79^{b}	6.26^{ab}	0.22	0.003	0.004	0.67
Albumen index	0.089^{a}	0.090^{a}	0.092^{ab}	0.106^{b}	0.097^{ab}	0.002	0.01	0.01	0.59
Haugh unit	74.81^{a}	74.64^{a}	75.39^{ab}	81.35^{b}	77.80^{ab}	1.62	0.02	0.02	0.80
Yolk index	0.49	0.50	0.52	0.54	0.53	0.02	0.53	0.13	0.57
Egg shell thickness (mm)	0.43	0.41	0.46	0.44	0.42	0.014	0.14	0.83	0.19

abc Values bearing different superscripts in a row differ significantly ($P<0.05$).

the addition of L-threonine in diet of broilers enhanced titres of antibody against Ranikhet disease virus at the age of 21 and 42 day. The improved antibody titre may be attributed to the greater availability of threonine, a key component of immunoglobulins (Kim *et al.* 2007). The primary mechanism by which an increased dietary threonine level affects the immune system involves the stimulation of lymphocyte proliferation and the inhibition of apoptosis (Kidd *et al.* 2004, Li *et al.* 2007). Apoptosis can be alleviated through the stimulation of cell growth and enhancement of antibody production in lymphocytes by incorporating 2mM-threonine into the culture medium, facilitated by protein synthesis and cellular signalling mechanisms (Duval *et al.* 1991). Our findings are consistent with those of MacDuogall and Klasing (1998) and Debnath *et al.* (2019), who observed that threonine supplementation significantly, improved leukocyte proliferation in both broilers and chicks, respectively.

Intestinal Histo-morphometry: VH was improved ($p<0.001$) in the T_4 and T_5 group (relative to the T_2 group) in comparison to the other trial groups (Table 5). The average VH showed both linear as well as a quadratic improvement. In contrast, VW and CD were higher ($p<0.05$) in the T_4 group compared to the T_1 group. In this context, the mean VW showed a linear raise. The average CD also demonstrated a linear increase. Nevertheless, the VH:CD was not influenced ($p>0.05$) by the treatments applied. Although VSA (mm^2) and the goblet cells number per villus were greater ($p<0.001$) in the T_4 and T_5 group compared to the T_1 group, the density of goblet cells per mm^2 was not affected ($p>0.05$) by the treatments. The mean VSA improved linearly as well as quadratically. Additionally, the mean number of goblet cells per villus increased linearly as well as quadratically.

L-threonine exhibits the highest metabolic activity in the portal-drained viscera, as it surpasses other amino acids in this regard (Schaart *et al.* 2005). Approximately 30 to 50% of threonine, along with several other amino acids, is utilized directly by the small intestine, rendering it unavailable for any other tissues (Wu, 1998). The levels of dietary threonine might have ensured sufficient level of this essential amino acid to support the rapid yield of mucosal tissues. In the current study, the improved intestinal histomorphometry observed in the experimental layer birds might have attributed to the optimal threonine utilization within the gut. The development of crypts plays a crucial role in maintaining crypt-cell turnover rates thus facilitating maturation of intestine. A deeper crypt correlates with enhanced growth of villus in enterocytes, thereby maximizing the gut surface area available for absorption from the intestine (Geyra *et al.* 2001). The present findings are also in support of Min *et al.* 2017 and Debnath *et al.* 2019. The increased VSA in jejunum of birds receiving a high dietary L-threonine intake may be explained by the positive relationship between VH and VSA, as well as the extension of surface area associated with growth of villus, which accounts for the improved absorption capacity (Abbasi *et al.* 2014). The relatively increased number of goblet cells per villus in birds receiving a high dietary level of L-threonine may be linked to an enhancement in the absorptive surface area, characterized by increased VH and VW. In our investigation, the quantity of goblet cells appeared to rise in direct proportion to the augmented VSA, regardless of the experimental groups.

Consequently, elevating dietary L-threonine levels could lead to a notable increase in the number of goblet cells within the intestinal absorptive region, thereby enhancing mucin production for improved protection (Nichols and

Table 4. Influence of dietary L-threonine on immunological attributes of BV-300 laying hens

Attributes	T_1	T_2	T_3	T_4	T_5	SEM	P-value	Linear	Quadratic
Total Immunoglobulin (g/L)	9.10^{a}	9.28^{a}	10.53^{b}	11.31^{bc}	12.14^{c}	0.28	<0.001	<0.001	0.43
HA-HI ND Titre	2.45^{a}	2.45^{a}	2.70^{ab}	2.70^{ab}	2.80^{b}	0.06	0.013	0.001	0.90
Lymphocyte proliferation response	0.84^{a}	0.87^{ab}	0.93^{ab}	0.97^{ab}	1.08^{b}	0.05	0.04	0.003	0.56
Neutrophil Phagocytosis Activity Index	0.57^{a}	0.61^{a}	0.62^{a}	0.75^{a}	1.09^{b}	0.04	<0.001	<0.001	0.001

abc Values bearing different superscripts in a row differ significantly (P<0.05).

Table 5. Influence of dietary L- threonine on Jejunal Histomorphometry of BV-300 laying hen

Attributes	T_1	T_2	T_3	T_4	T_5	SEM	P-value	Linear	Quadratic
Villus height (µm)	1029.50^{a}	1110.50^{b}	1142.00^{b}	1208.2^{c}	1200.2^{c}	10.72	<0.001	<0.001	0.003
Villus width (µm)	132.00^{a}	140.50^{ab}	148.75^{ab}	151.50^{b}	149.00^{ab}	3.96	0.008	0.003	0.83
Crypt depth (µm)	147.25^{a}	155.25^{ab}	163.75^{ab}	165.75^{b}	163.50^{ab}	4.10	0.03	0.005	0.10
Villus height: crypt depth	6.99	7.16	6.99	7.26	7.41	0.17	0.39	0.10	0.53
Villus surface area (mm^2)	0.47^{a}	0.54^{ab}	0.59^{bc}	0.63^{c}	0.62^{bc}	0.02	<0.001	<0.001	0.021
Goblet Cell/Villus	82.50^{a}	96.25^{b}	105.75^{bc}	118.00^{c}	113.00^{c}	2.87	<0.001	<0.001	0.006
Goblet Cell Density/mm^2	175.55	179.35	180.00	187.33	183.39	6.70	0.78	0.28	0.73

abc Values bearing different superscripts in a row differ significantly (P<0.05).

Table 6. Influence of dietary L-threonine on anti-oxidant status of BV-300 laying hens

Attributes	T_1	T_2	T_3	T_4	T_5	SEM	P value	Linear	Quadratic
Glutathione Peroxidase (µmol/L)	724.01[a]	902.29[ab]	1055.03[ab]	1041.55[ab]	1284.21[b]	104.07	0.02	0.001	0.75
Super-oxide dismutase (mU/mL)	118.41[a]	121.63[a]	120.44[a]	130.07[ab]	140.56[b]	3.90	0.009	0.002	0.04
Catalase (U/mL)	17.20	17.98	19.61	20.68	19.98	1.09	0.30	0.04	0.76

ab Values bearing different superscripts in a row differ significantly (P<0.05).

Table 7. Influence of dietary L- threonine on nutrient metabolizability and Economic Efficiency of BV-300 laying hens

Attributes	T_1	T_2	T_3	T_4	T_5	SEM	P value	Linear	Quadratic
Dry matter	70.22	71.22	70.93	71.96	70.59	0.98	0.77	0.64	0.37
Organic matter	73.51	74.42	74.79	75.58	74.61	0.77	0.47	0.19	0.26
Crude protein	53.40	53.74	55.41	56.10	55.60	0.78	0.09	0.02	0.36
Ether Extract	80.24	79.99	80.57	81.48	80.30	0.83	0.75	0.55	0.63
Crude fibre	13.55	13.18	13.52	13.06	13.33	0.59	0.90	0.64	0.70
Nitrogen-free extract	73.93	74.88	73.60	74.96	73.94	0.96	0.85	0.69	0.44
Feed Cost/hen/day (Rs.)	3.54[a]	3.55[a]	3.58[b]	3.57[b]	3.59[c]	0.001	<0.001	<0.001	0.87
Net Revenue (Rs.)/hen/day	0.41	0.43	0.44	0.48	0.43	0.03	0.61	0.34	0.40
EE	11.58	12.06	12.33	13.52	12.05	0.91	0.65	0.42	0.40
REE%	85.65	89.20	91.20	100	89.13	-	-	-	-

abc Values bearing different superscripts in a row differ significantly (P<0.05).

Bertolo, 2008) against the acidity of chyme, the action of enzymes of digestion and harmful organisms (Horn *et al.* 2009). Zaghari *et al.* (2011) and Debnath *et al.* (2019) reported that threonine supplementation had a significant impact on jejunal VH, goblet cell count, and CD of broilers aged 1 to 21 days. Additionally, Horn *et al.* (2009) noted no significant effect on the density of intestinal goblet cells (cells/micrometer² of VH) as dietary threonine levels were increased.

Anti-oxidative status: The T_5 group exhibited a higher ($p<0.05$) concentration of GSH-Px compared to the T_1 group (refer to Table 6). Additionally, GSH-Px levels increased in a linear fashion. Furthermore, the T_5 group demonstrated a greater concentration ($p<0.05$) of SOD than the other experimental groups. Serum SOD levels also showed a linear as well as quadratic increment.

The present findings corroborated with Azzam *et al.* (2012), who reported a higher ($p<0.05$) serum SOD activity in broiler chicks due to supplemental dietary L-threonine. In juvenile blunt snout bream, an increase in glutathione-peroxidase (GPx) was observed, rising from 1.58% to 2.08% as dietary L- threonine levels were elevated. This increase might be attributed to the up-regulated gene transcription for the anti-oxidant enzyme GSH-Px, which could have subsequently enhanced mRNA levels in response to higher L- threonine concentrations (Habte-Tsion *et al.* 2016).

Metabolizability and economic analysis: The dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP) and NFE metabolizability were found non-significant ($p>0.05$). Due to the additional expense associated with L-threonine, the daily feed cost per hen has risen. Nevertheless, the economic benefits observed in the groups supplemented with threonine may be linked to an increase in feed intake, an enhanced feed conversion ratio (per energy metabolized), and improved productive performance. Therefore, from both a nutritional and economic perspective, it was revealed that T_4 group exhibited superior performance and higher energy efficiency value compared to those fed a diet with lower threonine supplementation. This current trial was in tune with Gheisar *et al.* (2011), who showed in their economic analysis that the supplementation of dietary L- threonine at an increased level of 0.1% was the most economical.

The study concluded that to enhance laying performance, egg quality, gut health, and profitability, a recommendation of 120% threonine (relative to the BV-300 requirement, specifically 0.062 g/kg) is advisable. Nevertheless, during periods of outbreaks, further increase in threonine levels by 10% (i.e., 130% threonine of the BV-300 requirement) is suggested. Therefore, for optimal immune response, a recommendation of 130% threonine (of the BV-300 requirement) at 0.068 g/kg is warranted, which corresponds to a threonine/lysine ratio of 0.85. It is noteworthy that L-threonine supplementation does not diminish feed consumption in birds under high temperature and relative humidity conditions; instead, it enhances egg production, egg weight, egg quality and immunity, while also improving the intestinal epithelial structure and increasing goblet cell numbers.

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