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# Effects of Supplemental Threonine on Antioxidant Enzyme Activities and Haemato-biochemical Profile of Commercial Broilers in Sub-Tropics

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**ABSTRACT**

Present study was aimed at investigating the effects of threonine supplementation on antioxidant enzyme activities and haemato-biochemical profile of commercial broilers in sub-tropics. Three hundred thirty -day old straight run commercial broiler chicks (Vencobb-400) with initial average body weight of 44.04±0.42g were allocated into five experimental groups, in a completely randomized design (CRD) with 42 days experiment. Groups were formed according to the dose of supplemental L-threonine in various rations i.e. NRC specification, 100% of Vencobb-400 strain specification, 110% of Vencobb-400 specification, 120% of Vencobb-400 specification and 130% of Vencobb-400 specification group. The mean serum GSH-Px and serum catalase concentration increased linearly {(p=0.001) and (p=0.04), respectively} whereas the mean serum SOD level increased both linearly (p=0.002) and quadratically (p=0.04) with the increasing levels of supplemental L-threonine. Among the hematological parameters of blood, the H:L ratio decreased linearly (p=0.02) with the increasing levels of threonine. The serum glucose and total protein concentration increased linearly (p=0.002) with the increasing levels of supplemental L-threonine. There was a linear increment (P<0.001) in serum globulin level with a linear decrease (p<0.05) in albumin: globulin ratio on increased levels of supplemental L-threonine in the ration. There was a linear decrease (p<0.001) in cholesterol and VLDL level with the increasing levels of supplemental L-threonine, however, a linear increment (p=0.04) in the serum HDL level was noticed. It may be concluded that L-threonine supplementation at 130% threonine (of Vencobb-400 specification) has a better antioxidant function and better haemato-biochemical profile.

**Keywords:** Anti-oxidant enzyme, Catalase, GSH-Px, H:L ratio, L-threonine, SOD, Vencobb-400

Poultry birds are not capable of synthesizing threonine, the third limiting amino acid after methionine and lysine in corn and soybean meal based diets, thus making it nutritionally essential. Commercial L-threonine (98.5%) is generally added to the diet of broilers in order to exactly match the dietary amino acid balance to the unique nutritional requirements of the poultry birds. There are evidences that essential amino acids levels like threonine in the feed higher than NRC specifications are needed to achieve optimal growth performance, immune-competence and disease resistance in birds (Debnath *et al.,* 2019). In fact, the NRC requirement levels for poultry are mainly examined only in thermo-neutral environment, therefore, the dietary requirements as reported by the NRC (1994) may be insufficient for modern commercial poultry strains reared under heat and relative heat stress condition. Among the nutrients, the amino acids are susceptible to high temperature and humidity as some researchers noticed that higher temperature produces an effect on amino acid digestibility (Larbier *et al.*, 1993) or feed intake (Akyuz, 2009). Hence, threonine may be one of the essential amino acids liable to be limited when high-humidity heat stress would decrease feed intake On the other hand, even though Vencobb 400 specification of broiler birds is based on tropical or subtropical climatic conditions like Indian sub-continent, it may be necessary to conduct further investigation to know the actual threonine requirements for antioxidant enzyme activities and haemato-biochemical profile in order to know health status of birds reared under hot and humid climatic

condition. It is observed that during any stress condition, antioxidant enzyme activities and hemato-biochemical parameters mainly determine health status of birds. For example, a deviation of Heterophil/ lymphocyte ratio is a key indicator of physiological stress (Siegel, 1995).

There are reports that nutrient requirements established under temperate conditions may not be entirely satisfactory in the tropical environment characterized by high ambient temperature countries and low quality feedstuffs (Babangida and Ubosi, 2006). Summer season is very hot and humid in many subtropical with daily temperature averages above 30°C and 75% relative humidity. Amino acids requirements are influenced by many factors including dietary, environmental, genetics and production performances (Bahare *et al*., 2013). The detrimental effects of high temperatures are exacerbated by high relative humidity that ultimately leads to stress and reduced production in birds. Keeping in view the above facts, the present investigation was conducted to determine whether supplemental threonine can positively influence antioxidant enzyme activities and haemato-biochemical parameters in commercial broilers in stress condition of sub-tropical climate.

## MATERIALS AND METHODS

### Experimental Birds, experiment duration and climatic condition

Three hundred thirty (330) day old straight run commercial broiler chicks (Vencobb-400; Venkeys, Pune) with initial average body weight of 44.04±0.42g were allocated into five experimental groups, in a completely randomized design. Each group was sub-divided into six replicates where each replicate comprised of eleven birds, selected in random (Table 1). The experiment was carried out from the month of March to April, which is considered to be hot and humid period in West Bengal state. The temperature and humidity inside the shed were recorded twice daily (at 0800 and 1400 h). The average ambient relative humidity inside the shed was 75 ± 10% and the mean daily temperature was 33 ± 8°C. The experiment lasted for six weeks.

### Housing, light and diets

The broiler birds were housed in a conventional poultry shed, covered with asbestos roof, divided in 30 pens of 12sq. feet with rice husk bed, covered with corrugated paper for first three days. The floor space in the shed was 1.25 sq. ft. per broiler birds. The light programme used for broilers was 24 hours per day (natural and artificial light) during the experimental period of 42 days. Broilers were fed on the same basal diet based on maize, sybean meal, oil, minerals, vitamins, salts & premixes and crystalline amino acids (Table 2). The pooled feed samples were subjected for analysis of dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), nitrogen free extract (NFE) and ether extract (EE), Ca and P as per AOAC, 1995. Crystalline L-threonine (98.5% Threonine, Evonik) was added to the basal diet at various percentages. Prior to feed formulation, individual feed ingredients were analyzed for amino acids by HPLC in the Analytical laboratory. All the mash rations were also analyzed in the analytical laboratory for respective amino acid content. To determine the nutritional levels of threonine, iso-nitrogenous and iso-caloric diets were formulated for all experimental groups by supplementing L-glutamic acid in the premix. Feed in mash form and wholesome and clean drinking water was

**Table 1:** Allocation of different treatment groups and Threonine availability (%) and Threonine: Lysine Ratio (Thr/Lys) in Basal and Experimental iso-caloric and iso-nitrogenous Diets

| Group | Threonine level | 1-7 day | Thr/Lys | 8-21 day | Thr/Lys | 22-42 day | Thr/Lys |
|---|---|---|---|---|---|---|---|
| $T_1$ | Basal diet without supplemented threonine, (100% Threonine requirement of NRC'1994) | 0.80 | 0.57 | 0.74 | 0.62 | 0.68 | 0.61 |
| $T_2$ | Basal diet with supplemented threonine (100% Threonine of Vencobb-400 specification) | 0.87 | 0.62 | 0.76 | 0.63 | 0.73 | 0.65 |
| $T_3$ | 110% threonine of Vencobb-400 specification | 0.96 | 0.69 | 0.83 | 0.69 | 0.80 | 0.71 |
| $T_4$ | 120% threonine of Vencobb-400 specification | 1.04 | 0.74 | 0.91 | 0.76 | 0.88 | 0.79 |
| $T_5$ | 130% threonine of Vencobb-400 specification | 1.13 | 0.81 | 0.98 | 0.80 | 0.95 | 0.85 |

provided *ad libitum* to all experimental birds throughout the experimental period. All the experimental broiler birds were maintained in a brooder cum grower house with standard hygienic condition following all bio-security measures throughout the experimental period. Vaccination was performed in broilers against Newcastle disease on 5th and 21st day with $NDB_1$ and Lasota strain, respectively and Infectious Bursal Disease on 12th day.

**Table 2:** Composition of basal diets and chemical analysis (fulfilling 100% threonine requirement as per NRC'1994 without supplemental L-threonine)

| Attributes | 1-7 day | 8-21 day | 2-42 day |
|---|---|---|---|
| Yellow maize | 596 | 642 | 653 |
| Soybean meal | 360 | 310 | 275 |
| Oil | 5 | 10 | 34 |
| DCP | 16 | 16 | 16 |
| Limestone | 11 | 11 | 11 |
| Iodized Salt | 2 | 2 | 2 |
| L-Lysine HCl | 2.1 | 1.4 | 1.5 |
| DL-Methionine | 2.9 | 2.6 | 2.5 |
| Pre-mix[a] | 5.0 | 5.0 | 5.0 |
| Nutrient content DMB (%) | | | |
| CP[b] | 22.38 | 20.25 | 19.45 |
| EE[b] | 3.04 | 3.41 | 5.03 |
| TA[b] | 5.10 | 5.54 | 5.57 |
| AIA[b] | 1.10 | 2.71 | 3.45 |
| Crude fibre[b] | 3.15 | 3.18 | 3.16 |
| Calcium[b] | 0.91 | 0.88 | 0.88 |
| Total Phosphorus[b] | 0.75 | 0.74 | 0.73 |
| Lysine[b] | 1.40 | 1.20 | 1.12 |
| Methionine[b] | 0.62 | 0.57 | 0.56 |
| Cystine[b] | 0.37 | 0.34 | 0.32 |
| Methionine and Cystine[b] | 0.996 | 0.920 | 0.906 |
| Arginine[b] | 1.62 | 1.43 | 1.34 |
| Tryptophan[b] | 0.27 | 0.25 | 0.24 |
| Threonine[b] | 0.80 | 0.74 | 0.68 |
| ME (ME/Kg)[c] | 3001 | 3035 | 3180 |

[a] Per kg contained retinyl acetate 3.75 mg, 1,25-di-hydroxy-cholecalciferol 4 mg, DL-α-tochopheryl acetate 30 mg, menadione 4 mg, thiamine propyl disulfide 3 mg, riboflavin tetrabutyrate 8 mg, methylcobalamin 0.025 mg, sodium pantothenate 15 mg, pyridoxine 5 mg, niacin 60 mg, biotin 0.2 mg, folic acid 2 mg, manganese 40 mg, iron 30 mg, zinc 25 mg, copper 3.5 mg, iodine 0.3 mg, selenium 0.15 mg, choline chloride 200 mg. **Other premixes:** Phytase 5000 (0.1%), choline chloride (0.5%), toxin binder (0.5%), Maduramycine (0.5%), antioxidant (125ppm), sodium bi-carbonate (1%), liver tonic (0.5%); [b] analyzed values; [c] Calculated value.

### Collection and analysis of blood

Blood samples (about 5ml) from the representative twenty four experimental birds of each group (four birds/replicate) were collected at 42nd day from the wing vein of the individual broiler bird, respectively. Blood samples were placed in the centrifuge tube without anticoagulant. Centrifuge tubes were placed in refrigerator at $4^0$C. Serum was harvested by centrifuging the whole blood at 2500 rpm for 15 minutes and stored in deep freeze at -80 $^o$C for further analysis of biochemical parameters. The haematological parameters like total erythrocyte count (TEC), haemoglobin concentration (Hb), packed cell volume (PCV), total leukocytic count (TLC) and differential leukocytic count (DLC) were estimated by standard methods described by Jain, 1993. The serum biochemical parameters studied were glucose, total protein, uric acid, creatinine, albumin, globulin, albumin: globulin, AST, ALT, ALP, total cholesterol, HDL cholesterol, LDL cholesterol, VLD cholesterol. Analysis of blood serum samples was carried out with reagents and procedures supplied along with the kits (Trans Asia Bio-Medicals Ltd., Solan, HP, India). For anti-oxidant enzyme study, the serums were subjected to the measurement of SOD, GSH-Px, and catalase concentrations by spectrophotometric methods using a spectrophotometer (UV-2000, Unico Instruments Co. Ltd., Shanghai, China). All of the assays were done by chemical methods. Activity of SOD was measured by the epinephrine method at 480 nm, which monitors the inhibition of reduction of nitro blue tetrazolium by the sample (Misera and Fridovich, 1972). Activity of GSH-Px was detected with 5,50-dithiobis-p-nitrobenzoic acid, and the change of absorbance at 412 nm was monitored using a spectrophotometer (Griffith, 1980). Catalase activity was done as per Beers and Sizer, 1952 and the absorbance was recorded at 240nm.

### Statistical analysis

The data were subjected to one way analysis of variance (Snedecor and Cochran. 1994) in the Statistical Package for Social Sciences (2000) (SPSS 21.0, Chicago, IL, USA). Whenever significant differences were found (at $P<0.05$), the treatment means were compared using Tukey's test.

The main effects of threonine levels were further tested for linear and quadratic responses using polygonal contrasts. The linear and quadratic effects of the threonine levels were evaluated with both the NRC control group and Vencobb control group.

## RESULTS AND DISCUSSION

### Anti-oxidant enzyme activity

At various levels of L-threonine supplementation, group with 130% threonine (of Vencobb-400 specification) had higher ($p<0.05$) mean serum GSH-Px concentration than the NRC specification threonine group (Table 3). Serum GSH-Px also increased ($p=0.001$) linearly with the increasing levels of supplemental threonine. Group with 130% threonine (of Vencobb-400 specification) also showed higher ($p<0.05$) mean serum concentration of SOD than NRC specification, Vencobb 400 specification and 110% threonine (of Vencobb-400 specification) group. The mean serum SOD level increased both linearly ($p=0.002$) and quadratically ($p=0.04$) with the increasing levels of supplemental threonine. The mean serum catalase concentration also increased ($p=0.04$) linearly.

High temperature can disturb the balance between the production of reactive oxygen species (ROS) and the antioxidant system which may further stimulate the formation of ROS (Feng *et al*., 2008). Anti-oxidant enzymes like SOD, catalase and GSH-Px are the main anti-oxidant parameters used to assess oxidative status in the enzymatic system. Glutathione peroxidase (GPx) increased with increasing dietary threonine levels from 1.58% to 2.08% in juvenile blunt snout bream (Habte-Tsion *et al.*, 2016). Beneficial effect observed in the treatment groups of the present study might be due to an increased mRNA level on increased concentration of threonine supplementation which might have up-regulated the gene transcription of antioxidant enzymes GSH-Px (Habte-Tsion *et al.,* 2016). In contrary to present study, Azzam *et al.*, 2012 (at 0.47, 0.66 and 0.74% threonine) revealed that threonine supplementation had no effect ($p<0.05$) on serum GSH-Px in laying hens in summer. The divergent results quoted by the above researcher might be related to the differences in the genetic structure of broiler birds, their nutrition, management condition and health conditions (Kul and Seker, 2004). Therefore, adding threonine may maximize the concentration of GSH-Px in the serum and may protect cells of broiler birds from oxidative injury by clearing superoxide anions.

Threonine supplementation in broiler birds had significant effect ($p<0.05$) on serum SOD activity (Azzam *et al.*, 2012). Threonine is one of the amino acids that are able to carry a small fraction of copper (Cu) in the blood (Shils *et al*., 2006). Copper-zinc superoxide dismutase is known to have oxidation-retarding factor (Meyer *et al.,* 1994). The SOD is one of the main antioxidant enzymes in scavenging the oxygen free radicals (McCord, 1979). One of the important strategies in poultry farms during the summer months is to maximize antioxidant ability and minimize lipid peroxidation. Secondly, better effect in the threonine supplemented group might be due to increased mRNA level on increased concentration of threonine supplementation which might have up-regulated the gene transcription of antioxidant enzymes gene expressions of Cu-SOD (Habte-Tsion *et al.,* 2016). Therefore, adding threonine might have maximized the concentration of SOD in the serum and may protect cells from oxidative injury by clearing superoxide anions.

The better effect observed in the threonine supplemented groups might be due to increased mRNA level on increased concentration of threonine supplementation which might have up-regulated the gene transcription of antioxidant enzymes gene expressions of catalase (Habte-Tsion *et al.,* 2016). It may be concluded that L-threonine

**Table 3:** The Effects of Supplemental L-threonine on Serum Antioxidant Enzyme Activities of Vencobb-400 broilers

| Attributes | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | SEM | P-value | Linear | Quadratic |
|---|---|---|---|---|---|---|---|---|---|
| Glutathione Peroxidase (µmol/L) | $712.81^{a}$ | $852.49^{ab}$ | $1026.9^{ab}$ | $1061.8^{ab}$ | $1204.3^{b}$ | 107.17 | 0.02 | 0.001 | 0.74 |
| SOD (U/mL) | $122.71^{a}$ | $121.93^{a}$ | $120.74^{a}$ | $130.77^{ab}$ | $139.93^{b}$ | 3.92 | 0.009 | 0.002 | 0.04 |
| Catalase (Unit/mL) | 17.20 | 18.81 | 18.61 | 20.08 | 20.27 | 1.10 | 0.30 | 0.04 | 0.78 |

$^{ab}$Means bearing different superscripts within a row differ significantly ($p<0.05$).

supplementation tended to improve catalase concentration. Hence, threonine level in 130% threonine (of Vencobb-400 specification) group level might have a better antioxidant functions under the sub-tropical climate. Therefore, adding threonine may maximize the concentration of catalase in the serum and may protect cells from oxidative injury by clearing superoxide anions. However, further study is required to explore the effect of supplemental L-threonine on these anti-oxidant parameters under heat stress (climatic stress) condition.

**Haematological profile**

The hemoglobin and values of haematocrit level of blood among the experimental groups did not differ significantly ($p>0.05$) at increased levels of L-threonine supplementation (Table 4). At various levels of threonine supplementation, the values of MCV, MCH (pg) and MCHC percentage of blood did not differ significantly ($p>0.05$) among the experimental birds. The values of TEC (million/cc) and TLC (thousand/cc) alsodid not differ significantly ($p>0.05$) among the experimental broiler birds. However, the average heterophils percentage decreased linearly ($p=0.01$) whereas average lymphocyte percentage increased linearly ($p=0.05$) with the increasing levels of L-threonine in the diet. Hence, at various levels of threonine supplementation, Heterophils/Lymphocyte ratio decreased linearly ($p=0.02$).

The values obtained for packed cell volume and haemoglobin showed that at higher level of threonine, the treatment diets did not portend any danger to the animals. Our present study is in agreement with that of Rezeipour *et al.* (2012) who also observed that hemoglobin, hematocrit value, MCH (pg), RBCs and WBCs count of experimental birds were within the normal range and was not affected by dietary L-threonine supplementation. Present observation revealed that broiler birds with higher levels of supplemental L-threonine had lower ($p=0.01$) heterophils percentage linearly and higher ($p=0.05$) lymphocyte percentage linearly. An immunological challenge can raise the number of heterophils in the 6 to 12 h of an immune response because these cells participate in the first line of response in birds (Silva *et al.,* 2009). Lymphocytes are the main constituent of immune system defense against viruses, bacteria and fungi and adequate number of lymphocytes showed direct fights with antigens within the body (Kabir, 2013). In the majority of avian species, healthy animals have more lymphocytes than heterophils in circulation, which influences the heterophil/lymphocyte ratio, known to be an indicator of the presence of physiological stress. In stress condition, the corticoids when released in the blood, decreases the number of lymphocytes (Dasilva *et al*., 2010). It may be an indication that the birds on higher supplemental diet were not suffering from any stressful condition. Present observation revealed that broilers with higher level of supplemental threonine had higher lymphocyte percentage. The heterophils/ lymphocyte (H/L ratio) appears to be more reliable indicator of stress and higher value of H/L means the birds are in more stressful condition. Threonine level at 0.80, 0.87, 0.94 and 1.01% in the diet did not affect the H/L ratio (Heshmat *et al.,* 2013). However, it is evident from the present finding that broiler birds with higher level of supplemental L-threonine (110%, 120%

**Table 4:** The Effects of Supplemental L-threonine on Hematological Parameters of Vencobb-400 broilers

| Attributes | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | SEM | P value | Linear | Quadratic |
|---|---|---|---|---|---|---|---|---|---|
| Hb (%) | 10.53 | 10.63 | 10.48 | 10.34 | 10.48 | 0.38 | 0.99 | 0.73 | 0.95 |
| Haematocrit (%) | 32.17 | 32.50 | 31.67 | 31.33 | 31.67 | 0.55 | 0.60 | 0.23 | 0.81 |
| MCV (fL) | 129.58 | 127.45 | 129.64 | 130.02 | 130.36 | 1.53 | 0.70 | 0.40 | 0.59 |
| MCH (pg) | 42.42 | 41.66 | 42.80 | 42.83 | 43.07 | 1.12 | 0.91 | 0.49 | 0.83 |
| MCHC (%) | 32.75 | 32.68 | 33.02 | 32.91 | 33.03 | 0.71 | 0.99 | 0.73 | 0.98 |
| TEC ($10^6$/cc) | 2.48 | 2.55 | 2.44 | 2.41 | 2.43 | 0.04 | 0.19 | 0.09 | 0.92 |
| TLC (000/cc) | 21.72 | 21.70 | 22.10 | 22.17 | 22.23 | 0.35 | 0.71 | 0.19 | 0.90 |
| Heterophils (%) | 27.83 | 27.83 | 27.67 | 27.50 | 26.83 | 0.27 | 0.09 | 0.01 | 0.21 |
| Lymphocyte (%) | 67.67 | 67.50 | 67.83 | 68.00 | 68.67 | 0.37 | 0.25 | 0.05 | 0.29 |
| Heterophils: Lymphocyte | 0.411 | 0.411 | 0.408 | 0.405 | 0.391 | 0.003 | 0.16 | 0.02 | 0.26 |

and 130% threonine of Vencobb-400 specification) groups tended to lower (p=0.02) H/L ratio linearly indicating that the birds were not suffering from stressful condition in the sub-tropical climate. Nevertheless, specific mechanisms regarding the effect of threonine on the blood hematology in birds need further study.

**Biochemical profile**

The average serum glucose concentration was higher (p<0.05) in 120% threonine (of Vencobb-400 specification) group when compared with NRC specification group (Table 5). The serum glucose concentration also linearly increased (p=0.002) with the increasing levels of supplemental L-threonine. However, the uric acid level decreased linearly (p=0.03). The average blood creatinine level decreased linearly (p=0.05) with the increased levels of supplemental L-threonine. The average serum total protein was higher (p<0.05) in 130% threonine (of Vencobb-400 specification) group when compared with NRC specification threonine group. There was also a linear increase (P=0.002) in serum total protein level with the increasing levels of supplemental L-threonine in the ration. The average serum globulin level was (p<0.001) higher in 130% threonine (of Vencobb-400 specification) group when compared with the NRC and Vencobb-400 specification group. There was a linear increment (P<0.001) in serum globulin level with a linear decrease in albumin: globulin ratio (p<0.05) with the increasing levels of supplemental L-threonine in the ration.

The values of average serum ALT, AST, ALP and triglyceride concentration did not differ (p>0.05) among the experimental groups. A lower concentration (p<0.05) of serum cholesterol level was observed in 130% threonine (of Vencobb-400 specification) group when compared either with NRC or Vencobb-400 specification group. There was a linear decrease (p<0.001) in cholesterol level with the increasing levels of supplemental L-threonine. The values of average serum HDL concentration had a linear increment (p=0.04) in the blood serum HDL level of broiler birds. The values of average serum LDL concentration did not differ (p>0.05) among experimental groups. The serum VLDL concentration decreased linearly (p<0.001) with increasing levels of supplemental L-threonine. The average serum VLDL level of broiler birds was (p<0.001) lower in 120% threonine (of Vencobb-400 specification) and in 130% threonine (of Vencobb-400 specification) group when compared with NRC and Vencobb-400 specification group.

The serum glucose concentrations linearly increased (p<0.002) with increasing levels of L-threonine (Weber *et*

**Table 5:** The Effects of Supplemental L-threonine on Serum Biochemical Parameters of Vencobb-400 broilers

| Attributes | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | SEM | P value | Linear | Quadratic |
|---|---|---|---|---|---|---|---|---|---|
| Blood Sugar (mg/dL) | $212.01^{a}$ | $220.65^{ab}$ | $223.14^{ab}$ | $246.16^{b}$ | $238.73^{ab}$ | 7.73 | 0.03 | 0.002 | 0.72 |
| Uric Acid (mg/dL) | 2.95 | 3.24 | 2.35 | 2.48 | 2.39 | 0.25 | 0.08 | 0.03 | 0.79 |
| Creatinine (mg/dL) | 1.61 | 1.57 | 1.44 | 1.45 | 1.33 | 0.11 | 0.38 | 0.05 | 0.94 |
| Total Protein (g/dL) | $3.27^{a}$ | $3.30^{ab}$ | $3.44^{ab}$ | $3.45^{ab}$ | $3.61^{b}$ | 0.08 | 0.03 | 0.002 | 0.65 |
| Albumin (g/dL) | 1.62 | 1.66 | 1.65 | 1.67 | 1.68 | 0.05 | 0.90 | 0.38 | 0.86 |
| Globulin (g/dL) | $1.65^{a}$ | $1.64^{a}$ | $1.79^{ab}$ | $1.78^{ab}$ | $1.93^{b}$ | 0.09 | <0.001 | <0.001 | 0.25 |
| Albumin: Globulin | $0.98^{ab}$ | $1.02^{b}$ | $0.93^{ab}$ | $0.94^{ab}$ | $0.87^{a}$ | 0.03 | 0.002 | <0.001 | 0.29 |
| ALT (IU/L) | 31.13 | 33.64 | 32.71 | 30.19 | 29.40 | 4.29 | 0.95 | 0.62 | 0.62 |
| AST (IU/L) | 212.78 | 213.87 | 220.52 | 213.99 | 215.47 | 3.59 | 0.58 | 0.63 | 0.37 |
| ALP (IU/L) | 161.87 | 166.07 | 164.42 | 147.55 | 151.85 | 6.98 | 0.27 | 0.09 | 0.57 |
| TGL (mg/dL) | 149.47 | 154.17 | 146.02 | 138.32 | 141.68 | 5.40 | 0.28 | 0.08 | 0.91 |
| Cholesterol (mg/dL) | $144.20^{b}$ | $143.57^{b}$ | $140.68^{ab}$ | $136.77^{ab}$ | $133.40^{a}$ | 2.10 | 0.005 | <0.001 | 0.42 |
| HDL (mg/dL) | 80.55 | 78.88 | 85.00 | 86.11 | 85.42 | 2.48 | 0.18 | 0.04 | 0.74 |
| LDL (mg/dL) | 37.72 | 36.81 | 38.53 | 35.71 | 35.23 | 1.85 | 0.70 | 0.31 | 0.60 |
| VLDL (mg/dL) | $25.93^{bc}$ | $27.87^{c}$ | $17.15^{ab}$ | $14.95^{a}$ | $12.75^{a}$ | 2.41 | 0<.0001 | <0.001 | 0.98 |

$^{ab}$Means bearing different superscripts within a row differ significantly (p<0.05).

*al.*, 2013). Abdel-Wareth and Esmail (2014) also revealed that serum glucose concentration significantly increased ($p<0.002$) with increased L-threonine supplementation. High concentration of serum glucose in the L-threonine supplemented groups might be due to catabolism of L-threonine in body. Catabolism results in glucogenic products like pyruvate and propionate that are needed for energy or glucose production. These biochemical processes might be responsible for increased serum glucose level in the treatment groups. In contrast, Azzam and El-Gogary (2015) revealed that serum glucose concentration was not significantly altered ($p>0.05$) in broilers with supplemental L-threonine levels (at 0.69, 0.71, 0.74, 0.76 and 0.79%). The divergent result observed by the above researchers might be due to presence of low levels of threonine in their experimental rations for which the full effect of threonine could not be observed.

In poultry, excess amino acids are metabolized to uric acid, which is then transported into the kidney and then it is excreted. In the present study, due to increased level of threonine there might have a modification of serum uric acid concentration by lowering its concentration. This indicated further that, with increasing threonine levels (increased Thr/Lys), protein metabolism may improve. Serum uric acid levels will increase when one or several amino acids are deficient or in excess. Azzam *et al.* (2011) observed no differences for uric acid among the threonine treatment groups in laying hens.

Total proteins, albumin and globulin were generally influenced by total protein intake (Njidda and Isidohomen, 2010) and high concentrations of total protein were associated with significant increases in levels of serum albumin and globulin (Azzam *et al*., 2011). In the process of protein anabolism and proteolysis, the serum protein level is always an indicator of the protein metabolism and immunity function situation *in vivo*. Avian total protein contains albumin and α, β and γ-globulin High concentrations of total protein are associated with significant increases in levels of serum albumin and globulin. The IgG concentration increased as the total protein concentration increased. Our present study is in agreement with that of Azzam *et al.* (2011) who revealed that the serum total protein concentration of broiler birds quadratically increased to supplemental L-threonine in laying hens.

The lowering of cholesterol level by supplemental threonine might be due to production of more amounts of bile acid/ bile salts in the liver. Twenty percent (20%) of excess threonine is converted to glycine which in turn synthesizes bile acids. Bile acids are synthesized from cholesterol, a major constituent of cholesterol metabolism. Hence, bile acids provide major excretory route of cholesterol. Bile acid sequestrants bind bile acids and prevent re-absorption of bile acids and hence cholesterol is lost in faeces. Hence, there may be disruption of enterohepatic circulation of bile acids and it may lower blood cholesterol level. The serum cholesterol decreased quadratically ($p<0.05$) in broilers with supplemental L-threonine levels (Azzam and El-Gogary, 2015) whereas it decreased linearly ($p<0.001$) and quadratically ($p=0.032$) in hens with supplemental L-threonine levels as reported by Abdel–Wareth and Esmail (2014). In contrast to the present trial, Rezaeipour and Gazani (2014) revealed that serum cholesterol concentration was not affected by dietary L-threonine supplementation in broilers. The present finding is in agreement with Rezaeipour and Gazani (2014) who revealed that serum LDL concentrations in broilers was not affected ($p>0.05$) however, serum VLDL levels reduced by dietary L-threonine supplementation ($p<0.05$) by the dietary threonine treatments.

## CONCLUSION

The present study assessed the effects of supplemental threonine on antioxidant enzyme activity and haemato-biochemical parameters of commercial broilers in the sub-tropics. From the results it may be concluded that L-threonine supplementation at 30% higher level (than Vencobb-400 specification) has a better antioxidant function and better haemato-biochemical profile under the subtropical climate.

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