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## Optimizing digestible threonine requirements for commercial broiler performance, immunological and carcass parameters

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### Abstract

A total of 250 commercial broiler birds (Ven Cobb) were used in the present research to assess the digestible threonine graded concentrations (100, 75, 70, 65, and 60% of the digestible lysine) in order to optimize the threonine requirements for improving the productive performance in commercial broiler diets. Using a completely random design (CRD) the chicks were divided into five treatments, each containing ten replicates and each with five chicks. Throughout the experiment, a corn soy based diet was prepared and supplemented with digestible threonine at graded concentration of digestible Lysine. The amount of digestible threonine in the broiler diet had no effect on feed intake and body weight gain in any of the growth phases. However, during the pre-starter and starting phases, FCR was significantly ( $p < 0.05$ ) influenced, with the supplemented groups at 65% and 60% showing better FCRs. While there was no difference in the slaughter parameters such as the heart and gizzard throughout treatment groups, the liver weight was considerably ( $p < 0.05$ ) lower in the 65 and 60% Thr groups than in all other threonine concentrations. Immune organ (spleen and bursa) weights, humoral immunity as measured by antibody titers to NDV, and cell mediated immunity as determined by PHA-P were unaffected by treatment groups. Thus, it can be concluded that in fast-growing commercial broilers, digestible threonine concentrations at 60% of the digestible lysine (0.708, 0.630, and 0.570%, respectively) during the pre-starter, starter, and finisher phases are ideal for improved growth performance without having any negative impact on carcass and immune parameters.

**Keywords:** Broilers, cell mediated immunity, giblets, humoral immunity, lymphoid organs, threonine

### Introduction

Approximately 70% of the expenses associated with producing broilers are related to feed alone. And broiler chicken diets usually include corn and soybean meal (SBM). Balanced and ideal nutritional feeding is necessary for precision nutrition. Protein is necessary for all animal tissues, and chicken growth is significantly impacted with it (Kamran *et al.*, 2004) [7]. Early marketing of broiler chickens requires a large intake of well-balanced nutrients, especially amino acids (AA), due to their rapid growth. These AA are essential for defence mechanisms in the body and structural building components, as well as for the normal functioning of enzymes and tissues (NRC, 1994). The digestible amino acids (DAA) concept may help reduce the amount of protein in the diet without sacrificing the best possible performance from chicken. In order to meet the requirements of current fast-growing broilers and allow them to reach their full genetic potential, these diets are inadequate in AA. Therefore, diets based on corn-SBM are supplemented with crystalline AAs to meet the AA needs. In order to achieve maximum efficiency in production, criterion AA has proven to be essential. Determining the optimal ratios for these potentially limiting essential amino acids requires careful consideration.

In low-CP poultry diets consisting of corn-soybean meal, dietary threonine (Thr) is the third limiting amino acid (Fernandez *et al.*, 1994) [6]. Since it combines with intestinal mucosal proteins, particularly mucins, and digestive enzymes in the lumen to encourage nutritional absorption and digestion, the amino acid threonine is necessary for maintaining the integrity of the gut mucosa and its barrier function (Dozier *et al.*, 2001; Mao *et al.*, 2011; Wang *et al.*, 2009) [3, 10, 15]. Research over the past 15 years has revealed that Thr should be expressed in response to the 0.63 to 0.70% of dietary Lys, with diet, age, gender, and strain acting as the

main determining factors (Kidd, 2002) <sup>[9]</sup>.

As a result, it is currently uncertain how much dietary protein can be substituted by AA supplementation without affecting broiler performance. Evaluating the performance, immunological profile, and carcass parameters of commercial broiler diets with varying Thr requirements for low-dietary-protein diets was the objective of the current biological investigation.

## Materials and Methods

In a CRD, 250 day-old male broiler chicks (Ven Cobb-400) were used in a six-week growth trial with five dietary treatments. After obtaining them, the chicks were weighed and their wings banded. After that, they were divided into five treatments at random, each of which had five chicks and ten replicates. For the duration of study, the chicks were kept in electrically heated battery brooders to provide ideal brooding conditions.

## Experimental diets

A control diet based on corn and soy was prepared in accordance with Cobb breeder's recommendations. Other experimental diets for the study were prepared by lowering crude protein levels by 2, 1, and 1% during the pre-starter, starter, and finisher phases, respectively. Graded concentrations of threonine during the pre-starter period (0-14 days) were 0.885, 0.826, 0.767, and 0.708; during the starter period (15-28 days), they were 0.787, 0.735, 0.682, and 0.630; and during the finisher period (29-42 days), they were 0.712, 0.665, 0.617, and 0.570. (Table. 4)

## Collection of data

Up to six weeks of age, each bird's body weight was recorded once at the end of each phase, which included the pre-starter (0-14 days), starter (15-28 days), and finisher phase (29-42 days). For each phase, the feed intake per bird was calculated replicate-wise. At each phase, the feed conversion ratio (FCR) was calculated as feed intake per unit body weight gain from 0 to 6 weeks of age. Ten blood samples were collected at random from each replicate for each dietary group at 28 and 42 days of age in order to study immunity parameters. The hemagglutination inhibition (HI) test was used to determine NDV-specific antibodies in the chicks' serum samples, which were then expressed as log<sub>2</sub> titers (Allan *et al.*, 1978) <sup>[1]</sup>. The measurement of cutaneous basophilic hypersensitivity (CBH) to phytohemagglutinin phosphate (PHA-P) was used to assess the cell mediated immune (CMI) response. One bird was selected at random from each replication on the 42<sup>nd</sup> day of the trial to test the CMI. A micrometer was used to measure the thickness of the web between the third and fourth interdigital spaces of the left and right feet. The left web (control) had an injection of 0.1 ml of phosphate buffer saline (PBS), while the right foot's web had an injection of 100 µg of PHA-P suspended in 0.1 ml of PBS. A micrometer was used to measure the web thickness of both feet 24 hours after injection, and the CBH was then calculated using the formula (Edelman *et al.*, 1986). In order to evaluate the giblets' characteristics (liver, heart, and gizzard) and the weight of their immune organs (spleen and bursa), which were expressed in grams per kilogram of live

body weight, ten birds from each dietary group were slaughtered at the end of the experiment. The data was analyzed using the one-way ANOVA in the 15<sup>th</sup> version of the Statistical Package for Social Sciences (SPSS). Duncan's multiple range test (Duncan, 1955) <sup>[4]</sup> was used to analyze the difference between the treatment means at  $p < 0.05$ .

## Results and Discussion

### Growth performance

The findings of the present study on growth performance viz, body weight gain, feed intake and feed conversion ratio has been depicted in Table.1 Significantly higher body weight gain was recorded with the addition of digestible threonine at 0.708, 0.630, and 0.570 percent (60 percent of the digestible lysine) during the pre-starter, starter, and finisher phases, respectively. The BWG consistently demonstrated an increasing trend throughout the various phases. In contrast, Mehri *et al.* (2012) <sup>[11]</sup>, Corzo *et al.* (2009) <sup>[2]</sup>, and Kidd *et al.* (1999) <sup>[8]</sup> found that dThr (%) 0.78, 0.73, and 0.66, respectively, significantly ( $p < 0.05$ ) improved the body weight gain in commercial broilers.

The concentration of digestible threonine in the broiler diet had no effect on feed consumption throughout the pre-starter, starter, or finisher phases ( $p > 0.05$ ). In contradiction to the findings of this investigation, Star *et al.* (2010) found that feed intake was significantly ( $p < 0.05$ ) higher in the treatment group when the dietary Thr:Lys ratio was higher (0.70) than in broilers with a Thr:Lys ratio of 0.65.

The control diet (T<sub>1</sub>) had a significantly ( $p < 0.05$ ) better FCR during the pre-starter phase when the Cobb digestible lysine recommendations was 0.77 and 0.885 percent (75 percent). Corzo *et al.* (2009) <sup>[2]</sup> found that dig. Thr (0.76%) had a significant effect on the FCR of male broilers aged 14-28 days. In this present study, all experimental groups showed comparable FCR during the starter phase, with the exception of the 0.682% dig. Thr (65% of dig Lys) supplemented group. However, during the study period the FCR did not reveal any significant ( $p > 0.05$ ) differences in any of the growth phases of commercial broilers.

### Carcass parameters (Giblet weights)

The results of the present biological study on carcass parameters has been presented in Table.2 The broilers' liver weight decreased significantly ( $p < 0.05$ ) in the 65 and 60% Thr supplemented dietary groups, although when different amounts of digestible threonine were added to their diet, there was no discernible change in their heart or gizzard ( $p > 0.05$ ). However, the liver weight of 75% of the Thr-fed group and the control dietary group was comparable.

### Immune parameters

The findings of the immune organ weights and NDV titer values (Log<sub>2</sub>) are documented in Table.3. When digestible threonine was added to commercial broiler diets consisting of maize and soybean meal, the spleen's and bursa's weights did not alter. The CMI response at day 42 and the humoral immunity (NDV titers) evaluated at days 28 and 42 were unaffected by the dietary treatment groups. However, Rezeipour and Fononi Irani (2012) <sup>[13]</sup> found that adding dietary threonine to broiler diets resulted in significantly ( $p < 0.05$ ) higher HI (NDV titers) at 42 days of age.

**Table 1:** Effect of digestible threonine supplementation on phase wise growth performance of commercial broilers.

| Treatment             | Body weight gain (gm/bird) |                   |                    | Feed Intake (gm/bird) |                   |                    | FCR                  |                    |                    |
|-----------------------|----------------------------|-------------------|--------------------|-----------------------|-------------------|--------------------|----------------------|--------------------|--------------------|
|                       | Pre-starter (0-14 d)       | Starter (15-28 d) | Finisher (29-42 d) | Pre-starter (0-14 d)  | Starter (15-28 d) | Finisher (29-42 d) | Pre-starter (0-14 d) | Starter (15-28 d)  | Finisher (29-42 d) |
| T <sub>1</sub> (100%) | 337                        | 700               | 832                | 393                   | 1096              | 1644               | 1.167 <sup>b</sup>   | 1.566 <sup>b</sup> | 1.986              |
| T <sub>2</sub> (75%)  | 329                        | 685               | 871                | 395                   | 1079              | 1637               | 1.200 <sup>a</sup>   | 1.574 <sup>b</sup> | 1.882              |
| T <sub>3</sub> (70%)  | 325                        | 654               | 855                | 397                   | 1030              | 1610               | 1.222 <sup>a</sup>   | 1.575 <sup>b</sup> | 1.885              |
| T <sub>4</sub> (65%)  | 334                        | 675               | 863                | 404                   | 1084              | 1671               | 1.211 <sup>a</sup>   | 1.604 <sup>a</sup> | 1.943              |
| T <sub>5</sub> (60%)  | 333                        | 701               | 877                | 406                   | 1101              | 1682               | 1.219 <sup>a</sup>   | 1.570 <sup>b</sup> | 1.919              |
| SEM                   | 2.048                      | 6.071             | 10.97              | 2.416                 | 8.450             | 16.27              | 0.005                | 0.004              | 0.014              |
| N                     | 10                         | 10                | 10                 | 10                    | 10                | 10                 | 10                   | 10                 | 10                 |
| P-Value               | 0.395                      | 0.075             | 0.756              | 0.324                 | 0.059             | 0.669              | 0.002                | 0.036              | 0.109              |

<sup>ab</sup>Mean bearing at least one common superscript in a column do not differ significantly ( $p < 0.05$ ).

**Table 2:** Effect of digestible threonine supplementation on carcass parameters (Giblets) of commercial broilers.

| Treatment             | (g / kg slaughter live weight) |       |         |
|-----------------------|--------------------------------|-------|---------|
|                       | Liver                          | Heart | Gizzard |
| T <sub>1</sub> (100%) | 18.62 <sup>a</sup>             | 4.491 | 14.72   |
| T <sub>2</sub> (75%)  | 18.63 <sup>a</sup>             | 4.773 | 15.91   |
| T <sub>3</sub> (70%)  | 17.37 <sup>ab</sup>            | 5.150 | 16.10   |
| T <sub>4</sub> (65%)  | 16.55 <sup>b</sup>             | 4.443 | 15.11   |
| T <sub>5</sub> (60%)  | 16.14 <sup>b</sup>             | 4.992 | 15.22   |
| SEM                   | 0.294                          | 0.104 | 0.238   |
| N                     | 10                             | 10    | 10      |
| P-Value               | 0.010                          | 0.128 | 0.324   |

<sup>ab</sup>Mean bearing at least one common superscript in a column do not differ significantly ( $p < 0.05$ ).

**Table 3:** Effect of digestible threonine supplementation on humoral, cell mediated immunity and immune organ weights of commercial broilers.

| Treatment             | NDV titer values (Log <sub>2</sub> ) |                      | PHA-P thickness index (%) 6 <sup>th</sup> Wk | Weight of immune organs (g / kg slaughter live weight) |        |
|-----------------------|--------------------------------------|----------------------|--|--|--------|
|                       | 28 <sup>th</sup> day                 | 42 <sup>nd</sup> day |  | Bursa  | Spleen |
| T <sub>1</sub> (100%) | 1.5                                  | 3.7                  | 93.80  | 1.72   | 1.43   |
| T <sub>2</sub> (75%)  | 1.5                                  | 3.4                  | 101.00                                       | 1.66   | 1.59   |
| T <sub>3</sub> (70%)  | 1.1                                  | 3.7                  | 88.66  | 1.49   | 1.82   |
| T <sub>4</sub> (65%)  | 2.1                                  | 3.5                  | 94.09  | 1.60   | 1.58   |
| T <sub>5</sub> (60%)  | 1.7                                  | 4.0                  | 81.96  | 1.50   | 1.38   |
| SEM                   | 0.143                                | 0.207                | 2.367  | 0.080  | 0.067  |
| N                     | 10                                   | 10                   | 10   | 10   | 10     |
| P-Value               | 0.276                                | 0.919                | 0.123  | 0.872  | 0.255  |

<sup>abcde</sup>Mean bearing at least one common superscript in a column do not differ significantly ( $p < 0.05$ ).

**Table 4:** Nutrient composition threonine supplemented diets in commercial broilers

| Pre starter phase (0-14 days) |                                 |                      |                      |                      |                      |
|-------------------------------|---------------------------------|----------------------|----------------------|----------------------|----------------------|
| Nutrient                      | T <sub>1</sub> (Cobb std. 100%) | T <sub>2</sub> (75%) | T <sub>3</sub> (70%) | T <sub>4</sub> (65%) | T <sub>5</sub> (60%) |
| ME (k cal/kg)                 | 3035                            | 3035                 | 3035                 | 3035                 | 3035                 |
| CP (%)                        | 22.00                           | 20.00                | 20.00                | 20.00                | 20.00                |
| Ca (%)                        | 0.90                            | 0.90                 | 0.90                 | 0.90                 | 0.90                 |
| Avl. Phosphorus (%)           | 0.449                           | 0.449                | 0.449                | 0.449                | 0.45                 |
| Lysine (%)                    | 1.18                            | 1.18                 | 1.18                 | 1.18                 | 1.18                 |
| M+C (TSAA) (%)                | 0.88                            | 0.88                 | 0.88                 | 0.88                 | 0.88                 |
| Threonine (%)                 | 0.770                           | 0.885                | 0.826                | 0.767                | 0.708                |
| Valine (%)                    | 0.89                            | 0.89                 | 0.89                 | 0.89                 | 0.89                 |
| Starter phase (15-28 days)    |                                 |                      |                      |                      |                      |
| ME (k cal/kg)                 | 3108                            | 3108                 | 3108                 | 3108                 | 3108                 |
| CP (%)                        | 20.0                            | 19.0                 | 19.0                 | 19.0                 | 19.0                 |
| Ca (%)                        | 0.84                            | 0.84                 | 0.84                 | 0.84                 | 0.84                 |
| Avl. Phosphorus (%)           | 0.42                            | 0.42                 | 0.42                 | 0.42                 | 0.42                 |
| Lysine (%)                    | 1.05                            | 1.05                 | 1.05                 | 1.05                 | 1.05                 |
| M+C (TSAA) (%)                | 0.80                            | 0.80                 | 0.80                 | 0.80                 | 0.80                 |
| Threonine (%)                 | 0.690                           | 0.787                | 0.735                | 0.682                | 0.630                |
| Valine (%)                    | 0.84                            | 0.81                 | 0.81                 | 0.81                 | 0.81                 |
| Finisher phase (29-42 days)   |                                 |                      |                      |                      |                      |
| ME (k cal/kg)                 | 3180                            | 3180                 | 3180                 | 3180                 | 3180                 |
| CP (%)                        | 19.0                            | 18.0                 | 18.0                 | 18.0                 | 18.0                 |
| Ca (%)                        | 0.76                            | 0.76                 | 0.76                 | 0.76                 | 0.76                 |
| Avl. Phosphorus (%)           | 0.38                            | 0.38                 | 0.38                 | 0.38                 | 0.38                 |
| Lysine (%)                    | 0.95                            | 0.95                 | 0.95                 | 0.95                 | 0.95                 |
| M+C (TSAA) (%)                | 0.740                           | 0.666                | 0.666                | 0.666                | 0.666                |
| Threonine (%)                 | 0.650                           | 0.712                | 0.665                | 0.617                | 0.570                |
| Valine (%)                    | 0.80                            | 0.76                 | 0.76                 | 0.76                 | 0.76                 |

## Conclusion

According to present findings from experiments, the ideal digestible threonine concentrations for improved growth performance in fast-growing commercial broilers are 60% of the digestible lysine during the pre-starter, starter, and finisher phases (0.708, 0.630, and 0.570%, respectively). These concentrations have no adverse effect on carcass and immune parameters.

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