Effect of L-Threonine Supplementation to Diet on Some Productive and Physiological of Traits Broiler Chickens Under Heat Stress Conditions

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Article history:
Received: 23 January 2022
Accepted: 9 May 2022
Published: 30 June 2022

Keywords: *L-Threonine, physiological traits, broilers, heat stress*

Abstract

This experiment was conducted in Erbil, Iraq, from July 7 to August 17, 2021, for determine the effects of L-Threonine supplementation on several productive and physiological traits Under heat stress conditions (36.04 ± 2 °C), A total of 200 broiler chicks were randomly distributed into five treatments: T1 control treatment, T2, T3, T4 and T5 standard diet with L-Threonine 250, 500, 750 and 1000 mg/kg, respectively. The results showed that the addition of L-Threonine to the ration improved broiler production performance under heat stress conditions such as body weight it increased significantly (P≤0.05) in T3 and T5 and reached 2187 and 2235 g, respectively compared to 2145 g in the T1 and reduce H/L ratio in T4 and T5 compared with T1 and did not affect effect (P≥0.05) on physiological traits such as red and white blood cell count, hematocrit, cholesterol, glucose, total protein, aspartate aminotransferase and alanine aminotransferase activities.

https://dx.doi.org/10.52951/dasj.22140106

Introduction

Tropic hot weather during summer is one of the significant factors that negatively on broiler performance (Dalolio *et al.*, 2021). Broiler chickens eat less and spend more time drinking water, panting, and resting around the waterer and other cool locations in the pen when the heat stresses them. Also, the broiler production performance was affected negatively (Wasti *et al.*, 2020).

Furthermore, heat-stressed broilers have shown an increase in abdominal fat and a loss in carcass protein due to heat stress (Mohamed *et al.*, 2019).

It also increases the need for nutrients and amino acids such as methionine, lysine and Threonine. So The growth rates of broiler under heat stress conditions depend on a sufficient supply of amino acids to produce proteins involved in various physiological functions, such as signal transduction, hormone signaling, cell structure, and antioxidant systems (Maharjan, 2020). Therefore, nutritional and management therapies can be introduced to minimize the heat stress due to high environmental temperatures in broiler production.

L-Threonine is often the third limiting amino acid, and it is important for gut health, morphology, and function, as well as the optimal utilization of total sulfur amino acids and lysine, immunity, carcass traits, structural protein synthesis, antibody, uric acid, and pancreatic enzymes, intestinal barrier maintenance, and mucin synthesis. The intestinal mucosa is protected by the mucus layer, which contains mucins, and glycoproteins that need Threonine for production (Al-Hayani, 2017; Al-Sagan *et al.*, 2018).

Estalkhzir *et al.*, (2013) found that supplementing broiler diets with threonine improved body weight, feed conversion, and dressing percentage. According to Rezaeipour *et. al.*, (2014) reported that

"threonine supplementation for the first 42 days improved Feed Conversion in broiler" and based on NRC (1994) requirement for threonine is 0.48% of the diet.

More research is needed to quantify amino acid needs under heat stress conditions, This experiment aimed to study the effects of L-Threonine Supplementation on some productive and physiological traits broiler (Ross 308) Under Heat Stress Conditions.

Materials and methods

Ethic Approval

The study proposal presented was accepted by the Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala.

Experimental design

This experiment was conducted in (The sako field) in (Gurato/Mergasor/Erbil/Iraq) from the period (5 / 7 / 2021 to 17 / 8 / 2021) and aimed to determine the effects of the use L-Threonine in the broiler as on some productive and physiological traits of broiler chickens Under Heat Stress Conditions. A total of 200 one-day old unsexed (Ross-308) broilers chicks were randomly distributed into five treatments and each treatment was further subdivided into fourth replicates:

- **T1: a standard diet without L-Threonine (control treatment)**
- **T2: a standard diet with L-Threonine 250 mg/kg**
- **T3: a standard diet with L-Threonine 500 mg/kg**
- **T4: a standard diet with L-Threonine 750 mg/kg**
- **T5: a standard diet with L-Threonine 1000 mg/kg**

The chicks of each treatment were housed in Full Environmental Control Houses and all of the experimental diets were designed to suit the nutritional needs of broiler chicks according to the NRC (1994) (See table 1). Feeds and water were supplied *ad-libitum*, the temperature and humidity were measured inside the breeding farms by using electronic thermometers distributed in different parts of the farm.

Table 1. Chemical analysis of the basal die

Ingredients	starter (1-10days)	Grower (11-24days)	Finisher (25-35days)
Protein %	22.50	20	18
Energy (Kcal/kg)	3069	3185	3250
Fat%	4.45	5.61	5.95
Lysine %	1.33	1.21	1.21
Methionine %	0.64	0.56	0.50
Ca %	0.98	0.95	0.90
P %	0.62	0.58	0.54

The preventive vaccination program was against the most common infectious diseases such as Newcastle disease and infectious bronchitis as shown in Table 2.

Table 2. Vaccination program of broiler chicks during the experimental period

Age (days)	Vaccine	Route of vaccination
1-3	Newcastle disease + Infectious Bronchitis	eye drop
10-12	Infectious bronchitis	eye drop
16-17	Hantavirus Pulmonary Syndrome	Sub cut.1/2 cc
22	Infectious bronchitis	drinking water
28	Newcastle disease	drinking water

Productive traits

Live body weight (LBW): live body weight of each replicate and treatment was individually measured at the one day old and weekly in the morning by weighing the broilers of one replicate individually as follows (Miah *et al.,* 2022):

LBW (g) = sum of broiler weights per replicate/number of broiler per replicate

Body weight gain (BWG): The BWG was calculated using the following equation according to (Miah *et al.,* 2022):

BWG = LBW2- LBW1/ number of bird

Feed intake (FI): The weekly feed intake for each repeater was calculated at the end of each week of the experiment by weighing a certain amount of diet provided to the birds for each repeater during the week and removing from it the weight of the remaining diet at the end of the week (Miah *et al.,* 2022).

Feed conversion ratio (FCR): FCR was measured weekly as Kg of FI / Kg of BWG **(**Miah *et al.,* 2022).

Physiological traits

Five ml of blood was collected from five birds (male) from each experimental treatment at 42 days of age, blood count parameters were determined: Red blood cell (RBC), weight blood cell (WBC), Hemoglobin (Hb), Hematocrit (PCV) and H/L ratio according to Archer (1965) and Natt and Herick (1952). Then another portion of the blood was centrifuged for ten minutes at 1000 rpm. All blood serum biochemical features, including cholesterol, glucose, total protein, albumin and globulin, uric acid, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, were measured photometrically using commercially available testing kits.

Statistical analysis

The data were statistically analyzed by the General Linear Models procedure of the SPSS software (SPSS, 2011). Statistically significant differences observed among treatments were separated using Duncan's multiple range test with 5% probability level.

Results and Discussion

Table 3 shows the impact of threonine supplementation on body weight (BW), body weight gain (BWG), and other production traits in the broiler chickens. It can be seen from the table 3 that threonine treatments have a significant effect ($p \leq 0.05$) on BW when compared to the control treatment. In T3 and T5, it reached BW 2187 and 2235 g, respectively, compared to 2145 in T1 and T5. The current study also found that broiler BWG was considerably greater ($p \leq 0.05$) in T3 and T5 than in T1, T2, and T4, but had no effect on FI or FCR.

Table 3. Effect of L- threonine supplementation on productive performance in broiler chickens (Mean±SE)

Treatment	IBW (g)	FBW (g)	BWG(g/ bird)	FI (g/ bird)	FCR
T1	41.89 ± 0.35	2145 ± 22.73 b	2104 ± 45.92 b	3187 ± 68.28	1.51 ± 0.01
T2	41.23 ± 0.27	2128 ± 21.37 b	2086 ± 37.17b	3173 ± 79.01	1.52 ± 0.08
T3	41.69 ± 0.13	2187 ± 10.15 ab	2145 ± 16.23 ab	3172 ± 61.22	1.47 ± 0.06
T4	41.30 ± 0.21	2144 ± 47.97 b	2103 ± 94.22 b	3179 ± 98.75	1.51 ± 0.03
T5	41.29 ± 0.29	2235 ± 22.94 a	2194 ± 43 a	3177 ± 13.22	1.44 ± 0.03
P-value	**NS**	*****	*****	**NS**	**NS**

- **T1 control treatment, T2, T3, T4 and T5 standard diet with L-Threonine 250, 500, 750 and 1000 mg/kg respectively**
- **The different letters within the same column indicate a significant difference between the means according to Duncan's test**
- *** means that there is a significant effect of treatment and NS means non-significant effect of treatment in the ANOVA table.**

Table 4 shows that using L-Threonine supplement has no significant effect ($P \geq 0.05$) on the broiler red and white blood cells (RBC and WBC) compared to T1 (control treatment), In comparison to T1 and the remainder of the L-Threonine treatments, the H/L ratio was much lower ($P \leq 0.05$) in T4 and T5, indicating that heat stress was less of an effect.

Table 4. Effect of L- threonine supplementation on physiological performance in broiler chickens (Mean±SE)

Treatment	RBC (*10^6/mm^3)	WBC (*10^3/mm^3)	H/L
T1	2.11 ± 0.04	21.75 ± 0.17	0.34 ± 0.01 a
T2	2.12 ± 0.04	21.90 ± 0.36	0.31 ± 0.00 ab
T3	2.10 ± 0.07	22.09 ± 0.13	0.32 ± 0.02 ab
T4	2.17 ± 0.05	21.38 ± 0.17	0.28 ± 0.00 b
T5	2.07 ± 0.11	22.03 ± 0.14	0.27 ± 0.01 b
P-value	**NS**	**NS**	*****

- **T1 control treatment, T2, T3, T4 and T5 standard diet with L-Threonine 250, 500, 750 and 1000 mg/kg respectively.**
- **The different letters within the same column indicate a significant difference between the means according to Duncan's test .**
- *** means that there is a significant effect of treatment and NS means non-significant effect of treatment in the ANOVA table.**

As shown in tables 5 and 6, the addition of L- threonine did not affect on the biochemical traits of blood serum (Glucose, Total protein, Cholesterol and Uric acid) or the activity of liver enzymes (AST and ALT) when compared to the control treatment T1.

Table 5. Effect of L- threonine supplementation on biochemical traits of blood serum in broiler chickens (Mean±SE)

Treatment	Glucose (mg/dl)	Total protein (mg/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)
T1	182.3 ± 5.9	2.23 ± 0.13	143.78 ± 5.30	7.12 ± 0.27
T2	191.42 ± 7.2	2.48 ± 0.09	149.34 ± 11.64	6.40 ± 0.22
T3	190.9 ± 6.6	2.56 ± 0.10	147.53 ± 7.59	6.78 ± 0.14
T4	191.2 ± 4.6	2.66 ± 0.18	147.74 ± 1.09	6.24 ± 0.52
T5	187.8 ± 10.6	2.79 ± 0.10	145.56 ± 2.87	6.54 ± 0.10
P-value	**NS**	**NS**	**NS**	**NS**

- **T1 control treatment, T2, T3, T4 and T5 standard diet with L-Threonine 250, 500, 750 and 1000 mg /kg respectively and NS means non-significant effect of treatment in the ANOVA table.**

Table 6. Effect of L- threonine on the activity of liver enzymes in broiler chickens (Mean±SE)

Treatment	AST (U/L)	ALT (U/L)
T1	117.50 ± 3.97	13.68 ± 0.46 b
T2	112.83 ± 1.53	13.19 ± 1.01 b
T3	110.51 ± 1.04	12.44 ± 0.70 b
T4	110.53 ± 0.82	11.85 ± 1.07 a
T5	112.18 ± 1.27	11.69 ± 0.21 a
P-value	**NS**	**NS**

- **T1 control treatment, T2, T3, T4 and T5 standard diet with L-Threonine 250, 500, 750 and 1000 mg /kg respectively**
- **NS means non-significant effect of treatment in the ANOVA table.**

The digestive system is lined with mucous membranes, and threonine is required for mucous membrane function, which includes adjusting the pace at which food travels through the digestive tract for optimum nutrient absorption and maintaining appropriate production of digestive enzymes (Dalolio et al., 2021), also the L -threonine maintain appropriate secretion of digestive enzymes which leads to increased feed digestibility and improved production traites (BW and BWG) (Rao et. al., 2011 and Johannsen *et. al.,* 2012) .

According to Rezaeipour *et al,*. (2012), increasing dietary L-threonine improved morphological aspects of the small intestine, resulting in greater development rate, whereas birds fed threonine-deficient diets exhibited decreased nutrient absorption in the digestive tract, "and Boosts in the number of heterophils are an essential measure of immune system function, and threonine increases globulin levels in blood and lymphocytes. (Azzam et. al., 2011).

Conclusion

Our findings show that L-Threonine supplementation can improve broiler production performance such as live body weight and body weight gain, and reduce the negative effects of heat stress. Still, it has no effect on feed intake, feed conversion ratio, blood serum biochemical traits, or liver enzyme activity.

Conflict of interests

The author's name appears below. He declares that he has no financial or non-financial interests (such as personal or professional ties, affiliations, expertise, or beliefs) in the subject matter or materials covered in this manuscript.

Acknowledgments

Thank you to the Saco field manager and his team for assisting with the study.

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