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Research Article
Effect of Threonine Supplementation on Broiler Chicken Productivity Traits

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Abstract

Objective: This study aimed to examine the impact of different amounts of threonine supplementation (0, 300, 600 and 900 mg kg^{-1}) on physiological and productivity traits of broiler chickens. **Materials and Methods:** A total of 300 one day-old unsexed Ross 308 broilers were raised to the age for market (35 days). At the end of the experimental period, the Body Weight (BW), Weight Gain (WG), Feed Intake (FI) and feed conversion ratio (FC) were calculated. The Carcass Weight (CW), Dressing Percentage (DP), relative breast weight (BRp), percentage of carcass parts and internal organs were also assessed. Physiological and chemical blood tests were conducted on a sample of 12 birds per treatment (6 males, 6 females) at 21 and 35 days of age. **Results:** Significant improvement was observed in BW, WG, DP and BRp of birds fed diet supplemented with 900 mg kg^{-1} threonine compared to those of fed diet without threonine supplementation. This concentration of threonine also significantly increased of red blood count cells (RBC) as well as hematocrit (PCV%), calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) and Growth Hormone (GH) levels, with a significant decrease in the H/L ratio. **Conclusion:** Supplementation of broiler chicken diets with 900 mg kg^{-1} threonine improved productivity traits.

Key words: Threonine, growth hormone, broiler, productivity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The growth rates of chickens depend on the availability of adequate amount of amino acids that can be used to synthesize proteins involved in many physiological processes, including signal transduction, hormone signaling, cell structure and antioxidant systems[1].

Amino acids are also important for energy metabolism, urinary system function and sexual maturation[2]. Due to the rapid growth of commercial chicken strains, the availability of amino acids for optimal growth, particularly muscle growth as well as physiological function is critical[3,4].

In addition to methionine and lysine, the aliphatic amino acid threonine is an important growth factor in broilers[5] and is one of the basic amino acids needed for growth of domesticated birds[6]. Chickens cannot synthesize several amino acids, including threonine and thus threonine supplementation is required[7]. Amino acids play many important physiological roles[8], particularly in thyroid function[9] and diet lack of threonine as well as other amino acids can lead to diminished physiological function[10-12]. Indeed, Azzam *et al*.[13,14] stated that leucine supplementation improved humoral immune responses by increasing γ-globulin levels as well as antioxidant levels in chickens[4]. Moreover, threonine helps maintain the integrity of the intestinal mucosal barrier and thus can enhance nutrient uptake by broiler chickens[15].

Estalkhzir *et al*.[16] found that the addition of threonine to broiler chicken diets increased productivity in terms of Body Weight (BW), Feed Conversion (FC), Dressing Percentage (DP), relative breast weight (BRp) and thigh weight (THp). In addition, Rezaeipour *et al*.[17] reported that threonine supplementation together with feed particle size improved FC, whereas threonine supplementation for the first 42 days after birth improved FC to levels above those seen with the addition of probiotics[18].

To date, these studies have not shown a direct role of threonine in improving chicken growth performance, especially in broilers. Therefore, in this study the diets of broiler chickens were supplemented with various threonine concentrations in order to better characterize the role of threonine in growth and productivity traits and to determine the optimal supplementation concentration.

MATERIALS AND METHODS

This study was conducted at the poultry farm at the Animal Production Department, College of Agriculture, University of Baghdad, Abu Ghraib, between March 19, 2016 and April 24, 2016. The study involved 300 one day-old, unsexed Ross 308 broiler chicks. The birds were randomly divided into four treatment groups with 75 birds per treatment and 25 birds per replicate.

Table 1: Study diet composition

Ingredients	Starter (%)	Finisher (%)
Maize	30	40
Wheat	28.25	24
Soybean meal (48% crude protein)	31.75	24
Protein concentration*	5	5.0
Sunflower oil	2.9	4.4
Limestone	0.9	0.6
Dicalcium phosphate	0.7	0.9
NaCl	0.3	0.1
Vitamin and mineral mixture	0.2	0.2
Total	100.0	100.0
Calculated analysis#		
Crude protein (%)	23	20
Metabolic energy (kcal kg^{-1})	3,027	3,195.3
Lysine (%)	1.20	1.10
Methionine (%)	0.49	0.46
Cysteine (%)	0.36	0.32
Methionine+cysteine (%)	0.85	0.78
Ca (%)	0.85	0.76
Phosphorus available (%)	0.45	0.49
P/C ratio	131.61	159.77

*Brocon-5 Special W: 40% crude protein, 3.5% fat, 1% fiber, 6% Ca, 3% available P, 3.25% lysine, 3.5% methionine, 3.90% methionine + cysteine, 2.2 Na, 2,100 kcal ME, 200,000 IU A, 40,000 IU D_3, 500 mg E, 30 mg K_3, 15 mg B_1, 15 mg B_2, 150 mg B_3, 20 mg B_6, 300 mg B_{12}, 10 mg folic acid, 100 Mcg biotin, 1 mg Fe, 100 mg Cu, 1.2 Mn, 800 mg Zn, 15 mg I, 2 mg Se, 6 mg Co, 900 mg antioxidant (BHT). #NRC[20]

The chicks were housed in a hall that was divided into 12, 2×2 m pens where the floors were covered with a 5 cm layer of sawdust. A continuous lighting system (23 h of light and one hour of darkness) was used.

The chicks were given two diets *ad libitum*, starter and finisher, which contained 3,027 and 3,195.3 kcal kg^{-1} metabolic energy and 23 and 20% crude protein[19], respectively (Table 1).

Threonine was added to the diet at 0, 300, 600 and 900 mg kg^{-1} to yield Th_0, Th_{300}, Th_{600} and Th_{900} treatments, respectively.

Productivity traits, including BW, WG, FI and FC, were measured weekly and at the end of the experimental period (35 days). At the end of the experimental period, 12 birds (6 males and 6 females per treatment) were slaughtered, cleaned and weighed to determine the Carcass Weight (CW) and Dressing Percentage (DP). The carcasses were then cut to determine the relative weight of the main (breast, thigh) and secondary (back (Bap), wing (Wp) and neck (Np)) regions of the carcass as well as the relative weight of the heart (Hp), liver (Lp) and spleen (SP). The weight of the bursa of Fabricius (BF) and amount of abdominal fat (Fp) were also measured.

Blood samples from 6 birds per replicate (3 males and 3 females) were collected on days 21 and 35. The blood samples were divided into tubes containing anticoagulants for blood tests to determine the Red Blood Cell (RBC) and White Blood Cell (WBC) counts, using a hemocytometer, (with an improved Neubauer ruling Chinese-made), H/L ratio[20] and PCV%[21]. Hemoglobin (Hb) was estimated using the Darbkin method as described by Varley *et al.*[22]. The remaining blood samples were placed into 6 ml Gel tubes and centrifuged at 4,000 rpm for 10 min to separate serum from cellular components. The serum was stored at -20°C prior to analysis. Glucose (Glu) concentrations were estimated according to a method described by Asatoor and King[23]. Total Protein (TP) concentrations were estimated according to the biuret method[24] and the albumin concentration was estimated using a method described by Henry *et al.*[25]. Globulin levels were estimated based on a formula that involved the TP value and albumin levels[26]. Alkaline phosphatase (ALP) activity was determined using a method by Kind and King[27], whereas aspartate transaminase (AST) and alanine transaminase (ALT) levels were estimated according to Reitman and Frankel[28]. Blood serum tests were performed using kits produced by Biomaghreb.

All statistical analyses were performed using the Statistical Package for Social Science (SPSS)[29] version 21.0 for windows (SPSS Inc., Chicago, IL, USA) according to a Complete Randomized Design. Means were compared by Duncan's Multiple Range Test[30] with a significance level of 5%.

RESULTS

The addition of threonine at 600 and 900 mg kg^{-1} (Th_{600}, Th_{900}) resulted in significant increases ($p<0.01$) in BW of 14 day-old birds compared to birds that received 0 (Th_0) or 300 mg kg^{-1} threonine (Th_{300}) and this difference persisted through the end of the third week (21 days) (Table 2). By week four (28 days), the BW of birds receiving Th_{600} and Th_{900} differed significantly from those of Th_0 and Th_{300}, whereas birds in the Th_{900} group had higher BW than birds in the Th_{600} group (Table 2). By the end of the experimental period (35 days), Th_{900} birds showed a significant increase ($p<0.05$) in BW compared to birds receiving the other three treatments (Table 2).

During the first 7 days, there were no significant differences in Weight Gain (WG) among the threonine treatments (Th_{300}, Th_{600} and Th_{900}) compared with the control treatment (Table 3). However, during the days 8-14, diet supplemented with threonine significantly increased WG of birds of Th_{600} and Th_{900} groups compared to birds fed diet without threonine (Table 3). For the period between days 22-28 there was no significant difference between the Th_0 and Th_{300} groups but significant increase in weight ($p<0.01$) was

Table 2: Effect of threonine supplementation on broiler live body weight (g $bird^{-1}$) (Mean±SE)

	Live body weight (g)				
	Treatments				
Age (days)	Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
07	171.58±3.18	175.88±3.55	177.13±2.98	178.75±3.68	NS
14	429.83±12.45^{B}	423.38±7.66^{B}	466.25±11.37^{A}	467.50±9.91^{A}	0.05
21	972.50±7.61^{AB}	942.00±18.20^{B}	995.75±27.12^{A}	1012.25±10.45^{A}	0.05
28	1531.58±11.07^{C}	1549.00±36.22^{C}	1645.25±25.01^{B}	1717.75±22.02^{A}	0.01
35	2173.42±11.47^{B}	2191.88±35.15^{B}	2166.75±49.33^{B}	2290.38±31.28^{A}	0.05

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, $^{A-C}$Means within a row lacking a common superscript differ significantly

Table 3: Effect of threonine supplementation on body weight gain of broiler chickens (g $bird^{-1}$) (Mean±SE)

	Body weight gain (g $bird^{-1}$)				
	Treatments				
Period (days)	Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
01-07	131.00±3.180	135.30±3.550	136.55±2.980	138.17±3.68	NS
08-14	258.25±12.15^{AB}	247.50±10.71^{B}	289.13±12.66^{A}	288.75±6.33^{A}	0.05
15-21	542.67±9.170	518.63±15.11	529.50±15.85	544.75±15.99	NS
22-28	559.08±14.43^{C}	607.00±26.51^{BC}	649.50±20.09^{AB}	705.50±29.92^{A}	0.01
29-35	641.83±16.55	642.88±43.80	521.50±70.69	572.63±29.87	NS
01-35	2132.84±11.47^{B}	2151.30±35.15^{B}	2126.17±49.33^{B}	2249.80±31.28^{A}	0.05

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, $^{A-C}$Means within a row lacking a common superscript differ significantly

Table 4: Effect of threonine supplementation on broiler feed intake (g/bird/week) (Mean±SE)

	Feed intake (FI, g/bird/week)				
	Treatments				
Periods (days)	Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
01-07	132.41±1.210	137.00±3.54	141.94±6.15	140.88±6.16	NS
08-14	385.38±11.16	380.63±3.56	412.69±5.31	412.19±15.03	NS
15-01	686.80±9.200	676.56±15.91	710.25±18.22	681.94±7.330	NS
22-28	1033.00±23.66^{B}	1079.38±23.17^{AB}	1149.94±20.57^{A}	1102.38±38.49^{AB}	0.05
29-35	1189.70±11.93^{A}	1108.44±22.84^{B}	1157.57±33.68^{AB}	1187.13±21.41^{A}	0.05
01-35	3427.28±23.11^{BC}	3382.00±48.56^{C}	3572.38±36.91^{A}	3524.51±34.85^{AB}	0.01

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, $^{A-C}$Means within a row lacking a common superscript differ significantly

Table 5: Effect of threonine supplementation on feed conversion ratio of broiler chickens (g feed/g gain) (Mean±SE)

	Feed conversion ratio (g feed/g gain)				
	Treatments				
Period (days)	Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
01-07	1.01±0.03	1.01±0.02	1.04±0.07	1.02±0.05	NS
08-14	1.51±0.07	1.55±0.06	1.44±0.08	1.43±0.05	NS
15-21	1.27±0.03	1.31±0.03	1.34±0.02	1.25±0.04	NS
22-28	1.85±0.02^{A}	1.79±0.10^{A}	1.77±0.05^{A}	1.57±0.08^{B}	0.05
29-35	1.86±0.06^{AB}	1.75±0.12^{B}	2.35±0.33^{A}	2.09±0.12^{AB}	0.05
01-35	1.61±0.02^{AB}	1.57±0.04^{B}	1.68±0.04^{A}	1.57±0.03^{B}	0.05

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, ^{A,B}Means within a row lacking a common superscript differ significantly

Table 6: Effect of threonine supplementation on carcass weight, dressing percentage and relative weight of heart, liver and gizzard (%) of broiler chickens (Mean±SE)

	Treatments				
Variables	Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
Live body weight (g)	2160.12±58.50	2145.00±60.19	2160.00±65.60	2169.07±78.85	NS
Carcass weight (g)	1599.12±56.50	1605.12±46.00	1628.45±53.54	1657.71±61.78	NS
Dressing percentage	73.95±0.78^{B}	74.83±0.33^{AB}	75.37±0.65^{AB}	76.42±0.63^{A}	0.05
Heart (%)	0.52±0.02^{A}	0.52±0.01^{A}	0.53±0.01^{A}	0.44±0.01^{B}	0.01
Liver (g)	2.43±0.08	2.31±0.05	2.35±0.03	2.09±0.08	NS
Gizzard (g)	1.46±0.05	1.39±0.03	1.48±0.09	1.51±0.08	NS

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, ^{A,B}Means within a row lacking a common superscript differ significantly

observed in Th_{600} and Th_{900} birds compared to Th_0 birds. The Th_{900} group had the highest WG across the entire experimental period (1-35 days) compared to the other three groups (Table 3).

Meanwhile, during the days 22-28, Feed Intake (FI) was increased significant ($p<0.05$) in the Th_{600} compared to Th_0 groups but there was no significant difference between Th_{300} and Th_{900} treatment groups (Table 4). There were also no significant differences between treatments Th_{600}, Th_{900} and Th_0 during the days 29-35 but there was a significant decrease ($p<0.05$) in FI for birds in the Th_{300} group compared with those in the Th_0 and Th_{900} groups. Over the entire experimental period (1-35 days), the FI was significantly higher in the Th_{600} group ($p<0.01$) compared to the Th_0 and Th_{300} groups.

The Th_{900} group had the lowest feed conversion ratio (FC) compared to the Th_0, Th_{300} and Th_{600} groups between the days 22-28 ($p<0.05$, Table 5). Between days 29-35, there were no significant differences in FC for any of the threonine treatment groups compared to Th_0, although FC decreased significantly in the Th_{300} group ($p<0.05$) compared to that of the Th_{600} group. With respect to the overall experimental period (1-35 days), there were no significant differences between Th_{300}, Th_{600}, Th_{900} and Th_0 in FC, although FC was significantly decreased ($p<0.05$) in the Th_{300} and Th_{900} groups compared to the Th_{600} group (Table 5).

Threonine supplementation did not affect the BW or Carcass Weight (CW) as well as the relative weights of the liver (LP) and gizzard (GP) across the treatment groups (Table 6).

Table 7: Effect of threonine supplementation on the relative weight of breast, thigh, back, wings and neck (%) in broiler chickens (Mean±SE)

Relative weight (%)	Treatments Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
Breast	35.66±0.05^B	36.37±0.23^A	36.66±0.13^A	36.49±0.59^A	0.05
Thigh	28.79±0.15	28.98±0.81	28.89±0.84	28.46±0.68	NS
Back	18.65±0.23	19.24±0.60	19.06±0.58	19.64±0.41	NS
Wings	9.74±0.08	9.41±0.27	9.08±0.22	9.14±0.11	NS
Neck	6.55±0.07^A	5.55±0.12^B	5.87±0.19^B	5.86±0.26^B	0.01

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, ^{A,B}Means within a row lacking a common superscript differ significantly

Table 8: Effect of threonine supplementation on blood values for broiler chickens (Mean±SE)

Variables	Age (days)	Treatments Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
Red blood cells	21	3.17±0.11	3.37±0.10	3.11±0.12	3.41±0.10	NS
(RBC, n×10^6 cells μL^{-1})	35	3.09±0.08^B	3.22±0.11^{AB}	3.36±0.16^{AB}	3.54±0.10^A	0.05
	Average	3.13±0.09^B	3.29±0.09^{AB}	3.24±0.12^{AB}	3.48±0.07^A	0.05
Hematocrit (PCV, %)	21	37.25±1.08	34.61±1.39	35.59±0.99	37.77±1.08	NS
	35	33.29±1.08	33.74±1.33	35.96±0.93	36.35±1.51	NS
	Average	35.27±0.84^{AB}	34.18±0.44^B	35.77±0.79^{AB}	37.06±1.06^A	0.05
Hemoglobin (Hb, g dL^{-1})	21	12.25±0.35	11.37±0.51	11.53±0.36	11.74±0.24	NS
	35	11.96±0.46	11.88±0.45	12.44±0.30	11.52±0.50	NS
	Average	12.10±0.31	11.62±0.21	11.99±0.31	11.63±0.35	NS
White blood cells	21	20.60±0.41^{AB}	20.21±0.59^{AB}	20.95±0.52^A	19.29±0.50^B	0.05
(WBC, n×10^3 cells μL^{-1})	35	20.29±0.53	21.09±0.86	20.91±0.54	20.40±0.50	NS
	Average	20.45±0.42	20.65±0.68	20.93±0.46	19.85±0.41	NS
H/L ratio*	21	0.36±0.02	0.34±0.03	0.31±0.03	0.29±0.03	NS
	35	0.38±0.02^A	0.35±0.03^{AB}	0.36±0.02^A	0.29±0.02^B	0.05
	Average	0.37±0.02^A	0.35±0.03^{AB}	0.34±0.02^{AB}	0.29±0.02^B	0.05

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, ^{A,B}Means within a row lacking a common superscript differ significantly, *Heterophil/lymphocyte ratio

However, the DP was significantly increased in birds from the Th_{900} group ($p<0.05$) compared to the Th_0 group. This change was accompanied by a significant decrease ($p<0.05$) in the relative weight of the heart (HP) compared to the Th_0, Th_{300} and Th_{600} groups (Table 6).

Threonine supplementation significantly improved the relative breast weight (BRp) in the Th_{300}, Th_{600} and Th_{900} groups compared to the Th_0 group (Table 7). On the other hand, the Th_{300}, Th_{600} and Th_{900} treatments had a significant decrease ($p<0.01$) in the neck weight (Np) compared to the control group. There were no significant differences among the threonine supplemented groups in weights of the wing (Wp), back (Bap) and thigh (THp) (Table 7).

Analysis of blood samples showed a significant increase ($p<0.05$) in the average number of RBCs on day 35 for the Th_{900} group compared to the other groups (Table 8). There were no differences in PCV% among the different treatment groups compared to the control on days 21 and 35 but the Th_{900} group showed significant increase ($p<0.05$) compare to the Th_{300} group in terms of the averages of the blood parameters assessed.

Despite changes in RBCs, threonine supplementation had no effect on Hb levels on the days assessed (Table 8).

Threonine treatments (Th_{300}, Th_{600} and Th_{900}) also did not significantly affect WBC levels. The Th_{900} group showed a significant decrease ($p<0.05$) in WBCs compared to the Th_{600} group on day 21 but there were no significant differences between Th_0 and the experimental treatment groups (Th_{300}, Th_{600} and Th_{900}) on day 35 or on average (Table 8).

The Th_{900} group showed a significant decrease ($p<0.05$) in the heterophil/lymphocyte (H/L) ratio, which is a measure of long-term stress in chickens, on day 35 and on average compared to the control group. However, the H/L ratio was not significantly different among the three treatment groups.

There were no significant differences among the threonine treatment groups for serum glucose (Glu) on day 21, day 35 or on average compared with the control group (Table 9).

The Th_{300}, Th_{600} and Th_{900} groups had higher total serum protein (TP) ($p<0.05$) compared with the Th_0 group on day 21 and on average, although values on day 35 were similar, with the exception of the Th_{900} group ($p<0.05$).

Table 9: Effect of threonine supplementation on blood glucose, protein, calcium and phosphorous levels in broiler chickens (Mean±SE)

		Treatments				
Adjectives	Age (days)	Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
Glucose (mg dL^{-1})	21	277.21±21.33	280.49±15.74	269.26±11.18	278.49±13.14	NS
	35	311.01±24.04	314.94±22.43	289.16±15.17	298.41±15.51	NS
	Average	294.11±22.19	297.72±18.00	279.21±12.62	288.45±13.68	NS
Total protein (g dL^{-1})	21	3.73±0.27^{B}	4.39±0.12^{A}	4.53±0.08^{A}	4.85±0.13^{A}	0.01
	35	4.21±0.31^{B}	4.68±0.22^{AB}	4.59±0.19^{AB}	4.98±0.17^{A}	0.05
	Average	3.97±0.22^{B}	4.53±0.11^{A}	4.56±0.10^{A}	4.91±0.14^{A}	0.01
Calcium (mg dL^{-1})	21	9.36±0.41^{B}	9.45±0.73^{B}	10.13±0.75^{AB}	12.16±0.89^{A}	0.05
	35	8.39±0.16^{B}	11.50±1.09^{A}	9.74±0.65^{AB}	11.77±1.02^{A}	0.05
	Average	8.87±0.22^{B}	10.47±0.82^{AB}	9.94±0.69^{AB}	11.96±0.81^{A}	0.05
Phosphorus (mg dL^{-1})	21	8.27±0.14	8.61±0.46	9.19±0.64	9.99±1.01	NS
	35	7.76±0.57^{B}	7.92±0.47^{B}	7.54±0.48^{B}	9.94±1.06^{A}	0.05
	Average	8.01±0.33^{B}	8.26±0.35^{AB}	8.37±0.42^{AB}	9.97±1.01^{A}	0.05

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and mg kg^{-1}, respectively. NS: Non-significant, ^{A,B}Means within a row lacking a common superscript differ significantly

Table 10: Effect of threonine supplementation on liver enzyme activity in blood serum from broiler chickens (Mean±SE)

		Treatments				
Enzymes	Age (days)	Th_0	Th_{300}	Th_{600}	Th_{900}	p-values
ALP (King Armstrong unit)	21	115.26±9.91^{B}	146.12±15.42^{AB}	176.37±20.48^{A}	185.79±21.11^{A}	0.05
	35	208.38±33.75	190.51±25.04	245.04±27.44	273.77±24.81	NS
	Average	161.82±21.65^{B}	168.32±19.58^{AB}	210.71±22.95^{AB}	229.78±21.58^{A}	0.05
AST (IU L^{-1})	21	141.15±3.08	139.15±2.85	138.15±2.67	139.91±3.40	NS
	35	166.76±3.58	164.32±3.31	162.62±3.12	163.81±4.12	NS
	Average	153.96±3.33	151.74±3.08	150.39±2.89	151.86±3.73	NS
ALT (IU L^{-1})	21	3.82±0.19^{A}	3.33±0.10^{AB}	3.69±0.17^{B}	3.90±0.09^{A}	0.05
	35	4.48±0.22^{A}	3.90±0.11^{B}	4.40±0.16^{A}	4.53±0.11^{A}	0.05
	Average	4.15±0.21^{A}	3.61±0.11^{B}	4.05±0.16^{A}	4.22±0.10^{A}	0.05

Th_0, Th_{300}, Th_{600} and Th_{900}: Threonine supplementation to 0, 300, 600 and 900 mg kg^{-1}, respectively. NS: Non-significant, ^{A,B}Means within a row lacking a common superscript differ significantly

There was also a significant increase ($p<0.05$) in serum calcium (Ca) for the Th_{900} group compared with the control on days 21 and 35. The Th_{300} group had significantly improved Ca levels ($p<0.05$) compared to the Th_0 group on day 35 but on day 21 and on average, the Th_{600} and Th_0 groups were similar (Table 9). In addition, the Th_{300}, Th_{600} and Th_{900} groups all had significantly higher ($p<0.05$) phosphorous (P) levels compared to the Th_0 group on day 35.

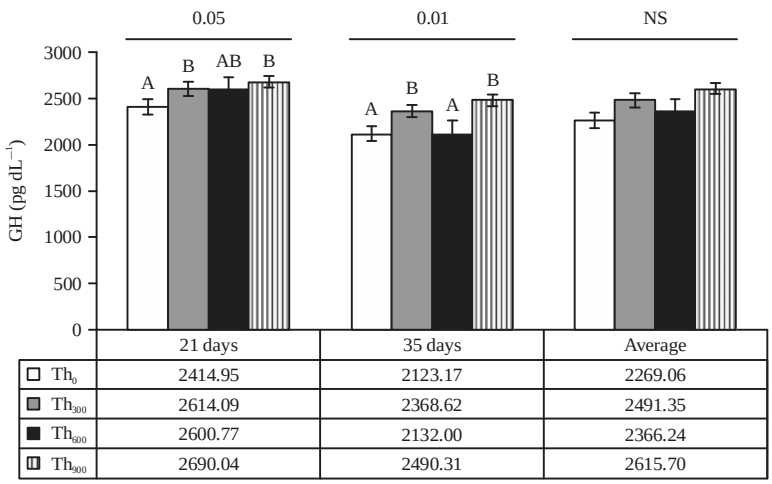
Broiler chickens in the Th_{600} and Th_{900} groups showed significant increases ($p<0.05$) in ALP activity at day 35 but there were no significant differences ($p<0.05$) between the Th_{300} and Th_0 groups. However, the Th_{900} group showed significant increase in ALP activity ($p<0.05$) (Table 10). In contrast, there were no significant differences in AST activity among the four groups (Table 10).

For ALT activity, the Th_{600} group showed a significant decrease ($p<0.05$) compared to the Th_0 and Th_{900} groups but no significant differences were observed in Th_{300} group on day 21, whereas the value of ALT in Th_{900} group was higher than the other groups (Table 10). On day 35 and for the overall average, the Th_{300} group had a significant decrease ($p<0.05$) in ALT levels compared to the Th_0, Th_{600} and Th_{900} groups.

Threonine supplementation did not affect Growth Hormone (GH) levels on day 21 (Fig. 1). Moreover, the Th_{300} and Th_{900} groups showed significant improvements ($p<0.01$) in GH levels on day 35 compared to the Th_0 and Th_{600} groups. In addition, the Th_{900} group had significantly higher average GH levels ($p<0.05$) compared to the Th_0 and Th_{600} groups but there were no significant differences between the Th_{300} and Th_0 groups (Fig. 1).

DISCUSSION

In this study, supplementation of broiler chicken diets with 900 mg kg^{-1} threonine produced significant improvements in BW and WG (Table 2 and 3), which underscores the important role of this amino acid in promoting growth and muscularity[31]. Earlier studies showed that increasing the amount of dietary threonine increases nitrogen retention and reduces nitrogen loss in chickens[32,33]. Mucous membranes line the majority of the digestive tract and mucous membrane function is dependent on threonine for modulating the speed at which food passes through the digestive tract for effective nutrient absorption[34]. Moreover,



	21 days	35 days	Average
Th_0	2414.95	2123.17	2269.06
Th_{300}	2614.09	2368.62	2491.35
Th_{600}	2600.77	2132.00	2366.24
Th_{900}	2690.04	2490.31	2615.70

Fig. 1: Effect of threonine supplementation on growth hormone concentration in blood from broiler chickens (Mean±SE)
Growth hormone values in blood samples were measured on 21 and 35 days and the average was taken across the experiment period

threonine also maintain adequate secretion of digestive enzymes. Rezaeipour *et al.*[35] demonstrated that increasing the amount of dietary threonine improved morphological traits of the small intestine in broiler chickens and these traits are reflected in an increased growth rate, whereas chickens fed diets lacking threonine had reduced nutrient absorption in the digestive tract.

Increased rates of nutrient absorption in the small intestine lead to increases in the availability of amino acids that can be used for protein synthesis, which is reflected in the increased levels of total protein (TP, Table 9) and activity of liver enzymes involved in metabolism[36]. These effects were associated with increased DP and relative breast weight and are reflected in the Th_{900} group (Table 6, 7).

The improvement in performance of birds fed the Th_{900} treatment highlight the role of threonine in increasing thyroid gland activity, including stimulation of hormone production. Azzam and El-Gogary[37] and Wu[38] reported that increasing the amount of dietary threonine led to increases in the concentrations of thyroxine (T_4) and triiodothyronine (T_3) hormones in chickens, which was likely due to the presence of threonine receptors in the thyroid gland important for the synthesis of T_3. Thyroxine T_4 is important for energy and protein metabolism[39,40] and induces increased concentrations of the IGF-I hormone[41]. Appropriate amounts of T_4 are needed for protein synthesis and maintenance of muscular mass as well as for enhanced function of GH[42-44]. The results of this study indicate that supplementation of diets with 600 or 900 mg kg^{-1} threonine increased GH levels (Fig. 1).

In addition to the pivotal roles of GH in muscularity and stimulation of T_3, T_4 and hormone IGF-I production[36], GH also has roles in amino acid metabolism and protein synthesis that increase protein production[45]. Threonine supplementation may have improved feed palatability and this improvement is reflected by the increased FI for the Th_{600} and Th_{900} treatments (Table 4). The increased FI may also be related to hormonal control, since increased metabolic rates are associated with increased T_4 concentrations. Moreover, GH levels can also affect the relationship between T_4 and FI[36,45].

The function of threonine in improving muscle growth and mass[32] was evidenced by the significant improvement in DP (Table 6) and BRp (Table 7), which was likely manifested through increased absorption of digestive enzymes in the small intestine as well as increased availability of amino acids and rate of absorption of feed through the small intestine. This increase was associated with the increased concentration of protein in the blood (Table 9), T_4[37] and GH (Fig. 1). Changes in these parameters are reflected in the increased body weight (Table 2) as well as DP (Table 6).

The improvement in productivity (Table 2, 3) and carcass traits (Table 6, 7) were associated with improvements in several physiological parameters, including RBCs and Hb (Table 8). The positive relationship of RBCs with BW and WG is reflected in increased metabolic rates manifested by T_4 and GH levels[43,46]. T_4 levels are also affected by erythropoietin, which increases productivity rates[47] and simulates both RBCs and estrogen production[48,49].

The significant decrease in the H/L ratio observed in this study is a strong indicator of increased amino acid levels[50], whereas increases in the number of heterophils is an important indicator of immune system function[51]. Threonine increases globulin levels in the blood by increasing lymphocyte ratios[14]. The significant improvement in TP of birds that received threonine treatments (Table 9) is indicated

by increased metabolic rates, especially if it is accompanied by body weight increases (Table 2) and high concentrations of Ca and P (Table 9). Threonine supplementation also increased ALP activity (Table 10), which is associated with an increased metabolic rate and muscle growth as well as increased GH levels (Fig. 1)[49]. Therefore, increased metabolic rates are reflected in increased ALP activity in the liver[52].

The results of the present study reveal that diet supplemented with threonine can increase GH levels in the blood of the birds (Fig. 1). The GH mediates body and muscle growth through a variety of pathways, including stimulation of T_4 and IGF-I production[37].

CONCLUSION AND FUTURE RECOMMENDATION

Increased in GH levels improved growth and productive performance of broiler chickens. Use of threonine in broiler diets at a level of 900 mg kg^{-1} is recommended. Future studies should explore the effects of threonine supplementation on the expression of growth hormone genes.

SIGNIFICANCE STATEMENTS

This study found that threonine supplementation improves broiler production by increasing metabolic rates as well as total protein, calcium and phosphorus levels. It has been shown that threonine stimulates the pituitary gland to produce growth hormone, which contributes to an increase in thyroid activity and hormone production, resulting in improved broiler production. Threonine contributes to the maintenance of physiologic homeostasis, which is underscored by an improved H/L ratio. Increasing broiler production is important for improving the current economic situation in Iraq and developing countries as well as reducing the reliance on imported meat and eggs.

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