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Effect of Excess Dietary Tryptophan on Performance, Plasma Hormone Levels and Immune Function of Broiler Chickens Reared Under Hot and Humid Summer Conditions

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Abstract

The effects of L-Tryptophan supplementation on growth performance, hormone levels, and immune function of heat-stressed broiler chickens were investigated. For 42 days, 480 male chicks were exposed to repeated cycles of hot temperatures and fed one of four diets: a basic diet (control group) or the same diet with extra L-Tryptophan added at three different levels (0.2, 0.4, and 0.6 g/kg diet). L-tryptophan supplementation did not significantly improve overall growth performance under heat stress. However, broilers fed 0.2 and 0.6 g/kg L-Tryptophan had a better feed conversion ratio at 10 days old. There were no significant differences in white blood cell counts. Interestingly, L-tryptophan supplementation increased antibody production against Influenza and Newcastle disease viruses at 14 days. Additionally, L-tryptophan influenced hormone levels, slightly decreasing growth hormone and increasing thyroxin at 10 days, with further increases in triiodothyronine observed at 24 and 42 days. While L-tryptophan supplementation did not enhance overall growth performance under heat stress, it positively impacted blood parameters like thyroxin, triiodothyronine, and immune response against specific viruses.

Introduction

Countries and cities located in humid subtropical regions, like the western part of Iran, experience extremely hot and humid summers with daily temperature averages above 30°C and 75% relative humidity. The poultry industry faces a major challenge in the form of heat stress, which is often underestimated and poses a threat to the health, growth, and productivity of broilers. The impact of climate change on livestock is becoming more evident, particularly in continuously unsuitable environments, where chickens suffer from reduced growth rates, impaired feed efficiency, and higher mortality, resulting in economic losses (Azad *et al.*, 2010; Loyau *et al.*, 2015; Fouad *et al.*, 2021). Studies have shown that birds facing thermal stress spend less time feeding and more time drinking, panting, and flapping their wings. They also tend to rest more and move or walk less to dissipate heat (Lara and Rostagno, 2013). Huang *et al.* (2016) demonstrated that the performance and meat quality of broilers are negatively affected by high environmental temperatures. Recently, it was reported that birds exposed to high ambient temperatures experience a decrease in protein and amino acid digestibility (Habashy *et al.*, 2017; Qaid and Al-Garadi, 2021). Although studies on these effects in birds are still relatively limited, it has been found that high temperatures affect the metabolism of various amino acids, including methionine (Met), lysine (Lys), arginine (Arg), and tryptophan (Trp)(Balnave and Oliva, 1991; Brake., 1998). In other studies, the administration of various amino acids has been found to reduce the psychological effects of stress in chicks (Erwan *et al.*, 2012; Fouad *et al.*, 2021). Trp is a necessary amino acid for chickens which contributes to various metabolic functions when provided in sufficient amounts. Recent research has shown that Trp has a calming effect as it affects serotonin (5-hydroxytryptamine) levels in the brain (Emadi *et al.*, 2010). Serotonin is a neurotransmitter that helps regulate mood, stress response, and social adaptability. Martin *et al.* (2000) have reported that Trp acts as a precursor of serotonin, indicating that it is essential for the production of this crucial neurotransmitter. In addition, Yahav and McMurtry

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(2001) have found that high ambient temperatures lead to metabolic changes and reduced serum concentrations of triiodothyronine (T3) and thyroxine (T4), which are important growth promoters in animals, and suppressed immune system function. Furthermore, Gershoff *et al.* (1968) have stated that a deficiency of Trp decreases antibody production in rats. Despite this, there is still a lack of information regarding the effects of excess Trp intake on the performance of broilers and immune function during hot weather. To address this issue, an experiment was conducted to investigate whether excessive Trp intake could enhance growth performance, immune function, and blood hormone levels under heat-stress conditions.

Materials and methods

Birds and housing

This study adhered to the animal welfare guidelines set forth by the ethics committee at Razi University (Kermanshah, Iran). Four hundred and eighty male broiler chicks (Ross 308) with an average body weight of 41 ± 0.35 g were bought from a commercial hatchery. After arriving at the house, they were randomly assigned to one of four treatments, with six replicates of 20 chickens per each. The chicks were reared in metal batteries for 42 days with four compartments each (dimensions: 127 x 87 x 45 cm). These cages were situated in open-sided housing, and environmental conditions within the housing mimicked natural summer conditions, with no controls implemented. As a result, temperature and humidity fluctuated throughout the day. Due to consistently high housing temperatures exceeding 31°C, artificial brooding was not employed. Ambient temperature and relative humidity were monitored within the poultry house using nine thermometers-hygrometers positioned at bird head level. These parameters were measured at least six times daily throughout the experiment, with temperature readings fluctuating between 26°C and 36°C (averaging 31°C) and relative humidity levels ranging from 77% to 88% (averaging 79%). A constant ventilation rate of 0.12 meters per second was maintained throughout the experiment. Light with an intensity of approximately 20 lux was continuously provided. The chicks were granted unrestricted access to both feed and water for the entire study duration. Vaccination was carried out in accordance with the established regional vaccination program, with timing optimized based on maternal antibody levels. Notably, no medications were administered during the experiment.

Diets

A basal diet formulated from corn and soybean meal was provided to the broilers. A single source provided the corn and soybean meal used in all four diets. The broilers were assigned to four treatment groups through a process of random allocation. Three varying dietary levels of L-Trp (Evonik Degussa GmbH, Hanau-Wolfgang, Germany) were incorporated into the control diet at 0.2, 0.4, and 0.6 g/kg, respectively, across three age groups (starter, grower, and finisher). Analysis of the corn and soybean meal used in formulating the experimental diets included DM, CP, and amino acids content, performed using near-infrared spectroscopy (Paya Amin Mehr, Tehran, Iran). The metabolizable energy content of the corn and soybean meal was estimated using regression models established by the NRC (NRC, 1994). The basal diet was formulated to meet the nutrient requirements for broiler chickens, as recommended by the Ross.

308 broiler management guide (Aviagen, 2012). Details regarding the ingredients and chemical composition of the basal diet can be found in Table 1.

Measurements

The weight of the broilers was measured at the beginning and end of every phase (10, 24, and 42 days). The feed intake (FI) and feed conversion ratio (FCR) were measured at the pen level using data obtained from feed residual and body weight gain (BWG) at the end of each phase (10, 24, 42 days) and for the entire experimental period (0–42 days). Every day, the mortality rate was monitored, and the number of birds was adjusted for subsequent analyses of FI, FCR and average daily gain (ADG). The performance index (PI) of each experimental group was estimated using the following formula: PI = Live weight (g) / Feed conversion ratio (g:g) ×100, from 0 to 42 days (Bird, 1955).

Blood leucocyte profiles

Blood samples were collected from eight birds per dietary treatment at 42 days old. EDTA-anticoagulant vials were used to collect 0.3 ml of blood from the bronchial wing vein. Following collection, samples were centrifuged for 15 minutes at 1008 g. The resulting plasma was then preserved at -20°C until analysis of the desired parameters. An optical microscope was utilized to count one hundred leucocytes per sample and separate heterophils from lymphocytes, as specified in the protocol outlined by Lucas (1961), to determine the blood leucocyte profiles. The heterophil-to-lymphocyte ratio was then computed.

Table 1. Ingredients and nutritional composition of the basal diets

Age, d	Starter	Grower	Finisher
	0-11	12-24	25-42
Ingredients, g/kg			
Corn 7.57 g of CP/kg	56.617	62.979	68.562
Soybean meal, 430.47 g of CP/kg	37.614	31.136	25.981
Soybean oil	0.513	1.190	0.960
Limestone (Caco3)	1.063	0.861	0.855
Dicalcium phosphate	2.579	2.298	2.137
Common salt	0.230	0.250	0.188
NaHco3 (Na. Bicarbonate)	0.159	0.136	0.227
Mineral premix [1]	0.250	0.250	0.250
Vitamin premix [2]	0.250	0.250	0.250
DL-Methionine (990 g/kg Met)	0.336	0.292	0.255
L -Lysine HCl (788 g/kg Lys)	0.257	0.233	0.219
L -Threonine (990 g/kg Thr)	0.072	0.065	0.056
Filler[3]	0.060	0.060	0.060
Nutrient composition (as fed basis)			
ME (Kcal/kg)[4]	2,874	2,993	3,040
Crude protein (%)	22,70	20,60	18,00
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42
Sodium (%)	0.16	0.16	0.16
Crude fiber (%)	3.998	3.133	3.046
DEB(meg/kg)[5]	255.725	210.863	200.000
Lys Total (Digestible Lys) %[6]	1.43 (1.27)	1.24 (1.10)	1.07(0.97)
MET Total (Digestible Met) %	0.63 (0.51)	0.56 (0.45)	0.53(0.51)
M+C Total (Digestible M+C) %	1.07 (0.94)	0.95 (0.84)	0.83(0.76)
THR Total (Digestible Thr) %	0.94 (0.83)	0.83 (0.73)	0.73(0.65)
TRP Total (Digestible Trp) %	0.23 (0.20)	0.20 (0.18)	0.21(0.17)
Arg Total (Digestible Arg) %	1.45 (1.38)	1.17 (1.15)	1.13(1.03)
Iso Total (Digestible Iso) %	0.97 (0.88)	0.76 (0.75)	0.74(0.67)
Leu Total (Digestible Leu) %	1.84 (1.69)	1.56 (1.54)	1.55(1.44)
Val Total (Digestible Val) %	1.09 (0.95)	0.96 (0.84)	0.84(0.75)

[1]Mineral premix provided per kilogram of diet: Mn (from MnSO 4 ·H 2 O), 65 mg; Zn (from ZnO), 55 mg; Fe (from FeSO 4 ·7H 2 O), 50 mg; Cu (from CuSO 4 ·5H 2 O), 8 mg; I [from Ca (IO 3) 2 ·H 2 O], 1.8 mg; Se, 0.30 mg; Co (from Co 2 O 3), 0.20 mg; Mo, 0.16 mg.

[2]Vitamin premix provided per kilogram of diet: vitamin A (from vitamin A acetate), 11,500 IU; cholecalciferol, 2,100 IU; vitamin E (from dl-α-tocopheryl acetate), 22 IU; vitamin B 12, 0.60 mg; riboflavin, 4.4 mg; nicotinamide, 40 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidine), 1.50 mg; folic acid, 0.80.

[3] Supplemental L-Trp was added at the expense of a filler (sand), to increase dietary Trp from 200 to 600 g/kg of diet.

[4]ME= Metabolizable energy

[5]DEB= represents dietary electrolyte balance and is defined as Na + K – Cl.

[6]Digestibility coefficients of the ingredients were obtained from *Evonik Degussa* GmbH, Hanau, Germany.

Assay for antibody response

Chicks were vaccinated for avian influenza (Nobilis Infuenza TRT; Merck Animal Health) and Newcastle disease (Nobilis G + ND; Merck Animal Health) at 7 days of age. Blood samples were then collected from the brachial veins at 14, 21, and 42 days post-immunization. Sera from these broiler chickens were subsequently analyzed to measure NDV-specific antibodies using both the hemagglutination inhibition (HI) test and enzyme-linked immunosorbent assay (ELIZA) (Marquardt *et al.*, 1985).

Chemical analysis of blood components

At the end of the experiment (42 days), blood was collected from the wing vein using a 25-gauge needle. Following blood collection, three samples were separated for subsequent analysis of various serum and plasma chemical constituents. Plasma T3, T4, and growth hormone (GH) measurements were designated for a single sample, which was then placed in heparinized tubes. Centrifugation at 3,000 rpm (1,008 g) for 15 minutes separated the plasma and stored at -20°C until analysis. Careful handling of the birds ensured blood collection within 2 minutes to minimize stress-induced hormone level alterations. Measurement of plasma T3, T4, and GH concentrations employed double antibody RIA with commercially available kits (China Institute of Atomic Energy, Beijing, China) following the methods outlined by Darras *et al.* (1992).

Statistical analyses

All data from the trials were analyzed using a completely randomized design implemented in the

Statistical Analysis System (SAS, 2015). Mean values are presented along with their corresponding pooled standard errors of the mean (*SEM*). Treatment means were compared using Duncan's multiple range test (Duncan, 1955) to identify significant differences. The LSMEANS option within SAS was employed to determine significance levels ($P < 0.05$). The following linear model was used to analyze the data: $Y_{ij} = \mu + \alpha_i + e_{ij}$, where Y_{ij} represents the measured characteristic, μ represents the overall mean, α_i represents the effect of experimental diet, and e_{ij} represents the random error term.

Results and Discussion

Growth performance

Table 2 displays the overall performance of broiler chickens, which were given varying levels of Trp while being raised under heat stress conditions. Despite all groups exhibiting satisfactory performance, their productivity was not up to the standard performance set by Aviagen for Ross 308 broilers (Aviagen, 2012). Our research indicates that the high temperature and humidity experienced in experimental housing had a detrimental effect on the growth performance of broiler chickens raised in tropical conditions. This observation is consistent with previous studies on heat-stressed broilers, as reported in Fouad *et al.* (2021). Broiler chickens are homeotherms and can only thrive in a narrow range of thermo-neutrality. Aviagen (2012) suggests that the most suitable temperature for the effective production of broiler chickens is 20°C. Based on research conducted by Austic (1985), it is believed that every 10 °C rise in ambient temperature above 20 °C leads to a 17% decrease in FI. The levels of Trp in the diet of broilers during heat stress conditions did not significantly impact FI, ADG, body weight and PI ($P > 0.05$) in our experiments. However, birds fed with a diet of 0.4 g/Kg Trp (1.06) had a significantly higher FCR at the age of 10 days ($P < 0.05$) compared to birds fed with diets of 0.2 g/Kg Trp (1.03), 0.6 g/Kg Trp (1.03), and control (1.02) ($P < 0.05$). At the age of 24 and 42 days, the FCR was not significantly affected by the experimental treatments ($P > 0.05$). Despite some studies reporting that Trp supplementation in broilers during stress conditions can increase FI and BWG (Harms and Russell, 2000; Corzo *et al.,* 2005; Yue *et al.,* 2017; Badakhshan *et al.,* 2021), we were unable to observe a positive effect of Trp supplementation on the performance of broilers. Fouad *et al.* (2021) propose that Trp can serve as an effective anti-stress additive in poultry feed. Bello *et al.* (2018) discovered that Trp can increase FI, but it does not significantly affect the growth performance of broilers that are under transportation stress. In contrast, some previous studies have shown that excessive dietary Trp and intraperitoneal administration of Trp can reduce FI in chickens (Pinchasov *et al.,* 1989; Rosebrough, 1996). The present research indicates that providing a diet with excessive Trp does not help alleviate heat stress in broiler chickens. This lack of benefit could be due to the inadequate dosage, duration of heat stress, or genetic factors of the birds (Geraert *et al.,* 1996). Furthermore, changes in the utilization of metabolic nutrients and reduced nutrient digestibility in birds during high ambient temperatures could be another reason for the lack of efficacy of Trp (Sahin *et al.,* 2002).

Table 2. Productive performance of broiler chickens fed on different dietary treatments.

Dietary treatments	L-tryptophan supplements, g/kg				*SEM*	P value
	0.0	0.2	0.4	0.6		
ADG, g						
0-10 d	16.41	16.54	16.02	16.12	0.20	0.265
11-24 d	34.46	34.15	34.04	33.65	0.43	0.609
25-42 d	86.84	87.66	85.60	87.18	1.49	0.789
0-42 d	51.76	52.20	51.58	51.97	1.68	0.930
FI, g/ chick/ day						
0-10 d	16.78	17.04	16.99	16.62	0.632	0.648
11-24 d	54.54	54.59	53.89	53.10	1.34	0.216
25-42 d	141.62	139.59	140.11	143.11	4.80	0.588
0-42 d	70.98	70.40	70.33	70.94	1.79	0.881
FCR, g/g						
0-10 d	1.02[b]	1.03[b]	1.06[a]	1.03[b]	0.01	0.047
11-24 d	1.59	1.59	1.58	1.57	0.01	0.632
25-42 d	1.63	1.59	1.63	1.64	0.02	0.387
0-42 d	1.37	1.35	1.36	1.37	0.01	0.298
BW, g/chick						
10 d	221.04	223.42	216.08	218.55	2.25	0.150
24 d	703.60	707.52	692.69	689.72	8.03	0.361
42 d	2266.80	2285.52	2258.58	2276.82	29.59	0.923
PI	69.53	65.22	67.25	66.49	1.50	0.575

[a,b,c] Means (n = 6) within the column with different superscripts are significantly different ($P < 0.05$), LSD test was applied to compare mean.

ADG = Average daily gain, FI = Feed intake, FCR= Feed conversion ratio, BW = Body weight, PI = Performance index from 1 to 42 days of age.

SEM = Standard error of mean.

Table 3 presents the thyroid plasma hormones and GH levels of broilers at 10, 24, and 42 days of age. The results suggest that Trp supplementation significantly altered plasma T3 concentrations ($P < 0.05$). The lowest plasma T3 levels were observed in the treatments of 0.4 and 0.6 g/kg of Trp at 10 days, while the control and 0.2 g/kg Trp treatments had the highest levels. At 24 days, the control treatment resulted in lower T3 levels than the other treatments ($P < 0.05$). At 42 days, the control diet and 0.2 g/kg Trp treatment had the lowest plasma T3 levels, while the 0.6 g/kg Trp treatment had the highest ($P < 0.05$).

The difference between the plasma T4 levels in different experimental treatments was not significant, except at 10 days of age, where the treatment containing 0.6 g/kg Trp had the highest hormone levels ($P = 0.056$). Increasing dietary Trp levels did not significantly affect the plasma GH concentration ($P > 0.05$), although it was nearly significant at 42 days of age ($P = 0.061$). At this age, the control treatment and the treatment containing 0.2 g/kg Trp resulted in the highest GH concentration. During heat stress, broiler chickens experience a decrease in the size and activity of their thyroid gland (Huston and Carmon, 1962). This decrease leads to reductions in the levels of hematic T3 and T4 observed in these birds (Rajaei-Sharifabadi *et al.,* 2017; Beckford *et al*., 2020). The consumption of a Trp-rich diet has been found to be associated with improvements in blood plasma parameters such as T3 and T4. This improvement is likely due to the fact that Trp plays a crucial role as an essential amino acid in various metabolic activities. Previous research by Kollmann *et al.* (2008) has indicated that Trp can serve as a precursor for the production of serotonin, a neurotransmitter. Studies conducted on Sanan goats by Khazali *et al.* (2005) have demonstrated that serotonin may increase the mean plasma concentration of T3 and T4. This increase could be attributed to the co-localizations of serotonin neurons with neurons that secrete the thyrotrophic-releasing hormone, as reported by Bujatti and Biederer, (1976) and Savard *et al.,* 1983, 1984). Recent research has shown that increasing the level of serotonin can help alleviate heat stress in animals (Koopmans *et al.,* 2006; Shen *et al.,* 2012a; Shen *et al.,* 2012b). Notably, dietary supplementation with Trp has been found to increase the concentration of serotonin in serum and hypothalamus, which has been shown to alleviate high-density feeding-induced stress (Liu *et al.,* 2015).

Table 3. Effect of level of dietary tryptophan on plasma growth hormone T3 and T4 concentrations.

Parameter	L-tryptophan supplements, g/kg				*SEM*	P value
	0.0	0.2	0.4	0.6		
GH, ng/mL						
10 d	15.71	15.65	15.72	15.86	0.06	0.104
24 d	15.91	15.83	15.80	15.82	0.11	0.909
42 d	16.52^{a}	16.39^{a}	16.05^{ab}	15.99^{b}	0.15	0.061
T3, ng/dL						
10 d	2.73^{a}	2.65^{a}	1.59^{b}	1.53^{b}	0.17	0.001
24 d	1.17^{b}	1.71^{a}	1.53^{a}	1.58^{a}	0.10	0.005
42 d	1.57^{c}	1.61^{c}	2.09^{b}	2.50^{a}	0.09	0.001
T4, µg/dL						
10 d	0.29^{b}	0.56^{ab}	0.38^{b}	0.70^{a}	0.11	0.056
24 d	1.38	1.65	1.38	1.39	0.09	0.101
42 d	1.32	1.18	1.32	1.43	0.10	0.419

^{a,b,c} Means (n = 6) within the column with different superscripts are significantly different ($P < 0.05$), LSD test was applied to compare the mean.
GH= growth hormone, T3= triiodothyronine, T4= thyroxine.
SEM= Standard error of the mean

As highlighted in previous research by Shea *et al.* (1990) and Zendehdel *et al.* (2012), serotonin synthesis plays a vital role in mood, behavior, and cognition. In a study by Badakhshan *et al.* (2021), the thyroid hormones of 7-day-old chicks exposed to heat stress were not affected by dietary Trp, which does not match our findings. Our study showed that an increase in Trp concentration in the diet led to a decrease in GH concentration at 42 days. Serotonin has a significant role in regulating GH secretion, and Rabii *et al.,* (1981) provided compelling evidence that it inhibits GH secretion. Carew Jr *et al.* (1983) reported that the most significant effects of Trp deficiency in their study were the 2- to 3-fold increases in plasma GH.

Table 4 provides an overview of the impact of Trp supplementation on the leukocyte profile and general antibody titers against Influenza and Newcastle viruses in broiler chickens during heat stress conditions. The dietary interventions did not produce any significant effect on heterophil (H) and lymphocyte (L) counts and the H/L ratio ($P > 0.05$).

Table 4. Leukocyte profile, hemagglutination inhibition (HI)-antibody titer against Newcastle disease (ND) and enzyme-linked immunosorbent assay (ELIZA) -antibody titer against Influenza of broiler chickens fed on different dietary treatments.

Parameter	L-tryptophan supplements, g/kg					
	0.0	**0.2**	**0.4**	**0.6**	***SEM***	**P value**
Heterophils, H; × 109/L	15.66	14.16	15.00	16.00	3.40	0.324
Lymphocytes, L; × 109/L	83.00	85.83	84.16	82.50	3.77	0.256
H/L ratio	0.18	0.17	0.17	0.21	0.04	0.401
HI-antibody titer against ND, log 10						
14 d	6.16[b]	7.50[a]	7.50[a]	7.33[a]	0.24	0.002
21 d	5.00	4.50	5.00	4.16	0.32	0.216
42 d	5.16	5.33	5.66	5.00	0.28	0.403
ELIZA-antibody titer against Influenza, log 10						
14 day	3.00[c]	5.66[b]	7.50[a]	3.66[c]	0.34	0.001
21 day	3.00	2.50	2.00	3.00	0.36	0.184
42 day	3.50	3.33	3.33	3.50	0.31	0.961

[a,b,c] Means (n = 6) within the column with different superscripts are significantly different ($P < 0.05$), LSD test was applied to compare the mean.
SEM = Standard error of mean.

H/L ratio is an indicator of the hypothalamic-adenohypophyseal-adrenocortical axis (HPA) activity and stress in poultry, according to Dávila *et al.* (2011). In recent research, Quinteiro-Filho *et al.* (2010) and Habibian *et al.* (2014) have found that chickens exposed to heat stress had glucocorticoid impact and a rise in the H/L ratio. The chicks fed 0.6 g/kg Trp had a slightly higher H/L ratio compared to the control group, although it was not significant. A higher H/L ratio could be attributed to improved protein synthesis in chicks, as indicated by Richards *et al.* (2005). The results of various studies may differ due to differences in the levels of Trp supplementation used, variations in the background of the targeted populations, age and genus of birds, overall farm hygiene, and severity of stress. At 14 days of age, supplementation of Trp had an impact on the overall antibody titers against Influenza and Newcastle viruses ($P < 0.05$). The chick's diet with different levels of Trp showed the most significant measurement of antibodies produced against the Newcastle viruses. Additionally, the diet supplemented with 0.4 g/kg Trp showed the most significant measurement of the antibody produced against the Influenza virus. This finding supports previous data from Kidd (2004), which demonstrated the significant effects of dietary amino acid levels on bird immunity. However, the current experiment did not show a consistent effect of Trp supplementation on Newcastle and Influenza antibody titers at both 21 and 42 days of age. Emadi *et al.* (2011) found that high levels of Arg and Trp have complementary effects on growth performance and systemic immune response in broiler chickens. The Trp metabolite melatonin induces the development of immune cells (Guo *et al.*, 2015; Chen *et al.*, 2016), which could explain why Trp plays a role in the modulation of avian immunity. Birds that were immunized against sheep red blood cells (SRBC) and fed with Trp had increased humoral immune response, particularly in immunoglobulin M and IgG levels, according to Carrillo-Vico *et al.* (2005). Trp was also found to have the potential to reduce the immune suppressive effects of live hot virus and to enhance specific protective immune responses against herpes simplex virus, as reported by Adams *et al.* (2004). Supplementation of Trp has the potential to improve antibody responses in birds under heat stress conditions.

Conclusion

To sum up, the findings imply that adding Trp to the diet did not affect the growth performance of broiler chickens in hot weather conditions. However, it did improve the blood plasma parameters such as T3, T4, and antibody titers against Influenza and Newcastle diseases.

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Conflict of interest

The authors have no conflict of interest to be declared.

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