Effects of Dietary Tryptophan Supplementation on Body Temperature, Hormone, and Cytokine Levels in Broilers Exposed To Acute Heat Stress

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Abstract

The present study was conducted to investigate the effects of tryptophan supplementation on the rectal temperature, hormone, and cytokine levels in broilers subjected to acute heat stress. A total of 300 female Arbor Acres broilers were randomly allocated to five dietary treatment groups with 6 replicates per treatment group and 10 birds per replicate. Broilers raised under thermoneutral (TN) conditions ($22 \pm 1^\circ\text{C}$) were fed a basal diet, and other broilers exposed to acute heat stress ($34 \pm 1^\circ\text{C}$) for 24 h were fed basal diets supplemented with 0%, 0.09%, 0.18%, and 0.27% tryptophan. Results indicated that acute heat stress increased the rectal temperature ($P < 0.05$), enhanced the concentrations of corticosterone, dopamine, adrenaline, adrenocorticotrophic hormone, and corticotropin-releasing hormone in serum ($P < 0.05$), and elevated the levels of serum tryptophan hydroxylase 1, tryptophan 2, 3-dioxygenase, and indoleamine 2, 3-dioxygenase, and kynurenate ($P < 0.05$), as compared with the TN group. Meanwhile, acute heat stress increased the levels of serum tryptophan, hypothalamic tryptophan, 5-hydroxytryptophan, and interleukin-22 ($P < 0.05$) relative to the TN group. However, dietary tryptophan supplementation decreased the rectal temperature, reduce the levels of hormones associated with heat stress, and promoted the immune functions of heat-stressed broilers. Moreover, dietary supplemented with 0.18% tryptophan was evaluated to be the optimal level in broilers reared under acute heat stress.

Introduction

Heat stress (HS) has been known to adversely affect the and reduce economic profit in poultry industry. Due to having lots of feathers and a lack of sweat glands on the shin, poultry is susceptible to heat stress (Gonzalez-Rivas et al. 2020), and commercial broilers are more sensitive to heat stress compared to local broilers (Yalcin et al. 2001). Over the last decennia, it has previously been suggested that reciprocal interactions between the immune and neuroendocrine systems coordinate their actions in regulating the host's response to stress, infection, and inflammation (Chrousos 1995; Wilder 1995). The hypothalamic-pituitary-adrenal (HPA) axis is activated by heat stress and results in an increased concentration of cortisol in circulation (Zhang et al. 2017; He et al. 2019). Compared to chronic heat stress, the intensity of these effects is relatively strong in acute heat stress (Gonzalez-Rivas et al. 2020). Emerging evidence demonstrated that heat stress increases rectal temperature, reduces feed intake, decreases immune function, contributes to electrolyte imbalance and endocrine disorders, and decreases reproduction in poultry (Sohail et al. 2010; Lara and Rostagno 2013; Nawab et al. 2018; Li et al. 2020). Advanced techniques, equipment and management conditions has been used in poultry breeding, which have in part mitigated the effect of high temperature on poultry. Moreover, nutrition manipulation has become an important method for alleviating the heat stress in poultry (Abdel-Moneim et al. 2021).

In addition to being involved in protein synthesis of the body, tryptophan is also engaged in multiple physiological functions. Tryptophan (TRP) has been considered a functional amino acid, which is important for maintenance, reproduction, and immunity (Hoglund et al. 2019; Sun et al. 2020; Mund et al. 2020). There are three main pathways of tryptophan metabolism, the serotonin-synthesis pathway, the kynurenine pathway, and the indole pathway (Ma et al. 2020). Serotonin (5-hydroxytryptamine, 5-HT) is one of the products of tryptophan metabolism formed by the hydroxylation and decarboxylation. Numerous studies

have shown that serotonin plays a important role in regulating physiological functions and neuroendocrine stress response in humans and animals (Bai et al. 2017; Calefi et al. 2019). In addition, tryptophan supplementation improves daily weight gain, feed utilization ratio, antioxidant status, and immune function in broilers (Biefer et al. 2017; Mund et al. 2020). Meanwhile, tryptophan supplementation has been reported to alleviates acute heat stress-induced impairment in pigs and steers (Koopmans et al. 2005; Madoka et al. 2018). However, there is scarce information regarding the effect of tryptophan supplementation on the physiological and immunological parameters in broilers exposed to acute heat stress. Therefore, the current study was conducted to assess the effects of tryptophan supplementation on body temperature, hormone levels, and cytokines in broilers reared under acute heat stress.

Materials And Methods

Experimental procedures were carried out in accordance with the Laboratory Animal Welfare and Ethics Censorship. The animal use and care protocol were reviewed and approved by the Laboratory Animal Ethics Committee of Jiangxi Agricultural University (Nanchang, Jiangxi, China).

Experimental design and diets

One-day-old female Arbor Acres broiler chicks were reared under standard environmental conditions and were fed a starter diet for 18 days. A total of 300 chicks with uniform body weight were then chosen and randomly allocated to five treatment groups. Each group had 6 replicate pens of 10 chicks per pen. The five treatment groups were as follows: the thermoneutral conditions (TN)group, heat stress (HS) group, HS + 0.09%Trp group, HS + 0.18%Trp group, and HS + 0.27%Trp group. After a 3-day adaptation period (19 to 21 days of age, $22 \pm 1^{\circ}\text{C}$), chicks in the TN group were continuously kept in the thermoneutral environment ($22 \pm 1^{\circ}\text{C}$). Chicks in the other four groups were subjected to acute heat stress ($34 \pm 1^{\circ}\text{C}$) for 24 h. Relative humidity was kept at 65–70% among all groups during the entire trial period. Chicks in TN and HS groups were fed with a basal diet. Chicks in the HS+0.09%Trp group, HS+0.18%Trp group, and HS+0.27%Trp group were fed with the basal diet supplemented with 0.09%, 0.18%, 0.27% tryptophan, respectively. The basal diet was formulated to meet the nutrient requirements recommended by National Research Council (NRC, 1994) (Table 1). L-Glutamic acid was added to the basal diet instead of L-Tryptophan to make all diets isonitrogenous. Chicks were allowed *ad libitum* access to feed and water.

Table 1
Ingredients and nutrient compositions of the experimental diets (as-fed basis)

Item	Day 1-18	Day 19-22				
		TN	HS	HS+0.09%Trp	HS+0.18%Trp	HS+0.27%Trp
Ingredient (%)						
Corn	52.50	57.00	57.00	57.00	57.00	57.00
Soybean meal	23.00	16.20	16.20	16.20	16.20	16.20
Corn gluten meal	10.00	10.00	10.00	10.00	10.00	10.00
Extruded soybean	6.00	8.00	8.00	8.00	8.00	8.00
Soybean oil	2.50	3.50	3.50	3.50	3.50	3.50
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Calcium hydrophosphate	1.70	1.50	1.50	1.50	1.50	1.50
Mineral premix ^a	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine HCl	0.20	0.31	0.31	0.31	0.31	0.31
DL-Methionine	0.15	0.10	0.10	0.10	0.10	0.10
Vitamin premix ^b	0.03	0.03	0.03	0.03	0.03	0.03
L-Tryptophan	0.00	0.00	0.00	0.09	0.18	0.27
L-Glutamic acid	0.00	0.39	0.39	0.26	0.13	0.00
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Zeolite powder	1.82	0.87	0.87	0.91	0.95	0.99
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient composition (%)						

ME: Metabolizable energy. ^a Mineral premix provided the following per kilogram of diet: Cu (Cu₂(OH)₃Cl), 14.5 mg; Mn (MnSO₄·H₂O), 124 mg; Se (Na₂SeO₃), 0.15 mg; Fe (FeSO₄·H₂O), 150 mg; Zn (ZnSO₄·H₂O), 100 mg and I (Ca(IO₃)₂), 0.30 mg. ^b Vitamin premix provided the following per kilogram of diet: Vitamin A, 6000 IU; Vitamin D₃, 600 IU; Vitamin E, 36 IU; Vitamin K₃, 0.9 mg; Vitamin B₁, 0.6 mg; Vitamin B₂, 2.4 mg; Vitamin B₆, 1.8 mg; Vitamin B₁₂, 15µg; D-biotin, 0.18 mg; D-pantothenic acid, 3 mg; Niacinamide, 18 mg; Folic acid, 0.75 mg. ^c Data in parentheses indicate the analyzed values.

Item	Day 1-18	Day 19-22				
		TN	HS	HS+0.09%Trp	HS+0.18%Trp	HS+0.27%Trp
ME (MJ/kg)	12.81	13.40	13.40	13.40	13.40	13.40
Crude protein	22.38	20.48	20.48	20.48	20.48	20.48
Tryptophan ^c	0.23	0.20(0.18)	0.20(0.18)	0.29(0.24)	0.38(0.33%)	0.47(0.39)
Lysine	1.16	1.11	1.11	1.11	1.11	1.11
Methionine	0.56	0.48	0.48	0.48	0.48	0.48
Ca	1.05	0.99	0.99	0.99	0.99	0.99
Available P	0.46	0.41	0.41	0.41	0.41	0.41
ME: Metabolizable energy. ^a Mineral premix provided the following per kilogram of diet: Cu (Cu ₂ (OH) ₃ Cl), 14.5 mg; Mn (MnSO ₄ ·H ₂ O), 124 mg; Se (Na ₂ SeO ₃), 0.15 mg; Fe (FeSO ₄ ·H ₂ O), 150 mg; Zn (ZnSO ₄ ·H ₂ O), 100 mg and I (Ca(IO ₃) ₂), 0.30 mg. ^b Vitamin premix provided the following per kilogram of diet: Vitamin A, 6000 IU; Vitamin D ₃ , 600 IU; Vitamin E, 36 IU; Vitamin K ₃ , 0.9 mg; Vitamin B ₁ , 0.6 mg; Vitamin B ₂ , 2.4 mg; Vitamin B ₆ , 1.8 mg; Vitamin B ₁₂ , 15µg; D-biotin, 0.18 mg; D-pantothenic acid, 3 mg; Niacinamide, 18 mg; Folic acid, 0.75 mg. ^c Data in parentheses indicate the analyzed values.						

Sample collection

Rectal temperature was measured with a mercury thermometer one hour before the end of heat stress treatment. After heat stress for 24 h, blood samples were collected from the wing vein, centrifuged at 3000 g for 10 min at 4°C to obtain serum, and stored at -20°C for further analysis. After that, the selected broilers were euthanized by cervical dislocation. Further, hypothalamic samples were collected and immediately frozen at -80°C awaiting further analysis.

Determination of the serum hormone levels related to heat stress

The levels of corticosterone, dopamine, adrenaline, adrenocorticotrophic hormone and corticotropin-releasing hormone in serum were determined using commercial kits (Shanghai Enzyme Link Biotechnology Co., Ltd, Shanghai, China) according to the manufacturer's instructions.

Determination of tryptophan metabolism enzymes and the metabolites

The concentrations of tryptophan (Trp), 5-hydroxytryptophan(5-HT), and 5-hydroxyindole acetic acid (5-HIAA), and the activities of kynurenate, tryptophan hydroxylase 1 (TPH1), tryptophan hydroxylase 2 (TPH2), tryptophan 2, 3-dioxygenase (TDO), and indoleamine 2, 3-dioxygenase (IDO) in serum and the hypothalamus samples were detected using ELISA Assay Kits (Shanghai Enzyme Link Biotechnology Co., Ltd, Shanghai, China) in accordance with the manufacturer's instructions. The total protein content in the hypothalamus

was determined using a commercial kit (Beyotime Institute of Biotechnology, Shanghai, China) following the manufacturer's instructions.

Determination of the serum cytokine levels

The levels of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-10, IL-22, and interferon (IFN)- γ in serum were determined using commercial ELISA kits (Shanghai Enzyme Link Biotechnology Co., Ltd, Shanghai, China) according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using SPSS statistical software (SPSS for Windows, version 25.0, Chicago, IL, USA). Data for the TN and HS groups were analyzed using independent students t-test. In contrast, data for the HS, HS+0.09% Trp, HS+0.18% Trp, and HS+0.27% Trp groups were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple comparisons. All data were expressed as mean $\pm$ standard error of the mean (SEM). Differences were considered to be statistically significant at $P < 0.05$ and trends at $0.05 < P < 0.10$.

Results

Rectal temperature

As shown in Fig. 1, heat stress increased the rectal temperature ($P < 0.05$), while L-tryptophan supplementation decreased the rectal temperature in broilers exposed to heat stress ($P < 0.05$).

The hormones related to heat stress

As presented in Fig. 2, heat stress increased the levels of corticosterone, dopamine, adrenaline, adrenocorticotrophic hormone, and corticotropin-releasing hormone in serum ($P < 0.05$). Compared with the HS group, the concentrations of corticotropin-releasing hormone in serum was decreased in the HS+0.09% Trp group ($P < 0.05$), the levels of dopamine, adrenaline, noradrenaline, and corticotropin-releasing hormone were reduced in the HS+0.18% Trp group ($P < 0.05$), the concentrations of dopamine and corticotropin-releasing hormone were decreased in the HS+0.27% Trp group ($P < 0.05$).

Tryptophan metabolic enzymes and metabolites

According to Fig. 3, the activities of TPH1, TDO, and IDO in the serum of the HS group were higher than those of the TN group ($P < 0.05$). Tryptophan supplementation reduced TDO activity in the serum of broilers reared under heat stress ($P < 0.05$), and dietary supplementation of 0.18% tryptophan decreased the activity of IDO compared with that in the HS group ($P < 0.05$).

As shown in Fig. 4, heat stress increased the concentrations of kynurenate and tryptophan in serum and tryptophan in the hypothalamus ($P < 0.05$), while reducing the concentration of 5-HT in the hypothalamus ($P < 0.05$). Tryptophan supplementation increased the level of tryptophan in serum ($P < 0.05$). Conversely, dietary supplementation of 0.18% tryptophan decreased the level of kynurenate in serum compared with that in the HS group ($P < 0.05$). In addition, the levels of tryptophan and 5-HT in serum and hypothalamus were

increased ($P < 0.05$), and the level of 5-HIAA/5-HT in the hypothalamus was decreased ($P < 0.05$) in the HS+0.18% Trp and HS+0.27% Trp groups compared with the HS group.

Serum cytokine profile

As presented in Fig. 5, the level of IL-22 in the serum of the HS group was lower than that in the TN group ($P < 0.05$). Tryptophan supplementation increased the level of IL-22 in the serum of broilers subjected to heat stress ($P < 0.05$). Meanwhile, dietary supplementation of 0.18% tryptophan tended to decrease ($P = 0.075$) the level of TNF- α in serum compared with that in the HS group.

Discussion

Heat stress is a critical challenge in animal health, and adversely affects animal growth performance, productivity, and meat quality (Lara and Rostagno 2013; Nawab et al. 2018; Gonzalez-Rivas et al. 2020). Traditionally, blood pH, rectal temperature, and respiratory rate were considered as the indicators of response to heat stress in broilers (Chen et al. 2013; Mutibvu et al. 2017; Shakeri et al. 2019). In the present study, the rectal temperature of broilers in the HS group was higher than that in the TN group, in agreement with previous studies (Zhang et al. 2018; Vesco et al. 2020). It suggested that the heat stress model was established successfully. Notably, it is urgent to develop nutritional strategies to prevent the detrimental effects of heat stress. Of note, the current study observed that tryptophan supplementation decreased the rectal temperature in broilers exposed to heat stress. Similarly, previous work indicated that intravenous administration of tryptophan reduced rectal temperature in steers reared under heat stress (Madoka et al. 2018), and dietary supplementation of Tryptophan improved the intestinal barrier and immune functions in other animals subjected to darkness, drive, and other stress (Yue et al. 2017). Acute heat stress activated the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis and increased the levels of cortisol, catecholamine, and glucocorticoids (Kadim et al. 2006; Gonzalez-Rivas et al. 2020). Meanwhile, an increase of cortisol affected the pituitary and hypothalamus and stimulated the levels of adrenocorticotrophic hormone and corticotropin-releasing hormone. In addition, higher cortisol level affected the HPA axis, and induced dysfunctions to increase the risk of diseases (Luo et al. 2019). In the present work, heat stress increased the levels of cortisol, dopamine, adrenaline, adrenocorticotrophic hormone, and corticotropin-releasing hormone in the serum of broilers. However, dietary supplementation of Tryptophan decreased the concentrations of dopamine, adrenaline, noradrenaline, and corticotropin-releasing hormone in the serum of broilers exposed to heat stress. Consistently, the effect of heat stress on these hormones was abolished by tryptophan treatment (Yue et al. 2017; Alhassan et al. 2018). Moreover, dietary supplementation of tryptophan may be a beneficial strategy against weaning stress by decreasing adrenaline, noradrenaline, and 5-HT (Liu et al. 2013). These indicated that tryptophan supplementation may promote the healthy function in broilers exposed to heat stress.

The TDO expression is under the control of glucocorticoids and glucagon (Castro-Portuguez and Sytphin 2020). Our results found that heat stress enhanced the level of glucocorticoid and was associated with an increase of TDO and IDO concentrations in serum. During inflammation, the expression of IDO increased and stimulated the kynurenine metabolic pathway (Breda et al. 2016; Sun et al. 2020). Indeed, the concentration of kynurenine in the HS group was higher than that in the TN group. An increase of kynurenine activated the

aryl hydrocarbon receptor, then suppressed the T cell proliferation and induced the apoptosis of T/B cells (Fuertig et al. 2016; Platten et al. 2019). Importantly, the current study found that tryptophan supplementation reduced the level of IDO, TDO, and kynurenine in the serum of broilers subjected to heat stress. 5-HT is a type of neurotransmitter involved in the HPA axis with hypothalamic corticotropin-releasing hormone (Dinan 1996; Winberg et al. 1997) and participated in the body behaviors and neuroendocrine system in response to stress. 5-HIAA is an indicator reflecting 5-HT conversion serotonin activity in the nervous system (Hierden et al. 2004). In this study, heat stress elevated serum 5-HIAA/5-HT and decreased hypothalamic 5-HT level significantly, which was consistent with the previous work (Calefi et al. 2019).

Emerging evidence reported that increasing the 5-HT level was a benefit for alleviating heat stress in animals (Koopmans et al. 2006; Shen et al. 2012a; Shen et al. 2012b). Noteworthy, dietary supplementation of tryptophan increased the concentration of 5-HT in serum and hypothalamus, which alleviated the high-density feeding-induced stress (Liu et al. 2015). In the present study, tryptophan supplementation, especially at the 0.18% level significantly enhanced the concentrations of tryptophan and 5-HT in serum and hypothalamus and decreased the 5-HIAA/5-HT in the hypothalamus. In addition, a previous study reported that heat stress damaged the intestinal barrier function by increasing the levels of TNF- α and IL-1 β (Siddiqui et al. 2020). Similarly, the current work found that heat stress reduced the serum IL-22 level in broilers. IL-22 is engaged in homeostasis balance between the immunity and microorganism, with involvement of antimicrobial peptide release based on microbial abundance and diversity (Zelante et al. 2013; Behnsen et al. 2014). Notably, tryptophan plays a critical role in the production of IL-22 in the intestine (Lee et al. 2011; Gao et al. 2018). Consistently, in this study, dietary supplementation of 0.18% tryptophan decreased the level of TNF- α in the serum of broilers. These findings suggested that tryptophan supplementation may alleviate the heat stress in broilers, and the optimal level was 0.18%.

In summary, dietary tryptophan supplementation could alleviate the heat stress in broilers, which is associated with decreasing rectal temperature, suppressing the kynurenine metabolic pathway, increasing the 5-HT synthesis, and enhancing the anti-inflammatory ability. Moreover, dietary supplementation of 0.18% tryptophan was the optimal level for broilers subjected to acute heat stress. These results demonstrate the beneficial effect of tryptophan on the healthy function in broilers reared under acute heat stress.

Declarations

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Data availability Data generated or analysed during this study are included in the current article.

Author contributions All authors contributed to the study conception and design. Conceptualization, Guanhong Li; Data curation, Qiufen Li, Hua Zhou, and Jingxin Ouyang; Formal analysis, Qiufen Li; Funding acquisition, Guanhong Li and Qiufen Li; Investigation, Qiufen Li, Hua Zhou, Jingxin Ouyang, Shuaipeng Guo, and Jun Zheng; Methodology, Qiufen Li, Hua Zhou, Jingxin Ouyang, Shuaipeng Guo, and Jun Zheng; Supervision, Guanhong Li; Writing – original draft, Qiufen

Li and Jingxin Ouyang; Writing – review & editing, Huan Zhou and Guanhong Li. All authors have read and approved the submitted version.

Statement of animal right Experimental procedures were carried out in accordance with the Laboratory Animal Welfare and Ethics Censorship. The animal use and care protocol were reviewed and approved by the Laboratory Animal Ethics Committee of Jiangxi Agricultural University (Nanchang, Jiangxi, China).

Conflicts of interest The authors declare no conflict of interest.

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Figures

Figure 1

Effect of dietary tryptophan supplementation on the rectal temperature in broiler exposed to heat stress. TN: chicks fed with a basal diet raised under thermoneutral conditions; HS: chicks fed with a basal diet raised under acute heat stress condition; HS + 0.09%Trp: chicks fed with a basal diet supplemented with tryptophan by 0.09% raised under acute heat stress condition; HS + 0.18% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.18% raised under acute heat stress condition; HS + 0.27% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.27% raised under acute heat stress condition. The rectal temperature was determined using a mercury thermometer. Mean values with * differ significantly ($P < 0.05$). Mean values of HS group, HS + 0.09% Trp group, and HS + 0.27% Trp group without common superscript letters differ significantly ($P < 0.05$)

Figure 2

Effect of dietary tryptophan supplementation on the serum corticosterone (A), dopamine (B), adrenaline (C), noradrenaline (D), adrenocorticotrophic hormone (E), and corticotropin-releasing hormone (F) levels in broiler exposed to heat stress. TN: chicks fed with a basal diet raised under thermoneutral conditions; HS: chicks fed with a basal diet raised under acute heat stress condition; HS + 0.09%Trp: chicks fed with a basal diet supplemented with tryptophan by 0.09% raised under acute heat stress condition; HS + 0.18% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.18% raised under acute heat stress condition; HS + 0.27% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.27% raised under acute heat stress condition. The levels of serum hormones related to heat stress were determined using commercial ELISA kits. Mean values with * differ significantly ($P < 0.05$). Mean values of HS group, HS + 0.09%Trp group, HS + 0.18%Trp group, and HS + 0.27%Trp group without common superscript letters differ significantly ($P < 0.05$)

Figure 3

Effect of dietary tryptophan supplementation on the serum tryptophan hydroxylase 1 (A), tryptophan hydroxylase 2 (B), tryptophan 2, 3-dioxygenase (C), and indoleamine 2, 3-dioxygenase (D) levels in broiler exposed to heat stress. TN: chicks fed with a basal diet raised under thermoneutral conditions; HS: chicks fed with a basal diet raised under acute heat stress condition; HS + 0.09%Trp: chicks fed with a basal diet supplemented with tryptophan by 0.09% raised under acute heat stress condition; HS + 0.18% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.18% raised under acute heat stress condition;

HS + 0.27% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.27% raised under acute heat stress condition. The levels of tryptophan metabolism enzymes were determined using commercial ELISA kits. Mean values with * differ significantly ($p < 0.05$). Mean values of HS group, HS + 0.09% Trp group, HS + 0.18% Trp group and HS + 0.27% Trp group without common superscript letters differ significantly ($p < 0.05$)

Figure 4

Effect of dietary tryptophan supplementation on the tryptophan metabolite levels in serum (A-E) and hypothalamic (F-I) of broiler exposed to heat stress. TN: chicks fed with a basal diet raised under thermoneutral conditions; HS: chicks fed with a basal diet raised under acute heat stress condition; HS + 0.09%Trp: chicks fed with a basal diet supplemented with tryptophan by 0.09% raised under acute heat stress condition; HS + 0.18% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.18% raised under acute heat stress condition; HS + 0.27% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.27% raised under acute heat stress condition. The levels of tryptophan metabolites were determined using commercial ELISA kits. Mean values with * differ significantly ($P < 0.05$). Mean values of HS group, HS + 0.09% Trp group, HS + 0.18% Trp group and HS + 0.27% Trp group without common superscript letters differ significantly ($P < 0.05$)

Figure 5

Effect of dietary tryptophan supplementation on the serum tumor necrosis factor (TNF)-α (A), interleukin (IL)-1β (B), IL-10 (C), IL-22 (D), and interferon (IFN)-γ (E) levels of broiler exposed to heat stress. TN: chicks fed with a basal diet raised under thermoneutral conditions; HS: chicks fed with a basal diet raised under acute heat stress condition; HS + 0.09%Trp: chicks fed with a basal diet supplemented with tryptophan by 0.09% raised under acute heat stress condition; HS + 0.18% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.18% raised under acute heat stress condition; HS + 0.27% Trp group: chicks fed with a basal diet supplemented with tryptophan by 0.27% raised under acute heat stress condition.

The levels of serum cytokines were determined using commercial ELISA kits. Mean values with * differ significantly ($P < 0.05$). Mean values of HS group, HS + 0.09%Trp group, HS + 0.18%Trp group and HS + 0.27%Trp group without common superscript letters differ significantly ($P < 0.05$)