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Nutritional strategies to alleviate heat stress in broiler chickens and laying hens: role of functional nutrients

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Abstract

This study was conducted to examine the negative impact of heat stress on broiler chickens and laying hens and to explore the potential of dietary functional nutrients in mitigating these effects. Heat stress in poultry was found to negatively influence productivity and immune response while simultaneously increasing mortality, internal nutrient requirements, and stress-related hormones levels. These physiological changes led to increase of blood glucose levels, respiration, muscle tension, and neural sensitivity. To address these heat stress-induced challenges, the inclusion of functional nutrients in poultry diets may offer several benefits, including: (i) attenuation of heat stress responses, (ii) enhancement of immune function, (iii) improvement of antioxidant capacity, (iv) maintenance of productivity, and (v) promotion of intestinal health. These functional effects are expected to enhance disease resistance and overall productivity. However, the effectiveness of dietary functional nutrients may differ based on the specific type of additive, the method of administration, and physiological and environmental conditions. Therefore, it is crucial to optimize the selection and application of functional nutrients to the particular needs and context of each poultry farming operation. In conclusion, this study provides foundational insight and strategic recommendations for practical use of dietary functional nutrients to reduce the impact of heat stress in broiler chickens and laying hens.

Keywords: Broiler chicken, Functional nutrient, Heat stress, Laying hen, Poultry

INTRODUCTION

Global average temperatures have been steadily rising due to climate change. In South Korea, the climate is gradually shifting from temperate to subtropical conditions [1,2]. This temperature increase has adversely affected various industries, with livestock being among the most severely impacted. As ambient temperatures rise, livestock are increasingly exposed to heat stress, which negatively affects their performance, fertility, milk production, and overall health [3–5]. Among livestock, poultry experiences

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consistent heat-related mortality, especially in the summer.

The poultry industry suffers an annual economic loss of approximately $128 million, largely attributed to heat stress exacerbated by global warming [6]. Additionally, with global population predicted to reach around 9.8 billion by 2050, the demand for protein-rich food sources is expected to increase substantially [7]. Globally, poultry such as broiler chickens, laying hens, ducks, and turkeys provide an essential supply of both meat and eggs. The poultry industry plays an essential role in the global economy, and poultry consumption in the United States has outpaced that of beef or pork [8]. These facts highlight that continued poultry mortality not only threatens sustainability of the industry but also can lead to broader public health challenges associated with protein shortages.

Chickens are classified as homeothermic animals, with body temperatures typically approximately 39℃ for chicks and 41℃ for mature birds [9]. Due to their sparse distribution of sweat glands and feather coverage, chickens are generally less tolerant of heat than other livestock. When the temperature in poultry houses exceeds 30℃, chickens may experience hyperventilation and heat stress, leading to deteriorated gut health, reduced productivity, oxidative damage, and increased mortality, ultimately resulting in significant economic losses [10,11]. Addressing heat stress in poultry, especially in broiler chickens and laying hens, which are the most commonly raised and heat-sensitive species, remains a critical challenge in poultry industry. In response, research efforts have focused on developing dietary interventions, including feed additives and functional ingredients, to mitigate adverse effects of heat stress [12–14]. These efforts emphasize an urgent need for effective nutritional strategies aimed at enhancing heat tolerance and resilience in broiler chickens and laying hens. Therefore, the objective of this review is to summarize physiological and productivity-related consequences of heat stress in broiler chickens and laying hens and to explore the efficacy of using dietary functional nutrients as a strategy to alleviate negative effects of heat stress.

ADVERSE EFFECTS OF HEAT STRESS ON BROILER CHICKENS AND LAYING HENS

Mortality

When exposed to heat stress, chickens would physiologically increase their respiration rates. Such response known as thermal tachypnea, polypnea, or panting results in thermal hyperpnea. This mechanism facilitates heat dissipation by promoting evaporative cooling through water loss from the respiratory tract [15,16]. However, prolonged elevation in respiration rate can lead to excessive loss of carbon dioxide in blood, resulting in respiratory alkalosis. This condition is often accompanied by a reduction in blood calcium level, which can impair eggshell mineralization and compromise growth performance [17,18]. Such electrolyte imbalance can lead to decreased productivity in broiler chickens and adverse effects in laying hens, including diminished eggshell quality and increased incidence of abnormal eggs. In severe cases of extreme heat stress, where birds are unable to regulate their body temperatures effectively through respiration, the condition may progress to unconsciousness. If prolonged, it ultimately leads to mortality.

Growth performance

The period immediately following hatching is the most crucial phase for muscle development in chicks. During this time, satellite cell proliferation is completed with terminal differentiation and fusion of muscle fibers, leading to an increase in breast muscle fibers and elevated expression of *myogenin* and *myogenic regulatory factor 4* [19]. Therefore, the first week post-hatch is particularly sensitive to environmental factors such as temperature, feed intake (FI; including energy and

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nutrient concentrations), water availability, humidity, and lighting, all contributing to muscle development [19]. Continuous exposure to heat stress during this early growth phase may severely impair muscle cell development, resulting in both short- and long-term reductions in body weight (BW) and muscle mass [20].

Upon exposure to temperatures exceeding their thermoneutral ranges, broiler chickens and laying hens typically reduce FI, body movement, and water consumption. These changes are often accompanied by signs of lethargy, dullness, and depression, ultimately increasing internal heat production [21,22]. One of the primary symptoms observed in poultry under these conditions is reduced FI under heat stress to prevent excess internal heat production. Reduced FI under heat stress has a critical impact on basic physiological metabolism of poultry, notably causing a decline in BW gain (BWG) and uniformity among flocks [23]. One of the major underlying mechanisms for this decline involves disruption of the leptin neuropeptide Y axis, a key neuroendocrine pathway regulating appetite [24]. Leptin secreted by adipocytes plays an essential role in maintaining energy balance and controlling BW by acting on hypothalamus to suppress appetite [25,26]. Increased leptin levels under heat stress conditions may inhibit FI, contributing to growth retardation in broiler chickens and reduced laying performance and egg quality of laying hens. Moreover, heat-stressed poultry often exhibit selective feeding behavior, preferentially consuming larger feed particles or coarse grains such as corn. This can result in nutrient imbalances and excessive abdominal fat deposition [27].

Water intake typically increases under heat stress as a compensatory mechanism for thermoregulation. However, excessive water intake can lead to rapid excretion, increasing incidence of diarrhea and impairing the absorption of microbiota-derived nutrients in the intestinal lumen [28]. Additionally, heat stress can induce oxidative damage and inflammatory responses in the intestinal mucosa, leading to reduced digestive enzyme activity and a shift in gut microbiota composition. Populations of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* are reduced, while harmful bacteria including *Coliforms* and *Clostridium* are increased [28]. Reduced FI under heat stress can also shorten gastrointestinal transit time, thereby limiting nutrient digestion and absorption while increasing fecal output [29]. These changes contribute to nutrient deficiencies, dehydration, and electrolyte imbalances in poultry. It also promotes malodorous feces and pest proliferation within the farm environment [29,30].

Immune response

Exposure to heat stress may enhance free radicals in poultry, disrupting the normal balance between oxidative activity and antioxidant defense. Such an imbalance can result in oxidative damage to proteins, lipids, and other vital cellular components [31]. Notably, poultry exposed to heat stress exhibit functional impairment in key lymphoid organs, including spleen, thymus, and bursa of Fabricius [32,33]. In poultry, the spleen is responsible for humoral immune response, the bursa of Fabricius plays a key role in B cell development, and the thymus is essential for T cell maturation [34]. Heat stress has been associated with increased expression of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-4, IL-6, and nuclear factor-kappa B (NF-κB). This proinflammatory response may suppress humoral immunity in broiler chickens and laying hens, potentially diminishing the efficacy of vaccines [35–38]. Indeed, stressed chickens have been shown to exhibit weakened immune response to vaccination and heightened susceptibility to infections such as Newcastle disease (ND) and infectious bronchitis (IB) [3,39,40]. These findings suggest that heat stress experienced during early phases may have long-term immunosuppressive effects, impairing vaccine-induced immunity and increasing vulnerability to pathogenic challenges in the environment. Moreover, a chicken's nasal cavity plays a vital role in filtering dust and airborne

bacteria. However, elevated respiration rates under heat stress may alter the respiratory tract microbiota, leading to a reduction in filtration capacity and increased risk of bacterial respiratory diseases [41,42]. Heat stress may also impair functions of various immune organs. Heat-stressed broiler chickens showed a reduction in $Bu1^+$ B cells and $CD3^+$ T cells in the spleen and bursa of Fabricius, along with a decrease in both immature and mature T cell populations in the thymus [34]. This has been reported to result from a decline in $CD45^+$ leukocytes, as well as structural changes and damage to the immune organs of broiler chickens caused by heat stress [34]. Thus, exposure to heat stress may lead to immunological disturbances in poultry, including reduced antibody production, altered immune cell profiles, and diminished immune organ weight, ultimately compromising disease resistance and overall health.

Hormones

In response to heat stress, both broiler chickens and laying hens exhibit an immediate reaction via their nervous systems, resulting in increases of blood glucose levels, respiration rate, muscle tension, and neural sensitivity [43,44]. In addition, heat stress can activate the hypothalamic-pituitary-adrenal axis, stimulating the release of adrenocorticotropic hormone (ACTH) by the pituitary gland [44]. Subsequently, ACTH stimulates corticosterone (CORT) production in the adrenal cortex, leading to elevated stress hormone levels in birds exposed to high temperatures [45]. Several studies have indicated that heat stress adversely influences the secretion of reproductive hormones. The levels of gonadotropin-releasing hormone, follicle-stimulating hormone, and luteinizing hormone are decreased under heat stress, consequently impairing reproductive performance of poultry [46,47]. Rozenboim et al. [48] observed that plasma progesterone and testosterone levels are significantly decreased after two days of heat exposure, suggesting potential disruption of ovarian function. Furthermore, Elnagar et al. [49] found that heat stress reduced concentrations of T3:T4 ratio, estradiol, and progesterone in blood of laying hens. Such hormonal reductions can negatively affect ovarian function, thereby decreasing egg production and quality [50]. In contrast, Li et al. [51] reported no statistically significant relationship of heat stress with changes in hormone levels except ACTH. This suggests that hormonal responses to heat stress may depend on the specific hormone and physiological conditions [51].

NUTRITIONAL STRATEGIES USING FUNCTIONAL NUTRIENTS TO MITIGATE HEAT STRESS IN BROILER CHICKENS AND LAYING HENS

The rise in summer temperatures, which contributes to heat stress in broiler chickens and laying hens presents, poses significant challenges for mitigation and management through changes in the rearing environment. Heat stress can accelerate nutrient depletion in poultry by inducing metabolic disorders, oxidative stress, and immune dysfunction [31,52]. These physiological disturbances are known to increase nutritional demands for amino acids, vitamins, and minerals [30,53,54]. Among various strategies, nutritional intervention through the use of functional nutrients is considered one of the most accessible and efficient strategies to mitigate negative effects of heat stress. Accordingly, extensive research is ongoing to identify effective dietary supplements that may mitigate heat-induced stress responses in broiler chickens and laying hens [32,55–57]. Therefore, this section aims to categorize and discuss key functional nutrients, such as amino acids, vitamins, minerals, and other feed additives, which have demonstrated efficacy in reducing the impact of heat stress in broiler chickens and laying hens.

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Amino acids

Glutamine (Gln), an amino acid involved in protein synthesis, is abundantly distributed in the blood and muscle tissues [58]. It plays a vital role as a precursor in the synthesis of glutathione (GSH), it is interconvertible with glutamate (Glu) [58]. Gln plays a critical role in cellular antioxidant defense by promoting GSH production, thereby reducing oxidative stress induced by heat stress [55]. Under heat stress, muscle Gln concentrations in poultry are depleted, leading to muscle loss. Dietary Gln supplementation has been shown to reduce muscle Gln catabolism and improve body composition in poultry [55]. Bai et al. [59] observed that dietary supplementation of Gln increases the expression of nuclear factor erythroid 2-related factor 2 (Nrf2), a central modulator of antioxidant response elements, and improves growth performance and antioxidant enzyme activity in livers of heat-exposed broiler chickens. Furthermore, Gln serves as a major energy source for intestinal mucosal cells. Supplementing Gln under heat stress conditions supports improved intestinal barrier function, as evidenced by increased villus height (VH) and upregulated mRNA expression levels of tight junction proteins such as *zonula occludens-1* (*ZO-1*), *claudin-1* (*CLDN1*), and *occludin* (*OCLN*) in the jejunum and ileum [60]. In laying hens, the inclusion of Gln in diets has been shown to enhance egg quality by increasing eggshell percentage and specific gravity under heat stress conditions [61]. Heat stress also induces the release of CORT by the adrenal cortex. Unlike mammals, poultry produce very low levels of cortisol, and CORT is considered the principal glucocorticoid regulating stress response in avian species [62,63]. CORT is known to increase levels of heterophil [64,65]. Kim et al. [66] indicated that dietary Gln supplementation reduces feather CORT concentrations and heterophil to lymphocyte (H:L) ratio in broiler chickens exposed to heat stress, indicating a stress-attenuating effect of Gln.

Previous studies evaluating the effects of dietary Gln concentrations in broiler chickens and laying hens under heat stress are summarized in Table 1. Dietary concentrations of 0.5, 1.0, and 1.5% Gln improved BW, BWG, FI, and feed conversion ratio (FCR) [59,67–70]. Dietary Gln concentrations also enhanced breast and thigh meat quality of broilers reared in a heat-stressed environment. Concentrations of 0.5, 1.0, 1.5, and 2.0% Gln in diets increased pH, water holding capacity (WHC), hardness, gumminess, chewiness, and concentrations of Gln, Glu, glutaminase, and Gln synthetase, but decreased cooking loss, drip loss, and water loss [55,66,70,71]. Dietary concentrations of 0.5, 1.0, 1.5, and 2.0% Gln enhanced levels of GSH, glutathione peroxidase (GPx), catalase (CAT), total antioxidant capacity (TAC), superoxide dismutase (SOD), and Nrf2 in breast meat, thigh meat, serum, intestinal mucosa, and liver and decreased malondialdehyde (MDA) and Kelch-like ECH-associated protein 1 in breast and thigh meat of broiler chickens exposed to high temperature conditions [55,59,68–72]. In addition, Gln at concentrations of 0.5, 1.0, and 1.5% in diet improved immune responses under heat stress conditions by increasing T and B lymphocytes, phagocytic rate, immunoglobulin (Ig), and p38 mitogen-activated protein kinase of broiler chickens [59,70]. Furthermore, dietary Gln improved gut health of broiler chickens subjected to heat stress. Dietary concentrations of Gln from 0.25 to 1.0% improved VH, crypt depth (CD), TNF-α, IL-10, digestive enzyme activities, the number of goblet cells, and Ig in the duodenum, jejunum, ileum, and intestinal mucosa of broiler chickens maintained under thermal stress conditions [60,67,69,70]. Moreover, Gln at concentrations of 0.5 and 1.0% elevated mRNA expression of *ZO-1*, *CLDN1*, and *OCLN*, while decreasing mRNA expression of *sodium/glucose cotransporter 1*, *L-fatty acid binding protein,* and *calcium-binding protein D28k*, in intestinal mucosa of broiler chickens raised under heat stress [60,69]. In laying hens, dietary Gln at a concentration of 1.0% enhanced egg quality by increasing eggshell weight and specific gravity under heat stress conditions [61]. Beneficial effects of dietary supplementation of Gln in broiler chickens and laying hens exposed to heat stress may be explained by its physiological mechanisms, including the

Table 1. Effect of dietary glutamine concentrations in broiler chickens and laying hens raised under heat stress conditions

Animals	inclusion level (%)	Optimal inclusion level (%)	Rearing conditions	Effects[1)]	References
Broiler chickens	0.5, 1.0, 1.5	1.5	Temperature: 34℃ for 8 h/d and 24℃ for 16 h/d Humidity: 60–70% for 8 h/d and 45–55% for 16 h/d	Thigh meat quality (↑pH, ↓cooking loss, ↓water loss rate, ↓MDA, ↑TAC, ↑GSH, ↑GPx, ↑SOD, ↑CAT, ↑Gln, ↑Nrf2, ↓Keap1), mRNA expression in thigh meat (↑*GPx*, ↑*SOD*, ↑*CAT*, ↑*Nrf2*, ↓*Keap1*)	[55]
	0.5, 1.0, 1.5	1.5	Temperature: 34℃ for 8 h/d and 24℃ for 16 h/d	Growth performance (↑BWG, ↑FI), liver characteristic (↓MDA, ↑TAC, ↑GSH, ↑GPx, ↑GST, ↑SOD, ↑Nrf2, ↑p38 MARK), mRNA expression in liver (↑*Nrf2*, ↑*p38 MARK*)	[59]
	0.5, 1.0	0.5	Temperature: 33 ± 1℃ for 10 h/d and 22 ± 1℃ for 14 h/d Humidity: 50–60%	Gut health (↑VH, ↓CD, ↑VH:CD, ↓sICAM-1, ↓TNF-α, ↑IL-10, ↓TNF-α/ IL-10), serum parameter (↓D-lactic acid, ↓DAO, ↓sICAM-1), mRNA expression in jejunum and ileum (↑*ZO-1*, ↑*CLDN1*, ↑*OCLN*)	[60]
	0.5	0.5	Temperature: 31–32℃ for 8 h/d and 27–28℃ for 16 h/d	Breast meat quality (↑pH), stress biomarker (↓H:L ratio, ↓feather CORT)	[66]
	0.25, 0.5, 1.0	0.5	Temperature: 32 ± 1℃ Humidity: 40%	Growth performance (↑BW), gut health (↑VH)	[67]
	0.5, 1.0, 1.5	1.5	Temperature: 34℃ for 8 h/d and 24℃ for 16 h/d	Growth performance (↑BW, ↑BWG, ↑FI, ↓FCR), blood parameter (↑total protein, ↓glucose), serum parameter (↑TAC, ↑GSH, ↑GPx, ↑SOD)	[68]
	0.5, 1.0	0.5	Temperature: 36 ± 1℃ for 10 h/d and 22 ± 1℃ for 14 h/d	Growth performance (↑BWG, ↑FI, ↓FCR), gut health (↑GSH, ↑trypsin, ↑lipase, ↑AKPase, ↑Na^+-K^+-ATPase, ↑Ca^{2+}-Mg^{2+}-ATPase, ↑D-xylose), mRNA expression in intestinal mucosa (↓*SGLT1*, ↓*CaBP-D28k*, ↓*L-FABP*)	[69]
	0.5, 1.0	0.5	Temperature: 33 ± 1℃ for 10 h and 22 ± 1℃ for 14 h/d Humidity: 55 ± 1%	Growth performance (↑BW, ↑ADG, ↑ADFI, ↓FCR), blood parameter (↑T lymphocyte, ↑B lymphocyte, ↑phagocytic rate, ↑IgA, ↑IgG, ↑IgM), gut health (↑goblet cell, ↑IEL cell, ↑sIgA, ↑IgA, ↑IgG, ↑IgM)	[70]
	1.0, 1.5, 2.0, 3.0	3.0	Temperature: 34 ± 1℃ Humidity: 60–65%	Breast meat quality (↓L*, ↑pH, ↓cooking loss, ↑WHC, ↑hardness, ↑gumminess, ↓MDA, ↑TAC, ↑GSH, ↑GPx, ↑Gln, ↑Glu, ↑GLS), thigh meat quality (↑pH, ↓cooking loss, ↑WHC, ↑hardness, ↑springiness, ↑gumminess, ↑chewiness, ↓MDA, ↑GSH, ↑GPx, ↑Gln, ↑Glu, ↑GLS, ↑GS)	[71]
	0.5, 1.0, 1.5	1.5	Temperature: 34℃ for 8 h/d and 24℃ for 16 h/d Humidity: 60–70% for 8 h/d and 45–55% for 16 h/d	Breast meat quality (↑pH, ↓cooking loss, ↓drip loss, ↓water loss rate, ↓MDA, ↑TAC, ↑GSH, ↑GPx, ↑CAT), mRNA expression in breast meat (↑*CAT*, ↑*GPx*, ↑*HSP70*)	[72]
Laying hens	1.0	1.0	Temperature: 27–32℃	Egg quality (↑eggshell weight, ↑specific gravity)	[73]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

MDA, malondialdehyde; TAC, total antioxidant capacity; GSH, glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; Gln, glutamine; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; BWG, body weight gain; FI, feed intake; GST, glutathione S-transferase; p38 MARK, p38 mitogen-activated protein kinase; VH, villus height; CD, crypt depth; , soluble intercellular adhesion molecule; TNF-α, tumor necrosis factor-α; IL, interleukin; DAO, diamine oxidase; ZO-1, zonula occludens-1; CLDN1, claudin-1; OCLN, occludin; H:L ratio, heterophil to lymphocyte ratio; CORT, corticosterone; BW, body weight; FCR, feed conversion ratio; AKPase, alkaline phosphatase; ATPase, adenosine triphosphatase; SGLT1, sodium/glucose cotransporter 1; CaBP, calcium-binding protein; FABP, fatty acid binding protein; ADG, average daily gain; ADFI, average daily feed intake; Ig, immunoglobulin; IEL cell, intestinal intraepithelial lymphocyte cell; sIg, secretory immunoglobulin; L*, lightness; WHC, water holding capacity; Glu, glutamate; GLS, glutaminase; GS, glutamine synthetase; HSP70, heat shock protein 70.

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enhancement of antioxidant capacity via GSH synthesis, preservation of muscle Gln concentrations, improvement of intestinal barrier function through tight junction regulation, and modulation of immune and stress responses [55,59,60,66,70]. Therefore, inclusion of additional Gln in diets may serve as a beneficial approach to improve performance, antioxidant defense, immune function, and intestinal health of broiler chickens and laying hens under heat stress.

Tryptophan (Trp), an essential amino acid and a fundamental component of protein synthesis, is a precursor for several physiological processes [73]. It is involved in the regulation of mood, behavior, sleep, appetite, gut motility, and bone formation [74]. Importantly, Trp serves as a precursor for serotonin, a neurotransmitter produced in both intestinal and nerve cells. It is known to possess stress-mitigating and antioxidant properties [75]. This function is particularly beneficial for broiler chickens and laying hens exposed to heat stress [76]. Serotonin is also a precursor for melatonin, which regulates circadian rhythms and reduces oxidative stress. Le Floc'h et al. [77] reported that several important molecules including serotonin, melatonin, quinolinic acid, and kynurenic acid are formed through metabolism of Trp. Notably, Trp may regulate the expression of heat stress-related biomarkers, including CORT, cortisol, and heat shock protein 70 (HSP70) [78–80]. Opoola et al. [81] demonstrated that dietary supplementation of Trp from 0.21 to 0.24% improved growth performance of broiler chickens raised during a hot season.

Results of previous experiment examining the effects of dietary concentrations of Trp in broiler chickens and laying hens raised under heat stress conditions are summarized in Table 2. In broiler chickens, dietary concentration of 0.23% total Trp (i.e., 0.38% digestible Trp) improved BW, BWG, average daily gain (ADG), FI, and FCR [81,82]. In addition, dietary concentration of 0.38% total Trp decreased levels of dopamine, noradrenaline, adrenaline, corticotropin-releasing hormone, kynurenic acid, and epinephrine of broiler chickens raised under heat stress [80,83]. Additionally, dietary concentration of total Trp at 0.38% increased levels of SOD, CAT, TAC, and aspartate aminotransferase (AST) in the serum and liver of broiler chickens exposed to heat stress [84,85]. Also, total Trp at 0.38% decreased IL-1β, IL-6, and IL-18 and increased IL-22, bursal index, IgA, and IgM in heat-stressed broiler chickens [80,82,85]. Dietary concentration of 0.38% total Trp increased mitochondrial membrane potential, number of goblet cells, and trans-epithelial electrical resistance (TEER) in broiler chickens subjected to heat stress [82,85]. Under heat stress, broiler chickens fed diets containing 0.38% digestible Trp showed reduced feather CORT concentrations and H:L ratio [82]. In the liver, ileum, and hypothalamus, dietary concentrations of 0.38% total Trp regulated mRNA expression levels of mitochondria-, metabolism-, and transcriptional regulator-related genes in broiler chickens exposed to heat stress [83–85]. In laying hens raised under heat stress, dietary total Trp at a concentration of 0.21% increased TAC and albumin in the serum [86]. Dietary Trp supplementation in broiler chickens and laying hens subjected to heat stress may exert positive effects by modulating stress responses and circadian regulation via serotonin and melatonin pathways, promoting antioxidant capacity, downregulating inflammatory cytokine production, and supporting gut integrity and mitochondrial functions [75–80,82,85]. Thus, these findings collectively suggest that additional dietary Trp may effectively improve performance, immune function, antioxidant capacity, and gut health of broiler chickens, and partially improve those of laying hens raised under heat stress conditions.

Arginine (Arg) promotes protein synthesis via the production of polyamines and contributes to creatine synthesis, which supplies energy in the form of creatine phosphate for muscle and brain functions [87]. Additionally, Arg as a precursor for nitric oxide plays key roles in relaxing vascular smooth muscle, regulating vasoconstrictor substances such as serotonin, and enhancing cell proliferation and immune responses [88]. These functions collectively contribute to pathogen eradication and improved gut health in broiler chickens [89]. Creatine is synthesized from

Table 2. Effect of dietary tryptophan concentrations in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level	Optimal inclusion level	Rearing conditions	Effects[1)]	References
Broiler chickens	19–22 d: 0.20, 0.29, 0.38, 0.47% total Trp	19–22 d: 0.38% total Trp	Temperature: 34 ± 1℃ Humidity: 65–70%	Serum parameter (↓dopamine, ↓adrenaline, ↓noradrenaline, ↓CRH, ↓TDO, ↓IDO, 5-HT, ↑Trp, ↓kynurenic acid, ↓IL-1β, ↑IL-22), hypothalamus characteristic (↑Trp, ↑5-HT, 5-HIAA/5-HT)	[80]
	Starter (1–28 d): 0.15, 0.19, 0.23, 0.27, 0.31% total Trp Finisher (34–56 d): 0.13, 0.17, 0.21, 0.25, 0.29% total Trp	Starter: 0.23% total Trp Finisher: 0.21% total Trp	Temperature: 29–45℃	Growth performance (↑BW, ↑BWG, ↑ADG, ↑FI)	[81]
	22–35 d: 0.19, 0.38% digestible Trp	22–35 d: 0.38% digestible Trp	Temperature: 30 ± 0.3℃ for 10 h/d and 23 ± 0.2℃ for 14h/d Humidity: 43.6 ± 4.62%	Growth performance (↓FCR), Stress biomarker (↓H:L raito, ↓CORT), Gut health (↑goblet cell, ↑TEER)	[82]
	19–42 d: 0.20, 0.38% total Trp	19–42 d: 0.38% total Trp	Temperature: 34 ± 1℃ for 8 h/d and 23 ± 1℃ for 16 h/d Humidity: 65–70%	Serum parameter (↓dopamine, ↓epinephrine, ↑serotonin, ↓CRH), organ trait (↑bursal index, ↑IgA, ↑IgM), mRNA expression in hypothalamus (↓*IDO-L1*, ↓*IDO-L2*, ↑*TPH2*, ↑*AANAT*), mRNA expression in liver (↓*IDO-L1*, ↑*AANAT*, ↓*MAO*, ↑*SLC6A14*), metabolite content in serum (↑serotonin)	[83]
	19–21 d: 0.20, 0.38% total Trp	19–21 d: 0.38% total Trp	Temperature: 34 ± 1℃ Humidity: 65–70%	Serum parameter (↑SOD, ↑CAT), liver characteristic (↓GPx, ↑SOD), gut health (↓CAT, ↓TAC, ↑mitochondrial DNA copy number), mRNA expression in liver (↑*PGC-1α,* ↑*CYT-c,* ↑*COX1,* ↑*COX5A,* ↑*SIRT1*)	[84]
	19–42 d: 0.20, 0.38% total Trp	19–42 d: 0.38% total Trp	Temperature: 34 ± 1℃ for 8 h/d and 23 ± 1℃ for 16 h/d Humidity: 65–70%	Serum parameter (↓total protein, ↑GPx, ↑SOD, ↑CAT, ↑TAC, ↓AST, ↓IL-1β, ↓IL-6, ↓IL-18), gut health (↑mitochondrial membrane potential), mRNA expression in ileum (↑*TFAM*, ↑*COX1*)	[85]
	Starter: 0.23, 0.35, 0.46% digestible Trp Finisher: 0.214, 0.32, 0.42% digestible Trp	Starter: 0.46% digestible Trp Finisher: 0.42% digestible Trp	Temperature: 25–36℃	Blood parameter (↑total protein, ↑globulin, ↓albumen:globulin ratio, ↓cholesterol), carcass trait (↑liver, ↑gizzard, ↑heart, ↑spleen)	[167]
Laying hens	40–48 wk: 0.17, 0.19, 0.21, 0.25% total Trp	40–48 wk: 0.21% total Trp	Temperature: 30 ± 5℃ Humidity: 85 ± 3%	Serum parameter (↑TAC, ↑albumin)	[86]
	47–55 wk: 0.15, 0.225% digestible Trp	-	Temperature: 30.7 ± 1.41℃ Humidity: 72.5 ± 11.61%	No significance	[99]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

Trp, tryptophan; CRH, corticotropin releasing hormone; TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; 5-HT, 5-hydroxytryptamine; IL, interleukin; 5-HIAA, 5-hydroxyindole acetic acid; BW, body weight; BWG, body weight gain; ADG, average daily gain; FI, feed intake; FCR, feed conversion ratio; H:L ratio, heterophil to lymphocyte ratio; CORT, corticosterone; TEER, trans-epithelial electrical resistance; Ig, immunoglobulin; TPH2, tryptophan hydroxylase 2; AANAT, aryl alkylamine N-acetyltransferase; MAO, monoamine oxidase; SLC6A14, solute carrier family 6 (amino acid transporter), member 14; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; TAC, total antioxidant capacity; PGC-1α, peroxisome proliferative activated receptor gamma coactivator 1 alpha; CYT-c, cytochrome c; COX1, cytochrome c oxidase subunit I; COX5A, cytochrome c oxidase subunit 5A; SIRT1, sirtuin 1; AST, aspartate aminotransferase; TFAM, mitochondrial transcription factor A.

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guanidinoacetic acid and L-ornithine through condensation of L-Arg and glycine [90]. Therefore, various amino acids including Arg, methionine (Met), lysine (Lys), and guanidinoacetic acid play important roles in maintaining poultry health. Dietary supplementation with these amino acids has been shown to significantly improve growth performance, breast muscle energy, physiological response, survival rate, nitric oxide production, and hepatic heat stress mitigation in broiler chickens [91–94]. Arg:Lys ratio is particularly important for optimal growth and health in poultry, as both are basic amino acids that share similar transport pathways in the intestine. An imbalance in this ratio may impair growth performance and alter plasma and muscle amino acid concentrations [91,92,95].

Previous studies evaluating the effects of dietary Arg concentrations in broiler chickens and laying hens raised under heat stress are summarized in Table 3. Esser et al. [96] observed that concentration of 1.88% digestible Arg in diets enhanced carcass weight and breast meat weight while reducing abdominal fat of broiler chickens raised under heat stress. However, Dao et al. [97] observed that feeding a low-protein diet containing 1.07% digestible Arg to broiler chickens under cyclic heat stress did not result in significant differences. Bozakova et al. [98] reported that laying hens fed diets containing 2.01% total Arg decreased blood levels of glucose, cholesterol, creatinine, triglyceride, and CORT of laying hens exposed to heat stress. Dietary Arg also influenced the behavior of heat-stressed laying hens. In laying hens raised under heat stress conditions, dietary concentration of 2.01% total Arg increased frequencies of feeding, egg laying, feather cleaning, dust bathing, and sexual behavior but decreased inactivity, resting, and aggression [98]. In contrast, 1.11% digestible Arg in diet had no significant effect on laying hens exposed to heat stress [99]. Under heat stress conditions, dietary Arg alleviates physiological disruptions in broiler chickens and laying hens by stimulating NO production, supporting creatine-based energy metabolism, and modulating immune and stress responses, thereby improving meat quality, metabolic functions, and behavioral stability [87–89,91,92,96,98]. In summary, additional supplementation of Arg in diets may effectively alleviate stress, enhance meat quality, and support immune and metabolic functions in poultry.

Vitamins

Vitamin C is an effective nutrient for alleviating heat stress in broiler chickens and laying hens. It functions as an antioxidant and electron donor. It also plays a role in amino acid and mineral metabolism and enhances immune responses [100,101]. Through the synthesis of carnitine, which facilitates conversion of fat into energy, vitamin C enhances lipid utilization, thereby promoting protein deposition and muscle development in broiler chickens exposed to heat stress [102]. Furthermore, dietary vitamin C supplementation has been reported to enhance nutrient digestibility in broiler chickens reared under heat stress conditions [103]. Heat stress typically induces increases of pro-inflammatory cytokines, suppresses antibody production, and elevates circulating CORT levels [52]. The inclusion of vitamin C in poultry diets decreases activities of CORT-synthesizing enzymes and scavenges free radicals with an antioxidant effect [104,105].

Previous studies examining the effects of dietary vitamin C supplementation in broiler chickens and laying hens raised under heat stress are summarized in Table 4. Dietary supplementation of vitamin C at 200, 250, and 300 mg/kg improved BW, BWG, ADG, FI, and FCR of broiler chickens raised under heat stress conditions [103,105–109]. Under heat stress conditions, 200 and 300 mg/kg vitamin C increased antibody titer of ND, infectious bursal disease (IBD), and IB, total protein, insulin-like growth factor 1, triiodothyronine (T_3), and thyroxin (T_4) and decreased heterophil [103,106,108]. Broiler chickens fed diets containing 200 and 250 mg/kg vitamin C showed improved antioxidant capacity of broiler chickens exposed to heat stress [105,109]. Serum

Table 3. Effect of dietary arginine concentrations in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level	Optimal inclusion level	Rearing conditions	Effects[1)]	References
Broiler chickens	Starter (1–21 d): 1.297, 2.097% digestible Arg Grower (22–37 d): 1.159, 1.959% digestible Arg Finisher (38–44 d): 1.08, 1.88% digestible Arg	Starter: 2.097% digestible Arg Grower: 1.959% digestible Arg Finisher: 1.88% digestible Arg	Temperature: 32℃	Growth performance (↑carcass weight), carcass trait (↑breast meat, ↓abdominal fat)	[96]
	Grower (7–21 d): 0.88, 1.17% digestible Arg Finisher (21–35 d): 0.78, 1.07% digestible Arg	Grower (7–21 d): 1.17% digestible Arg Finisher (21–35 d): 1.07% digestible Arg	Temperature: 33 ± 1℃ for 6 h/d and 24℃ for 18 h/d	No significance	[97]
Laying hens	42–65 wk: 1.01, 2.01% total Arg	42–65 wk: 2.01% total Arg	N/D	Blood parameter (↓CORT, ↓glucose, ↓total cholesterol, ↓creatinine, ↓triglyceride, ↑total protein), behavior (↑feeding, ↑egg laying, ↑feather cleaning, ↑dust bathing, ↑sexual behavior, ↓moving, ↓resting, ↓aggression)	[98]
	47–55 wk: 0.74, 1.11% digestible Arg	-	Temperature: 30.7 ± 1.41℃ Humidity: 72.5 ± 11.61%	No significance	[99]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

Arg, arginine; CORT, corticosterone.

levels of TAC, CAT, SOD, and GPx were increased in broiler chickens raised under heat stress conditions [105,109]. Supplementation of vitamin C at 200 and 250 mg/kg reduced H:L ratio and serum CORT concentrations [103,108,110]. Jahejo et al. [111] reported that dietary inclusion levels of 150 mg/kg vitamin C improved the weights and lengths of the jejunum, ileum, and large intestine in heat-stressed broiler chickens. In laying hens, dietary supplementation of vitamin C at 150, 200, and 250 mg/kg increased BW, FI, and crude protein digestibility and decreased FCR and mortality of laying hens raised under heat stress conditions [112–114]. Ajakaiye et al. [112] and Torki et al. [113] reported that supplementation of vitamin C at 150 and 250 mg/kg increased egg, yolk, albumen, and eggshell weights, yolk color, eggshell mass, and eggshell thickness of laying hens subjected to heat stress. Moreover, laying hens fed diets containing 200 mg/kg vitamin C exhibited enhanced laying rate and egg mass under heat stress [114]. In blood parameters, serum glucose, estrogen, progesterone, T_3, and T_4 concentrations in laying hens raised under heat stress conditions can be increased by 200 mg/kg vitamin C [114]. Additionally, 200 mg/kg vitamin C increased length of the oviduct and weights of liver, spleen, thyroid gland, ovary, large follicle, and oviduct of laying hens subjected to heat stress conditions [114]. Owing to improved antioxidant capacity, modulation of endocrine and immune functions, and support of energy metabolism, dietary supplementation of vitamin C helps broiler chickens and laying hens maintain physiological homeostasis and reproductive efficiency under heat stress, resulting in enhanced productivity, gut health, and stress tolerance [100–105,108,111,114]. In summary, dietary vitamin C supplementation effectively improves productivity, immunity, antioxidant capacity, gut health, and stress resilience in broiler chickens and laying hens reared in heat stress conditions.

Vitamin E, known for its lipophilic antioxidant properties, helps prevent oxidative stress by reducing reactive oxygen species [115]. It plays a vital role in preserving the structural stability of the cardiovascular and other physiological tissues [110]. Functioning as a chain-breaking antioxidant, vitamin E interrupts the propagation of free radicals in cell membranes and plasma lipoproteins [116]. Its oxidized form, the tocopheroxyl radical that is non-reactive with oxygen, can be regenerated to α-tocopherol by ascorbate [116].

Results of previous studies evaluating effects of dietary vitamin E supplementation in broiler chickens and laying hens raised under heat stress conditions are summarized in Table 5. In broiler

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Table 4. Effect of dietary vitamin C supplementation in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level (mg/kg)	Optimal inclusion level (mg/kg)	Rearing conditions	Effects[1)]	References
Broiler chickens	200	200	Temperature: 36 ± 2℃ for 7 h/d and 25 ± 3℃ for 17 h/d Humidity: 75–85%	Growth performance (↑BWG), blood parameter (↑PCV, ↑lymphocyte, ↑basophil, ↓heterophil, ↑albumin), stress biomarker (↓H:L ratio)	[103]
	250	250	Temperature: 35 ± 2℃ for 8 h/d and 22 ± 2℃ for 16 h/d	Growth performance (↑BWG), ↑serum parameter (↑GPx)	[105]
	300	300	Temperature: 25–34℃ for 3 h/d, 34℃ for 6 h/d, 34–25℃ for 3 h/d, and 25℃ for 12 h/d Humidity: 45.12–61.91%	Growth performance (↑BW, ↑FI, ↓FCR), blood parameter (↑ND antibody titer, ↑IBD antibody titer, ↑IB antibody titer, ↑total leucocyte, ↑lymphocyte, ↑monocyte), carcass trait (↑spleen, ↑BF, ↑thymus)	[106]
	200	200	Temperature: 23.9–38℃ for 3 h/d, 38℃ for 5 h/d, 38–23.9℃ for 4 h/d, and 23.9℃ for 12 h/d Humidity: 55%	Growth performance (↑BW, ↑ADG, ↓FCR), blood parameter (↑ascorbic acid)	[107]
	200	200	Temperature: 84.5–96.2°F Humidity: 68.5–76.5%	Growth performance (↑BW, ↑BWG, ↓FI, ↓FCR), serum parameter (↑IGF-1, ↑T_3, ↑T_4, ↑Hb, ↑total protein, ↑albumin, ↑globulin), antioxidant capacity in serum (↑HSP70, ↑TAC, ↑CAT, ↑SOD), immune response (↑ND antibody titer), stress biomarker (↓H:L ratio)	[108]
	100	100	Temperature: 26.95–36.71℃ Humidity: 85.24–93.95%	Growth performance (↓FCR)	[109]
	250	250	Temperature: 38 ± 1.4℃ Humidity: 47–51%	Stress biomarker (↓serum CORT)	[110]
	150	150	Temperature: N/D Humidity: N/D	Gut health (↑large intestine weight, ↑large intestine length, ↑jejunum weight, ↑jejunum length, ↑ileum weight, ↑ileum length)	[111]
	250	-	Temperature: 23.5–31℃ for 3 h/d, 31℃ for 7 h/d, 31–23.5℃ for 3 h/d, and 23.5℃ for 11 h/d	No significance	[121]
	300	-	Temperature: 34 ± 1℃ for 5 h/d Humidity: 65–70%	No significance	[168]
Laying hens	150	150	Temperature: 35.9℃ Humidity: 75%	Growth performance (↑BW), egg quality (↑egg weight, ↑yolk weight, ↑albumen weight, ↑eggshell weight)	[112]
	250	250	Temperature: 32℃ Humidity: 50%	Growth performance (↑FI), egg quality (↑yolk color, ↑eggshell mass, ↑eggshell thickness), serum parameter (↑Ca)	[113]
	200	200	Temperature: 38 ± 1℃ for 4 h/d and 22–24℃ for 20 h/d Humidity: 55–65%	Growth performance (↑BW, ↓mortality, ↑FI, ↓FCR, ↑CP digestibility), laying performance (↑laying rate, ↑egg mass), blood parameter (↑glucose, ↑estrogen, ↑progesterone, ↑T_3, ↑T_4), carcass trait (↑liver weight, ↑spleen weight, ↑thyroid gland weight, ↑ovary weight, ↑large follicle weight, ↑oviduct weight, ↑oviduct length)	[114]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

BWG, body weight gain; PCV, hematocrit volume; H:L ratio, heterophil to lymphocyte ratio; GPx, glutathione peroxidase; BW, body weight; FI, feed intake; FCR, feed conversion ratio; ND, Newcastle disease; IBD, infectious bursal disease; IB, infectious bronchitis; BF, bursa of Fabricius; ADG, average daily gain; IGF-1, Insulin like growth factor 1; T_3, triiodothyronine; T_4, thyroxin; Hb, haemoglobin; HSP70, heat shock protein 70; TAC, total antioxidant capacity; CAT, catalase; SOD, superoxide dismutase; CORT, corticosterone; Ca, calcium; CP, crude protein.

Table 5. Effect of dietary vitamin E supplementation in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level (mg/kg)	Optimal inclusion level (mg/kg)	Rearing conditions	Effects[1)]	References
Broiler chickens	100	100	Temperature: 36 ± 2℃ for 7 h/d and 25 ± 3℃ for 17 h/d Humidity: 75–85%	Growth performance (↑BWG), carcass trait (↑dressing yield, ↓abdominal fat). blood parameter (↑basophil, ↓AST)	[103]
	250	-	Temperature: 38 ± 1.4℃ Humidity: 47–51%	No significance	[110]
	125, 250	125	Temperature: 24–37℃ for 4 h/d, 37℃ for 8 h/d, 37–24℃ for 4h/d, and 24℃ for 8 h/d Humidity: 47–51%	Growth performance (↓FI), carcass trait (↓abdominal fat), breast meat characteristics (↓MDA, ↑Se)	[117]
	250	250	Temperature: 29.3–36.6℃ Humidity: 52.0–65.8%	Growth performance (↑BW, ↓FCR), blood parameter (↓heterophil), serum parameter (↑IBD antibody titer, ↑paraoxonase, ↓total oxidant status)	[118]
	100, 200	100	Temperature: 34℃ for 10 h/d and 18.5–22.5℃ for 14 h/d Humidity: N/D	Growth performance (↑BW, ↑BWG), breast meat characteristics (↑breast yield, ↓drip loss, ↓TBARS, ↑total tocopherols, ↑vitamin E equivalent), liver characteristics (↑retinol, ↑total tocopherols, ↑vitamin E equivalent)	[119]
	350, 600	350	Temperature: 35℃ Humidity: N/D	Growth performance (↑BW, ↑BWG, ↑FI, ↓FCR, ↓mortality, ↑productivity index, ↑economic index)	[120]
	200 IU	200 IU	Temperature: 23.5–31℃ for 3 h/d, 31℃ for 7 h/d, 31–23.5℃ for 3 h/d, and 23.5℃ for 11 h/d	Breast meat characteristic (↑α-tocopherol)	[121]
	250	250	Temperature: 29.3–36.5℃ Humidity: 52.0–65.8%	Serum parameter (↓AST, ↓glucose, ↑protein, ↓cholesterol, ↓triglyceride, ↑HDL, ↓LDL)	[122]
	350, 600	350	Temperature: 35℃ Humidity: N/D	Liver characteristics (↓MDA, ↓peroxide value, ↓free fatty acids, ↓LDL, ↓VLDL)	[123]
	300	300	Temperature: 35℃ Humidity: N/D	Gut health (↑VH in jejunum)	[124]
Laying hens	150	150	Temperature: 35.9℃ Humidity: 75%	Growth performance (↑BW), egg quality (↑egg weight, ↑yolk weight, ↑albumen weight, ↑eggshell weight)	[112]
	150	150	Temperature: 38 ± 1℃ for 4 h/d and 22–24℃ for 20 h/d Humidity: 55–65%	Growth performance (↑BW, ↑FI, ↓FCR, ↑CP digestibility), laying performance (↑laying rate, ↑egg mass), blood parameter (↑glucose, ↑estrogen, ↑progesterone, ↑T_3, ↑T_4), carcass trait (↑liver weight, ↑spleen weight, ↑thyroid gland weight, ↑ovary weight, ↑large follicle weight, ↑oviduct weight)	[114]
	250, 500	250	Temperature: 30.7–31.6℃ Humidity: 52.5–58.7%	Growth performance (↓FI, ↓FCR), blood parameter (↓heterophil) serum parameter (↓ALT, ↑total cholesterol, ↑globulin, ↑Ca)	[125]
	250, 500	-	Temperature: 30.7–31.6℃ Humidity: 52.5–58.7%	Growth performance (↓FI, ↓FCR), blood parameter (↑Hb, ↑T_4, ↓ALT)	[126]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

BWG, body weight gain; AST, aspartate aminotransferase; FI, feed intake; MDA, malondialdehyde; Se, selenium; BW, body weight; FCR, feed conversion ratio; IBD, infectious bursal disease; TBARS, Thiobarbituric acid reactive substances; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; VH, villus height; CP, crude protein; T_3, triiodothyronine; T_4, thyroxin; ALT, alanine aminotransferase; Ca, calcium; Hb, hemoglobin.

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chickens exposed to heat stress, dietary supplementation of vitamin E at 100, 125, 200, 250, 350, and 600 mg/kg improved BW, BWG, FI, FCR, mortality, productivity index, and economic index of broiler chickens [103,117–120]. Moreover, dietary inclusion levels of 100, 125, and 250 mg/kg vitamin E elevated dressing yield and reduced abdominal fat of broiler chickens raised under high temperature [103,117]. Dietary supplementation of vitamin E at 100, 125, 200, and 250 mg/kg enhanced breast yield, total tocopherols, vitamin E equivalent, and selenium (Se) concentrations but decreased drip loss, MDA, and thiobarbituric acid reactive substances in the breast of broiler chickens subjected to heat stress conditions [117,119,121]. Under heat stress, blood parameters were also affected by dietary vitamin E. Supplementation of 100 and 250 mg/kg vitamin E in diets increased basophil, total protein, paraoxonase, high-density lipoprotein (HDL), and IBD antibody titer but decreased heterophil, glucose, cholesterol, triglyceride, low-density lipoprotein (LDL), AST, and total oxidant status in the blood of broiler chickens raised under heat stress conditions [103,118,122]. In addition, vitamin E modulated hepatic characteristics under heat stress conditions. Dietary supplementation of 100, 200, 350, and 600 mg/kg vitamin E enhanced retinol, total tocopherols, and vitamin E equivalent and reduced MDA, peroxide value, free fatty acids, and LDL in the liver of broiler chickens subjected to heat stress [119,123]. Benefits of vitamin E to gut health have also been reported. Pirgozliev et al. [124] showed that dietary supplementation of 300 mg/kg vitamin E increased VH in jejunum of broiler chickens raised under heat stress. In laying hens exposed to heat stress, dietary inclusion levels of vitamin E at 150, 250, and 500 mg/kg enhanced BW, FI, FCR, and crude protein digestibility of laying hens [112,114,125,126]. Ajakaiye et al. [112] and Attia et al. [114] reported that dietary supplementation of 150 mg/kg vitamin E elevated laying rate, egg mass, and weight of egg, yolk, albumen, and eggshell of laying hens raised under heat stress, consequently improving productive performance and egg quality. Dietary supplementation of vitamin E at 150, 250, and 500 mg/kg increased hemoglobin, glucose, globulin, calcium concentrations, estrogen, progesterone, T_3, and T_4 levels but decreased heterophil and alanine aminotransferase (ALT) in the blood of laying hens subjected to heat stress conditions [114,125,126]. Additionally, dietary inclusion levels of 150 mg/kg of vitamin E enhanced the weight of liver, spleen, thyroid gland, ovary, large follicle, and oviduct of laying hens exposed to heat stress [114]. The beneficial effects of dietary vitamin E supplementation in broiler chickens and laying hens raised under heat stress conditions may be attributed to its roles in interrupting lipid peroxidation, preserving cellular membrane integrity, and regulating hepatic, immune, and reproductive functions, all of which contribute to improved productivity, liver health, and quality of meat and eggs [100,114–117,119,123]. In conclusion, vitamin E supplementation meaningfully improves productivity, antioxidant capacity, liver function, and gut health of broiler chickens and laying hens raised under heat stress conditions.

Minerals

Chromium (Cr), an essential trace mineral for poultry, is primarily known for enhancing insulin activity and playing a pivotal role in carbohydrate, fat, and protein metabolism [127]. Under heat stress conditions, Cr excretion is accelerated, leading to reduced Cr levels in the body. Dietary supplementation of Cr has been shown to facilitate glucose uptake by activating insulin receptors, lower blood glucose and CORT levels, and elevate the expression of antioxidant enzymes, leading to the mitigation of oxidative stress [128]. Moreover, dietary Cr supplementation enhances immune function by increasing lymphocyte count and antibody production, thereby counteracting immunosuppressive effects of heat stress [129]. In addition, Cr is continually utilized during egg production, which may lead to decreased insulin sensitivity and metabolic imbalance, conditions that are further exacerbated by heat stress [130]. Dietary supplementation of Cr has been reported

to improve feed efficiency and egg quality in heat-stressed laying hens, indicating its potential to replenish Cr losses and support metabolic stability [113].

Previous studies evaluating the effects of dietary Cr supplementation in broiler chickens and laying hens raised under heat stress are summarized in Table 6. In broiler chickens, dietary supplementation of Cr picolinate (CrPic), Cr propionate (CrPro), Cr histidinate (CrHis), and $CrCl_3$ at 0.4 to 20 mg/kg improved BW, BWG, ADG, FI, and FCR under heat stress conditions [36,40,131–134]. Inclusion levels of 0.4 mg/kg CrPic, CrPro, and $CrCl_3$ decreased cooking loss of broiler chickens exposed to heat stress [134]. Supplementation of 0.788 mg/kg CrHis in diets increased Cr concentrations in the breast meat and liver of broiler chickens subjected to heat stress [36]. Under heat stress conditions, broiler chickens fed diets containing 0.5 mg/kg CrPic, 1.0 mg/kg CrMet, and 0.788 mg/kg CrHis showed elevated total lymphocyte, protein, Cr concentrations, GPx, $CD8^+$, ND antibody titer, and IB antibody titer and reduced heterophil, glucose, cholesterol, LDH, and MDA in the blood [36,40,132]. Jahanian and Rasouli [40] and Huang et al. [134] reported that dietary supplementation of 1.0 mg/kg CrMet and 0.4 mg/kg CrPic, CrPro, and $CrCl_3$ increased dressing yield, bursa of Fabricius weight, and thymus weight and decreased abdominal fat of broiler chickens subjected to heat stress. Furthermore, dietary Cr improved stress-reducing effects, as evidenced by decreased H:L ratio and serum CORT concentrations in broiler chickens fed diets supplemented with 1.0 mg/kg CrMet [40]. Additionally, 0.788 mg/kg of CrHis upregulated the expression of Nrf2, glucose transporter 2, and 4 but downregulated NF-κB in the muscles of heat-stressed broiler chickens [36]. In laying hens reared under heat stress, supplementation of 0.2 mg/kg CrPic and 0.788 mg/kg CrHis in diets enhanced laying performance and egg quality by improving egg production, egg and eggshell weight, eggshell strength, eggshell thickness, eggshell mass, yolk color, Cr concentrations in the yolk, and Haugh unit [113,135]. Additionally, 0.788 mg/kg of Cr in diets also elevated the health status of heat-stressed laying hens by increasing serum Cr concentrations and decreasing glucose, cholesterol, and LDH levels [135]. Dietary supplementation of Cr in broiler chickens and laying hens subjected to heat stress may exert beneficial effects by enhancing insulin sensitivity and glucose utilization, activating antioxidant responses through Nrf2 signaling, suppressing inflammation and oxidative stress, and supporting immune function and metabolic balance [36,127–130,132,135]. Thus, dietary Cr is a functional nutrient that enhances productivity, meat quality, antioxidant capacity, and metabolic function of broiler chickens and laying hens exposed to heat stress.

Se, an essential trace mineral, contributes to various physiological functions, including cellular protection against reactive oxygen species-induced damage and attenuation of heat stress-related impacts [136]. In addition, Se promotes proliferation of T and B cells via increased expression of IL-2 receptors and decreases the production of pro-inflammatory cytokines by inhibiting the NF-κB signaling pathway [37,137]. In broilers and layers exposed to heat stress, dietary Se supports antioxidant defenses and limits oxidative damage [37,138]. Dietary Se supplementation helps maintain immune homeostasis, alleviates oxidative stress, and ultimately contributes to improved growth performance, laying performance, and stress resilience in poultry raised under heat stress conditions [37,139].

Results of previous studies examining effects of dietary Se supplementation in broiler chickens and laying hens raised under heat stress conditions are summarized in Table 7. In broiler chickens, dietary supplementation of Se-yeast at 0.15 mg/kg and nano Se at 0.1, 0.3, and 1.2 mg/kg improved BW, BWG, FI, and FCR of broiler chickens raised under heat stress conditions [138–141]. Regarding breast meat quality of broiler chickens raised under heat stress, 0.3 mg/kg sodium selenite, 0.2 and 0.5 mg/kg SeMet, 0.15 mg/kg Se-yeast, and 0.3 mg/kg nano Se in diets improved breast weight, WHC, Se concentrations, α-tocopherol, GSH, GPx, SOD, and sensory

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Table 6. Effect of dietary chromium supplementation in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level	Optimal inclusion level	Rearing conditions	Effects[1)]	References
Broiler chickens	1.6 mg/kg CrPic 0.788 mg/kg CrHis	0.788 mg/kg CrHis	Temperature: 34 ± 2℃ for 8 h/d and 22℃ for 16 h/d Humidity: N/D	Growth performance (↑BWG, ↑FI, ↓FCR), carcass trait (↓abdominal fat), serum parameter (↑Cr, ↓glucose, ↓cholesterol, ↓LDH), breast meat quality (↑Cr), liver characteristic (↑Cr), mRNA expression in muscle (↑*Nrf2*, ↓*NF-κB*, ↑*GLUT-2*, ↑*GLUT-4*)	[36]
	0.5, 1.0 mg/kg Cr-Met	1.0 mg/kg CrMet	Temperature: 35 ± 2℃ Humidity: N/D	Growth performance (↑BWG, ↑FI, ↓FCR), organ weight (↑BF, ↑thymus), immune response (↑ND antibody titer, ↑IB antibody titer), blood parameter (↓heterophil, ↑lymphocyte, ↓$CD8^+$), stress biomarker (↓H:L ratio, ↓plasma CORT)	[40]
	1 mg/kg CrPic	-	Temperature: 23.9–38.0℃ for 3 h/d, 38.0℃ for 5 h/d, 38.0–23.9℃ for 4 h/d, and 23.9℃ for 12 h/d Humidity: 55%	No significance	[131]
	0.5 mg/kg CrPic	0.5 mg/kg CrPic	Temperature: 31.5–39.4℃ Humidity: 65.9–88.3%	Growth performance (↑BW, ↑BWG), plasma parameter (↑GPx), serum parameter (↓MDA, ↑protein, ↓cholesterol, ↓glucose)	[132]
	20, 30, 50 mg/kg Cr-Met	20 mg/kg CrMet	Temperature: 32℃ Humidity: N/D	Growth performance (↑BWG, ↓FCR)	[133]
	0.4, 2.0 mg/kg CrPic 0.4, 2.0 mg/kg CrPro 0.4, 2.0 mg/kg $CrCl_3$	0.4 mg/kg CrPic 0.4 mg/kg CrPro 0.4 mg/kg CrC_l3	Temperature: 33 ± 2℃ Humidity: 67%	Growth performance (↑ADG), carcass trait (↑dressing yield, ↓abdominal fat), breast meat quality (↓cooking loss)	[134]
Laying hens	0.2, 0.4 mg/kg CrPic	0.2 mg/kg CrPic	Temperature: 32℃ Humidity: 50%	Egg quality (↑Roche yolk color, ↑eggshell mass, ↑eggshell thickness), serum parameter (↑Cr, ↑Ca)	[113]
	1.6 mg/kg CrPic 0.788 mg/kg CrHis	0.788 mg/kg CrHis	Temperature: 34 ± 2℃ for 8 h/d and 22℃ for 16 h/d Humidity: N/D	Growth performance (↑FI), laying performance (↑egg production), egg quality (↑egg weight, ↑eggshell weight, ↑eggshell strength, ↑Haugh unit, ↑yolk Cr), serum parameter (↑Cr, ↓glucose, ↓cholesterol, ↓LDH)	[135]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

CrPic, Chromium picolinate; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; Cr, chromium; LDH, Lactate Dehydrogenase; Nrf2, nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor kappa B; GLUT-2: glucose transporter type 2; GLUT-4, glucose transporter type 4; CrMet, chromium methionine; BF, bursa of Fabricius; ND, Newcastle disease; IB, infectious bronchitis; $CD8^+$, cytotoxic T cell; H:L ratio, heterophil to lymphocyte ratio; CORT, corticosterone; BW, body weight; GPx, glutathione peroxidase; MDA, malondialdehyde; CrPro, chromium propionate; $CrCl_3$, chromium chloride; ADG, average daily gain; CrHis, chromium histidinate; Ca, calcium.

Table 7. Effect of dietary selenium supplementation in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level	Optimal inclusion level	Rearing conditions	Effects[1)]	References
Broiler chickens	0.5, 1.0 mg/kg SeMet	0.5 mg/kg SeMet	Temperature: 24–37℃ for 4 h/d, 37℃ for 8 h/d, 37–24℃ for 4h/d, and 24℃ for 8 h/d Humidity: 47–51%	Breast meat quality (↓MDA, ↑Se)	[117]
	0.2 mg/kg SeMet	0.2 mg/kg SeMet	Temperature: 23.5–31℃ for 3 h/d, 31℃ for 7 h/d, 31–23.5℃ for 3 h/d, and 23.5℃ for 11 h/d	Breast meat quality (↑Se)	[121]
	0.6, 1.2 mg/kg nano Se	1.2 mg/kg nano Se	Temperature: 37 ± 1℃ for 6 h/d and 21 ± 1℃ for 18 h/d Humidity: 55%	Growth performance (↑BWG, ↓FCR), carcass trait (↓abdominal fat, ↑thymus weight), serum parameter (↓cholesterol, ↓LDL-C, ↓MDA, ↑GPx, ↑SOD), gut health (↑VH, ↑VH:CD), immune response (↑SRBC antibody response)	[138]
	0.1, 0.2 mg/kg nano Se	0.1 mg/kg nano Se	Temperature: 34 ± 2℃ for 12 h/d and 25 ± 2℃ for 12 h/d	Growth performance (↑BW, ↑BWG, ↓ADFI, ↓FCR), serum parameter (↓cholesterol, ↓triglyceride, ↓LDL-C, ↑glucose, ↑T_3, ↑IgA, ↑IgG, ↑IgM, ↓MDA, ↑GPx, ↑SOD)	[139]
	0.3 mg/kg nano Se 0.3 mg/kg sodium selenite	0.3 mg/kg nano Se	Temperature: 35 ± 1℃ for 9 h/d and 22 ± 1℃ for 15 h/d Humidity: 50–70%	Growth performance (↓FCR), carcass trait (↓abdominal fat, ↑BF weight, ↑thymus weight), breast meat quality (↑breast meat weight, ↓MDA, ↑α-tocopherol), liver characteristics (↓MDA), mRNA expression in liver (↑*GPx*, ↑*IL-2*)	[140]
	0.15 mg/kg sodium selenite 0.15 mg/kg SeMet 0.15 mg/kg Se-yeast	0.15 mg/kg Se-yeast	Temperature: N/D Humidity: N/D	Growth performance (↑FI, ↑BW, ↓FCR), breast meat quality (↑Se, ↑GPx), serum parameter (↑Se, ↓MDA, ↓total oxidant status, ↑GPx)	[141]
	0.3 mg/kg sodium selenite	0.3 mg/kg sodium selenite	Temperature: 31–38℃ for 5 h/d and 26–30℃ for 19 h/d Humidity: 60–80%	Breast meat quality (↑WHC, ↑L*, ↑a*, ↑b*, ↑Se, ↑GSH, ↑GPx, ↑SOD, ↓MDA), sensory trait (↑color, ↑chewingness, ↑overall acceptability), mRNA expression in breast meat (↑*GPX1,* ↑*GPX4,* ↓*HSP70*)	[142]
Laying hens	0.4 mg/kg Se-yeast	0.4 mg/kg Se-yeast	Temperature: 35℃ Humidity: N/D	Growth performance (↑FI, ↓FCR), laying performance (↑egg number), blood parameter (↑total leucocyte, ↑SRBC antibody titer, ↑TSI, ↑BSI, ↓IL-1β, ↓TNF-α, ↓MDA), stress biomarker (↓H:L ratio, ↓blood CORT)	[37]
	0.25, 0.50 mg/kg sodium selenite	0.25 mg/kg sodium selenite	Temperature: 30.7–31.6℃ Humidity: 52.5–58.7%	Growth performance (↓FI, ↓FCR), blood parameter (↑PCV, ↓heterophil, ↑lymphocyte) serum parameter (↑total cholesterol, ↑globulin, ↓ALT, ↑Ca)	[125]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

SeMet, selenium methionine; MDA, malondialdehyde; Se, selenium; BWG, body weight gain; FCR, feed conversion ratio; LDL-C, low-density lipoprotein cholesterol; GPx, glutathione peroxidase; SOD, superoxide dismutase; VH, villus height; CD, crypt depth; SRBC, sheep red blood cells; BW, body weight; ADFI, average daily feed intake; T3, triiodothyronine; Ig, immunoglobulin; BF, bursa of Fabricius; IL, Interleukin; Se-yeast, selenium-enriched yeast; WHC, water holding capacity; L*, lightness; a*, redness; b*, yellowness; GSH, glutathione; GPX1, glutathione peroxidase 1; GPX4, glutathione peroxidase 4; HSP70, heat shock protein 70; TSI, T-lymphocyte stimulation index; BSI, B-lymphocyte stimulation index; TNF-α, tumor necrosis factor α; H:L ratio, heterophil to lymphocyte ratio; CORT, corticosterone; PCV, packed cell volume; ALT, alanine aminotransferase; Ca, calcium.

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trait and decreased MDA [117,121,140–142]. El-Deep et al. [140] and Safdari-Rostamabad et al. [138] demonstrated that dietary supplementation of 0.3 and 0.12 mg/kg nano Se increased thymus and bursa of Fabricius weights while reducing abdominal fat of broiler chickens exposed to heat stress. Supplementation of 0.15 mg/kg Se-yeast and 0.1 and 1.2 mg/kg nano Se elevated glucose, Se concentrations, GPx, and SOD in the serum and reduced MDA, triglyceride, cholesterol, LDL cholesterol, and total oxidant status in the serum of broiler chickens subjected to heat stress [138,139,141]. Inclusion levels of nano Se at 0.1 and 1.2 mg/kg in diets increased T_3, IgA, IgG, IgM, and sheep red blood cell (SRBC) antibody response of broiler chickens raised under heat stress [138,139]. Moreover, 0.3 mg/kg of sodium selenite and nano Se increased *glutathione peroxidase 1*, *glutathione peroxidase 4*, *GPx*, and *IL-6* and decreased *HSP70* expression in liver and breast meat of broiler chickens exposed to heat stress [140,142]. Safdari-Rostamabad et al. [140] observed that 1.2 mg/kg nano Se improved VH and VH:CD ratio of heat-stressed broiler chickens. In laying hens exposed to heat stress, dietary supplementation of 0.25 mg/kg sodium selenite and 0.4 mg/kg Se-yeast improved FI and FCR [37,125]. In blood parameters of laying hens under heat stress conditions, 0.25 mg/kg sodium selenite and 0.4 mg/kg Se-yeast enhanced hematocrit volume, lymphocyte, leucocyte, globulin, T-lymphocyte and B-lymphocyte stimulation index, SRBC antibody titer, and Ca concentrations and reduced heterophil, ALT, MDA, IL-1β, and TNF-α in the blood [37,125]. Abbas et al. [37] observed that dietary supplementation of 0.4 mg/kg Se-yeast decreased H:L ratio and CORT concentrations in the blood of laying hens subjected to heat stress. Under heat stress, dietary Se enhances physiological stability and immune competence in broiler chickens and laying hens by activating antioxidant enzymes such as GPx and SOD, suppressing NF-κB mediated inflammatory responses, and improving intestinal morphology and stress indicators, thereby contributing to improved productivity, oxidative balance, and health [37,136–139]. Therefore, dietary Se may be beneficially utilized to improve productivity, immune function, stress tolerance, antioxidant defense, and gut health in broiler chickens and laying hens raised under heat stress conditions.

Zinc (Zn) is an essential trace mineral necessary for productivity, bone development, feather growth, immune function, appetite regulation, carbohydrate and energy metabolism, protein turnover, and nucleic acid synthesis [143,144]. It is also a component of the antioxidant enzyme SOD, which converts superoxide radicals into hydrogen peroxide and oxygen, thus interrupting chain reactions of reactive oxygen species [145]. Zn is not stored in the body. Therefore, it must be regularly replenished through diet. Dietary Zn supplementation for broiler chickens exposed to heat stress has been shown to increase productivity and positively influence cholesterol levels and fatty acid oxidation [146]. Moreover, dietary Zn has demonstrated immunomodulatory effects. It may increase immune-related organ weights and enhance antibody production in broiler chickens exposed to heat stress [106,147].

Previous studies evaluating the effects of dietary zinc in broiler chickens and laying hens raised under heat stress are summarized in Table 8. In broiler chickens raised under heat stress, dietary supplementation of 60 mg/kg Zn sulphate, 60 mg/kg Zn oxide, 100 mg/kg ZnMet, and 20 mg/kg nano Zn improved BW, BWG, FI, FCR, and digestibility of dry matter, crude protein, and ether extract [106,147–150]. Saleh et al. [148] reported that 100 mg/kg ZnMet elevated breast meat weight and Zn concentrations, and reduced MDA in the breast meat. Furthermore, dietary supplementation of 60 mg/kg Zn sulphate and 100 mg/kg ZnMet decreased abdominal fat and increased spleen, thymus, BF, pancreas, and small intestine weights of broiler chickens exposed to heat stress [106,147,148,150]. Shah et al. [147] observed that supplementation of 60 mg/kg Zn sulphate in diets improved length, width, and area of lymphatic follicle in the bursa of Fabricius in broiler chickens subjected to heat stress. In blood parameters, dietary supplementation of 60

Table 8. Effect of dietary zinc supplementation in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level	Optimal inclusion level	Rearing conditions	Effects[1)]	References
Broiler chickens	60 mg/kg Zn sulphate	60 mg/kg Zn sulphate	Temperature: 25–34℃ for 3 h/d, 34℃ for 6 h/d, 34–25℃ for 3 h/d, and 25℃ for 12 h/d Humidity: 45.12–61.91%	Growth performance (↑BW, ↑FI, ↓FCR), organ weight (↑spleen, ↑thymus, ↑BF), blood parameter (↑leucocyte, ↑lymphocyte, ↑monocyte, ↑ND antibody titier, ↑IBD antibody titier)	[106]
	30, 60 mg/kg Zn sulphate	60 mg/kg Zn sulphate	Temperature: 35 ± 1℃ for 8 h/d and 26℃ for 16 h/d Humidity: 75 ± 5% for 8 h/d and 65% for 16 h/d	Growth performance (↑BW, ↓FCR) organ weight (↑pancreas, ↑spleen, ↑BF, ↑small intestine), BF characteristic (↑length of lymphatic follicle, ↑width of lymphatic follicle, ↑area of lymphatic follicle)	[147]
	25, 50, 100 mg/kg ZnMet	100 mg/kg ZnMet	Temperature: 33–36℃ Humidity: 60–70%	Growth performance (↑BW, ↑ADG, ↓FI, ↓FCR, ↑dry matter digestibility, ↑CP utilization), carcass trait (↓abdominal fat), breast meat quality (↑breast meat, ↓MDA, ↑Zn), blood parameter (↓cholesterol, ↑GPx, ↑ND antibody titer, ↑IBD antibody titer)	[148]
	60 mg/kg Zn oxide	60 mg/kg Zn oxide	Temperature: 38℃ Humidity: 61%	Growth performance (↑BW, ↑FI, ↓FCR), blood parameter (↑RBC, ↑WBC, ↑Hb)	[149]
	20, 40, 60 mg/kg nano Zn	20 mg/kg nano Zn	Temperature: 37.8℃ for 1–10 days, 35.8℃ for 11–21 days, and 29.9℃ for 22–42 days Humidity: N/D	Growth performance (↑BW, ↑BWG, FCR), nutrient digestibility (↑CP, ↑ether extract, ↑crude fiber), serum parameter (↓SGPT, ↓SGOT, ↓creatinine, ↑Zn, ↑phosphorous), carcass trait (↑dressing yield, ↓fat)	[150]
	30, 60 mg/kg sulphate	60 mg/kg sulphate	Temperature: 35 ± 1℃ for 8 h/d and 26℃ for 16 h/d Humidity: 75 ± 5% for 8 h/d and 65% for 16 h/d	Gut health (↑VH, ↑VW, ↓CD, ↑VH:CD, ↑villus surface area, ↑lamina propria thickness, ↑goblet cell, ↑intraepithelial lymphocyte)	[151]
Laying hens	110 mg/kg Zn sulphate 110 mg/kg ZnPro	110 mg/kg ZnPro	Temperature: 32 ± 1℃ Humidity: N/D	Liver characteristic (↑Zn) pancreas characteristic (↑Zn)	[152]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

BW, body weight; FI, feed intake; FCR, feed conversion ratio; BF, bursa of Fabricius; ND, Newcastle disease; IBD, infectious bursal disease; ZnMet, zinc methionine; ADG, average daily gain; CP, crude protein; MDA, malondialdehyde; Zn, znic; GPx, glutathione peroxidase; RBC, red blood cell; WBC, white blood cell; Hb, hemoglobin; BWG, body weight gain; SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; VH, villus height; VW, villus width; CD, crypt depth; ZnPro, zinc propionate.

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mg/kg Zn sulphate, 60 mg/kg Zn oxide, 100 mg/kg ZnMet, and 20 mg/kg nano Zn elevated red blood cell, white blood cell, hemoglobin, lymphocyte, leucocyte, monocyte, Zn, phosphorous, GPx, ND antibody titer, IBD antibody titer and reduced serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, cholesterol, and creatinine of broiler chickens raised under heat stress [106,148–150]. Dietary 60 mg/kg Zn sulphate increased VH, villus width, VH:CD, villus surface area, lamina propria thickness, goblet cell count, and intraepithelial lymphocyte of broiler chickens exposed to heat stress [151]. In laying hens, dietary inclusion levels of 110 mg/kg ZnPro elevated Zn concentrations in the liver and pancreas of laying hens subjected to heat stress [152]. Dietary Zn supplementation enhances antioxidant capacity via SOD activation, supports nutrient metabolism and digestibility, and regulates immune and intestinal functions [106,143–147]. These physiological benefits help broiler chickens and laying hens maintain homeostasis and health under heat stress, ultimately leading to improved productivity and tissue integrity [150–152]. Therefore, under heat stress conditions, dietary Zn supplementation may support improved performance, immunity, and gut health in broiler chickens. It may partially offer benefits to laying hens.

Feed additives

Betaine serves primarily as a methyl donor in methyl transferase reactions, particularly in the Met-homocysteine methylation process, thereby supporting lipid metabolism as well as carnitine and creatine biosynthesis [153–155]. In addition to its methyl-donating role, betaine also acts as an osmolyte due to its uncharged nature and high solubility in water, enabling it to attract and retain water molecules [156]. Dietary inclusion of betaine in heat-stressed broilers and layers supports intracellular hydration, leading to improvements in resilience, productivity, and antioxidant capacity [114,157,158].

Results of previous studies examining effects of dietary supplementation of betaine in broiler chickens and laying hens raised under heat stress conditions are summarized in Table 9. In broiler chickens subjected to elevated temperatures, dietary supplementation of 1.0, 2.0, and 4.0 g/kg betaine improved BW, BWG, ADG, FI, average daily feed intake, and FCR [158–161]. Wen et al. [162] reported that dietary supplementation of betaine at 1.0 g/kg decreased MDA and increased redness, SOD, GSH, and GPx in the breast meat of broiler chickens under heat stress. In addition, inclusion levels of 2.0 and 4.0 g/kg betaine in diets enhanced dressing yield and reduced abdominal fat, intramuscular fat width, and subcutaneous fat thickness of broiler chickens raised under heat stress [159,160]. Dietary supplementation of betaine at 1.0, 2.0, and 4.0 g/kg increased lymphocyte, GPx, SOD, and ND antibody titer and decreased triglyceride, free fatty acids, total cholesterol, HDL, LDL, diamine oxidase, and MDA of broiler chickens reared under thermal stress [158–161,163]. Wen et al. [161] observed that dietary supplementation of 1.0 g/kg betaine improved MDA, GSH, GPx, and SOD in liver and mitochondria of broiler chickens exposed to heat stress. Dietary supplementation of 1.0 g/kg betaine enhanced the gut health of broiler chickens subjected to heat stress by decreasing IL-1β and increasing IL-10 and secretory IgA in the jejunal mucosa [163]. Furthermore, 1.0 g/kg betaine increased mRNA expression levels of *glutathione peroxidase 1* and *uncoupling protein* in the liver, while decreasing the expression levels of *HSP70* and increasing expression levels of *OCLN* in the jejunal mucosa of broiler chickens maintained in high-temperature environments [161,163]. In heat-stressed laying hens, dietary supplementation of 1.0 g/kg betaine enhanced BW, FI, FCR, crude protein digestibility, laying rate, and egg mass [114]. Attia et al. [114] also found that dietary inclusion levels of 1.0 g/kg betaine enhanced glucose, estrogen, progesterone, T_3, T_4, and weights of liver, spleen, thyroid, ovary, large follicle, and oviduct. The positive effects of dietary betaine supplementation in broiler chickens and laying hens exposed to heat stress may be attributed to its roles in donating methyl groups for metabolic regulation,

Table 9. Effect of dietary betaine supplementation in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level (g/kg)	Optimal inclusion level (g/kg)	Rearing conditions	Effects[1)]	References
Broiler chickens	1.0	1.0	Temperature: 33 ± 1℃ Humidity: N/D	Growth performance (↑BWG, ↓FCR), serum parameter (↓MDA, ↑GPx, ↑SOD)	[158]
	1.0, 2.0, 4.0	4.0	Temperature: 32 ± 1℃ Humidity: N/D	Growth performance (↑BWG, ↓FI, ↓FCR), carcass trait (↓abdominal fat, ↓intramuscular fat width, ↓subcutaneous fat thickness), serum parameter (↓triglyceride, ↓total cholesterol, ↓free fatty acids, ↓HDL-C, ↓LDL-C)	[159]
	1.0, 1.5, 2.0	2.0	Temperature: 25.4–35.8℃ Humidity: 52–87%	Growth performance (↑FI, ↑BWG, ↓FCR), carcass trait (↑dressing), blood parameter (↑lymphocyte, ↑ND antibody titer)	[160]
	1.0	1.0	Temperature: 34 ± 1℃ for 8 h/d and 22 ± 1℃ for 16 h/d Humidity: N/D	Growth performance (↑BW, ↑ADG, ↑ADFI), serum parameter (↓ALT), liver characteristics (↓MDA, ↑SOD, ↑GSH, ↑GPx), mitochondria characteristics (↑SOD, ↑GPx), mRNA expression in liver (↑*GPX1,* ↑*UCP*)	[161]
	1.0	1.0	Temperature: 34℃ for 8 h/d and 22℃ for 16 h/d Humidity: 65–75%	Breast meat quality (↑a*, ↑SOD, ↑GSH, ↑GPx, ↓MDA)	[162]
	1.0	1.0	Temperature: 33 ± 1℃ for 8 h/d and 22 ± 1℃ for 16 h/d Humidity: 50–60%	Stress biomarker (↓serum CORT), serum parameter (↓DAO), gut health (↓IL-1β, ↑IL-10, ↑SIgA), mRNA expression in jejunal mucosa (↓*HSP70,* ↑*OCLN,* ↑*ZO-1*)	[163]
Laying hens	0.1	0.1	Temperature: 38 ± 1℃ for 4 h in 3 days a week Humidity: 55–65%	Growth performance (↑BW, ↑FI, ↓FCR, ↑CP digestibility), laying performance (↑laying rate, ↑egg mass), blood parameter (↑glucose, ↑estrogen, ↑progesterone, ↑T_3, ↑T_4), carcass trait (↑liver weight, ↑spleen weight, ↑thyroid gland weight, ↑ovary weight, ↑large follicle weight, ↑oviduct weight)	[114]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

BWG, body weight gain; FCR, feed conversion ratio; MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase; FI, feed intake; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ND, Newcastle disease; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; ALT, alanine aminotransferase; GSH, glutathione; GPX1, glutathione peroxidase 1; UCP, uncoupling protein; a*, redness; CORT, corticosterone; DAO, diamine oxidase; IL, interleukin; SIg, secretory immunoglobulin; HSP70, heat shock protein 70; OCLN, occludin; ZO-1, zonula occludens-1; CP, crude protein; T_3, triiodothyronine; T_4, thyroxin.

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Table 10. Effect of dietary carnitine supplementation in broiler chickens and laying hens raised under heat stress conditions

Animals	Inclusion level (g/kg)	Optimal inclusion level (g/kg)	Rearing conditions	Effects[1)]	References
Broiler chickens	0.5	0.5	Temperature: 29.3–36.6℃ Humidity: 52.0–65.8%	Growth performance (↑BW, ↑FI), blood parameter (↓heterophil) serum parameter (↑IBD antibody titer, ↑paraoxonase, ↓total oxidant status, ↓glucose, ↑total protein)	[118]
	0.5	0.5	Temperature: 29.3–36.5℃ Humidity: 52.0–65.8%	Serum parameter (↓AST, ↓glucose, ↑protein, ↓cholesterol, ↓triglyceride, ↓LDL, ↑HDL)	[122]
	0.2	0.2	Temperature: 33.0–34℃ for 8 h/d and 24 ± 1℃ for 16 h/d Humidity: 60 ± 5%	Carcass trait (↑breast meat weight, ↓abdominal fat, ↓thigh fat, ↓breast fat), organ weight (↑thymus), blood parameter (↓heterophil, ↑SRBC antibody titer, ↑IB antibody titer), plasma parameter (↑catalase, ↓MDA)	[166]

[1)]The symbol '↑' represented an increase, while '↓' denoted a decrease.

BW, body weight; FI, feed intake; IBD, infectious bursal disease; AST, aspartate aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SRBC, sheep red blood cells; IB, infectious bronchitis; MDA, malondialdehyde.

maintaining cellular hydration as an osmoprotectant, enhancing antioxidant enzyme activity, and modulating immune and inflammatory responses [114,153–158]. These functions collectively support improved productivity, gut health, and physiological stability [114,153–159,163]. Therefore, dietary betaine supplementation may be effectively used to alleviate heat stress and improve performance, immunity, antioxidant capacity, and gut health in broiler chickens and laying hens raised under heat stress.

Carnitine, a derivative of amino acid, plays a key role in mitochondrial function by facilitating the β-oxidation of fatty acids, thereby contributing to cellular energy production [164,165]. By buffering excess acyl residues, carnitine contributes to an increased acetyl CoA/CoA ratio within the mitochondria [165]. Although poultry can synthesize carnitine endogenously, dietary supplementation of carnitine may be required under stress conditions. Carnitine supplementation in diets helps convert fat into energy in broilers raised under heat stress and oxidative stress, while also improving serum antibody production, and reducing oxidative stress [166].

Previous studies evaluating the effects of dietary carnitine on broiler chickens and laying hens raised under heat stress are summarized in Table 10. In broiler chickens subjected to high temperatures, dietary supplementation of 0.5 g/kg carnitine enhanced BW and FI [118]. Ghasemi and Nari [166] reported that 0.2 g/kg carnitine in diets increased breast meat and thymus weight and decreased abdominal fat, thigh fat, and breast fat of broiler chickens exposed to heat stress. In addition, dietary supplementation of 0.2 and 0.5 g/kg carnitine reduced heterophil, glucose, triglyceride, cholesterol, AST, LDL, MDA, and total oxidant status and elevated total protein, CAT, HDL, IBD antibody titer, IB antibody titer, and SRBC antibody titer in the serum and plasma of broiler chickens subjected to heat stress [118,122,166]. Rehman et al. [122] and Ghasemi and Nari [166] reported that dietary supplementation of 0.2 and 0.5 g/kg carnitine increased antibody titer of IBD, IB, and SRBC in the serum of broiler chickens raised under heat stress. Dietary supplementation of carnitine supports mitochondrial fatty acid oxidation and regulates metabolic and immune functions, which helps broiler chickens mitigate the physiological burden of heat stress and leads to improved growth performance, antioxidant status, and immune responses [118,164–166]. Therefore, under heat stress conditions, dietary carnitine may contribute to improved growth performance, immune response, and overall health in broiler chickens.

CONCLUSION

Heat stress induces diverse detrimental effects in broiler chickens and laying hens, including reduced productivity, suppressed immune function, hormone imbalances, and increased

requirements for internal nutrients. These physiological disruptions contribute to considerable economic losses in poultry production. Heat stress is recognized as a major constraint to poultry production during periods of elevated temperatures, particularly in the hot summer season. To mitigate adverse effects of heat stress, dietary supplementation of functional nutrients such as amino acids, vitamins, minerals, and other feed additives has been proposed as an effective strategy for both broiler chickens and laying hens. Recent research efforts focus on evaluating the effectiveness of these nutritional interventions in mitigating heat-induced stress and improving poultry health and performance. Despite promising findings, the demand for effective functional nutrients and the optimization of their levels in diets remains urgent. Therefore, continued investigation is essential to identify and develop novel compounds and strategies capable of attenuating heat stress under both current and changing environments for poultry production. Ultimately, successful development of innovative nutritional solutions may play a crucial role in enhancing poultry welfare and sustaining productivity in the poultry industry.

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