BMC Veterinary Research

RESEARCH

Open Access

Sodium butyrate alleviates high ambient temperature-induced oxidative stress, intestinal structural disruption, and barrier integrity for growth and production in growing layer chickens

Md Touhiduzzaman Sarker[1,2,3,4,5], Shenglin Wang[1,2,3,4,5], Shuang Wang[1,2,3,4,5], Weiguang Xia[7], Yanan Zhang[1,2,3,4,5], Chenglong Jin[1,2,3,4,5], Xuebing Huang[1,2,3,4,5], Kaichao Li[1,2,3,4,5], Abdelmotaleb Elokil[1,2,3,4,5,6], Yantai Lv[7], Chuntian Zheng[1,2,3,4,5*] and Wei Chen[1,2,3,4,5*]

Abstract

Background This study was conducted to evaluate the effects of dietary sodium butyrate (SB) supplementation on the antioxidant status, intestinal morphology, functional damage, and barrier integrity of heat-stressed Hy-Line Sonia (HYS) layer chicks. A total of 240 female HYS at 35 days of age with average body weights (415 ± 35 g) were divided into 6 groups with 10 replicates/group and 4 chickens per replicate. A 2 × 3 factorial design study was performed, including two conditions of ambient temperature (25 °C and 35 °C) and three dietary levels of SB (0, 0.5, and 1.0 SB g/kg diet).

Results HS decreased ($P < 0.05$) the performance parameters final body weight (FBW), average daily gain (ADG), and average daily feed intake (ADFI), and increased mortality; compared with the HS groups, supplementation with SB decreased mortality. Compared with thermoneutral conditions, the high-temperature conditions significantly decreased ($P < 0.05$) the thymus, liver, and heart weights, and the relative length of the jejunum, ileum, and cecum, whereas supplementation with 0.5 SB g/kg diet increased ($P < 0.05$) the weight of the spleen in growing layer chickens. High temperature decreased ($P < 0.05$) the villus height (VH) and VH/CD ratio, and increased the crypt depth (CD), and supplementation with SB and the T × SB interaction produced greater VH and VH/CD values in the LSB2 and HSB2 groups. SB decreased ($P < 0.05$) the concentration of serum malondialdehyde (MDA); however, high temperature decreased ($P < 0.05$) the activities of the catalase (CAT) and glutathione peroxidase (GSH-Px) antioxidant enzymes. The relative mRNA expression levels of the occluding, zonula occludens-1 (ZO-1), claudin-1, and interleukin-10 (IL-10) proteins were downregulated ($P < 0.05$) at high-temperatures, while that of transforming growth factor-β (TGFβ) was upregulated. Dietary supplementation decreased the expression of the inflammatory cytokines nuclear factor kappa B (NF-κB), transforming growth factor-β (TGFβ), and interferon-γ (IFNγ), and the T × SB interaction decreased

*Correspondence:
Chuntian Zheng
zhengcht@163.com
Wei Chen
cwei010230@163.com
Full list of author information is available at the end of the article

TGFβ gene expression in the LSB2 and HSB2 groups compared with that in the other groups of growing layer chickens.

Conclusion SB supplementation effectively alleviated HS-induced oxidative stress and structural and functional damage to the intestine in layer chickens in the growing phase.

Keywords Antioxidant, Growing layer chicken, Heat stress, Intestine integrity, Sodium butyrate

Background

Despite advancements in poultry farming design, housing facilities, and cooling systems, the health and overall productivity of farm animals are still significantly affected by high ambient temperatures. High temperatures have emerged as a significant environmental factor, that negatively affects poultry health and production, particularly in tropical and subtropical climate areas [1, 2]. The southern part of China, surrounding areas of Guangdong, has a subtropical monsoon climate; summer is hot with high humidity and heavy rainfall. In this context, nutritional regulation by dietary sodium butyrate (SB) supplementation may function as an alleviator of the effects of heat stress (HS) on antioxidant enzyme activities and gastrointestinal integrity during the growth and development of chickens. Several animal researchers have focused on different strategies to mitigate the adverse effects of HS and reduce production loss. In addition, chickens exposed to HS have altered intestinal structure, including villus height (VH), crypt depth (CD), and epithelial cell damage [3]. Several studies have reported that HS negatively impacts feed intake, energy efficiency, physiological function, the oxidative state, and the development of growing chickens [4]. The small intestine is extremely vulnerable to high temperatures, which hampers intestinal function by damaging its morphology, changing intestinal integrity, and downregulating the mRNA expression of tight junction-related genes [5, 6]. Heat stress may disrupt the gastrointestinal blockade in growing chickens by reducing the levels of internal junction proteins, such as zonula occludens-1(ZO-1) and occluding (OCLN) [7].

Dietary SB is used in growing broiler chicken as a feed additive or stress modulator because studies have reported that SB has beneficial effects on HS conditions in the poultry industry [8]. The addition of SB improved bird growth performance, feed intake efficiency, nutritional absorption capacity, and immunological response [9]. The protective forms of SB may enhance nutrient absorption by expanding the length, circumference, and villus area of the anterior part of the digestive tract during stress conditions [10]. Increasing research has shown that SB can reduce oxidative stress, intestinal inflammation, and microbial development and subsequently modulate the immune system; these properties have beneficial impacts on the intestinal structure, microbial cell proliferation, growth, and production of chickens [11, 12]. Under typical circumstances, the antioxidant system safeguards the animal from oxidative damage, mostly through the action of protective antioxidant enzymes, such as, total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), which help reduce oxidative stress at the cellular level [13]. Another study conducted on chickens revealed that adding dietary SB may alleviate oxidative damage and reduce the intensification of malondialdehyde (MDA) while increasing the activity of T-SOD [14]. We hypothesized that dietary supplementation with SB may have beneficial effects on the antioxidant capacity and intestinal barrier integrity of growing layer chickens under HS. Nevertheless, minimal research has been performed to evaluate the impacts of SB dietary supplementation on growing layer chickens under HS conditions. Therefore, the present study aimed to assess the alleviating effects of SB supplementation on the antioxidant capacity, intestinal structure, functional damage, and barrier integrity for the growth and development of growing layer chicks under HS conditions.

Methods

The procedure and protocol used in this research were authorized by the Committee of Institutional Animal Care of the Guangdong Academy of Agricultural Sciences, China, with approval number ACUCGAAC2022. The sample analysis was performed at the Animal Nutrition and Feed Science, the Key Laboratory Department of the Ministry of Agriculture and Rural Affairs, and the Laboratory of Molecular Biology, Institute of Animal Science. This experiment was conducted under the ARRIVE guidelines to minimize animal suffering.

Animals, experimental diets

At 35 d of age, a total of 240 female Hy-Line Sonia chicks (HYS) with average body weights (415 ± 35 g) were selected for this experiment. The experimental design was a 2×3 factorial arrangement, which included 6 dietary treatment groups (40 chickens per group), with each group consisting of 10 replicates ($n=4$ chickens per replicate). The experiment was conducted under two environmental conditions of 25 °C ambient temperature with 60% humidity (L) and 35 °C ambient temperature with

70% humidity (H) and three levels of dietary supplementations with SB, which included zero (SB1), 0.5 g/kg diet (SB2), and 1 g/kg diet (SB3) and the experimental period lasted for 30 days. The chicken groups of LSB1, LSB2, and LSB3 were reared under 25 °C of ambient temperature with 60% humidity and dietary supplemented with zero, 0.5 g/kg diet, and 1 g/kg diet, respectively. While, the chicken groups of HSB1, HSB2, and HSB3 were reared under 35 °C of ambient temperature with 70% humidity and dietary supplemented with zero, 0.5 g/kg diet, and 1 g/kg diet, respectively. Chickens were raised in each room containing a wire cage (dimensions: 30 × 35 × 35 cm^3) in each cage ($n = 4$ chickens/cage). For exposure to heat stress, the HSB1, HSB2, and HSB3 groups of chickens were kept for 10 h (9 am to 7 pm) under 35 °C and 70% humidity, and 16 h (7 pm to 9 am) of the day they were kept at room temperature (25 °C) and 60% humidity. Diet formulation was determined according to HYS commercial layers guide (https://www.hyline.com/literature/sonia). The ingredients and chemical composition of the diet are shown in Table 1.

Table 1 Composition and nutrient levels of the basal diet (as fed-basis, %)

Ingredients	Content (%)
Corn	55
Wheat bran	15.07
Corn protein flour	13.21
Soybean meal	1.13
Soybean oil	12.35
Lys-HCl (78%)	0.38
Limestone powder	1.56
Salt	0.3
Premix[a]	1
Total	100
Calculated nutrient levels	
ME (Mcal/kg)	3.56
CP (%)	15.03
Ca (%)	0.6
P (%)	0.35
Met (%)	0.27
Lys (%)	0.66
Met + Cys (%)	0.52
Thr (%)	0.45

[a] The premix provided the following per kilogram of diet: vitamin A 5,500 IU, vitamin D3 400 IU, vitamin E 10 IU, vitamin K 2.0 mg, vitamin B1 3.0 mg, vitamin B2 4.6 mg, vitamin B6 2.2 mg, vitamin B12 0.02 mg, choline 500 mg, D-calcium pantothenate 7.4 mg, folic acid 1.0 mg, biotin 0.08 mg, Fe 80 mg, Cu 10 mg, Mn 39 mg, Zn 52 mg, I 0.26 mg. Crude protein (CP) was measured values, metabolizable energy (ME), Ca, available P, Met, Lys, Met + Cys, and Thr are calculated values

Growth parameters of growing layer chickens

The growing chickens were weighed at the beginning (35 d) and end (65 d) of the experimental period. The feed intake (FI) was calculated on a cage basis, using supplied and residual feed. The following formulas were used to calculate the average daily total feed intake, ADFI (g/d) = sum of the feed intake (g)/feeding period (d); average daily gain, ADG (g/d) = (final live weight at 63 d of age – initial live weight at 35 d of age, (g)/feeding period (d); and the feed- to-gain ratio, F/G (g/g) = overall ADFI (g/d)/overall ADG (g/d).

Sampling and evaluation

At 65 d of age, following a 12-h fast, 9 chicks per treatment were selected for sample collection. The chicks were individually weighed, and blood samples (5 mL vacutainer tubes) were collected via the wing vein and then centrifuged at 3000 × g for 10 min at 4 °C to assess the serum indices [15]. The obtained serum samples were stored in frizz at 20 °C to assess the oxidative and antioxidant markers, and other parts of the samples were then kept at -80 °C until gene expression analysis was performed. Then, the chickens were euthanized by cervical dislocation, and the immune, reproductive, and intestinal tissue along with the thymus, liver, spleen, bursa of Fabricius, heart, gastrointestinal tract, ovary, duodenum (from where the pyloric junction and duodenal mesentery are connected), jejunum (from the confluence with Meckel's diverticulum, where the duodenal mesentery is jointed), ileum (from the junction with Meckel's diverticulum to the ileocecal junction) and cecum (from the opening to the tip of each) were collected [16]. The length of each section of the small intestine was subsequently assessed using a pliable measuring tape on a glass surface to avoid unintentional elongation. The reproductive and immune organ weights were determined as a percentage of the bird's weight (g/kg), and the lengths of the intestinal segments and cecum (the combined length of both sides) were measured relative to the live body weight (cm/kg). The measurement methods were based on previous studies [17]. The ileum segment of the intestine was collected and stored in 10% formaldehyde solution for further morphological analysis.

Serum antioxidant indices

The antioxidant enzyme activities in the serum were determined via the corresponding assay kits following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) using a spectrophotometer. Briefly, the malondialdehyde (MDA) level was measured at 532 nm (MDA, catalog. No. A003-1), hydrogen peroxide (H2O2) level was measured at 405 nm (H2O2, catalog. No. A064-1), the catalase (CAT) activity was measured at 405 nm (CAT, catalog. No. A007-1),

the glutathione peroxidase (GSH-Px) activity was measured at 412 nm (GSH-Px, catalog. No. A005-1), and the total antioxidant capacity (T-AOC) (T-AOC, catalog. No. A001-3) and total superoxide dismutase (T-SOD) (T-SOD, catalog. No. A015-2) activities were measured at 550 nm.

Ileum morphology

Approximately 5 cm of the ileum segment was excised, washed, dehydrated with an ethanol solution, and then embedded in paraffin wax for intestinal morphology analysis. The tissue sample of the ileum was then cross-sectioned at thickness of 5 µm via a microtom (Leica Microsystems, Wetzlar, Germany) and set on glass slides for morphological staining with hematoxylin and eosin (H&E) following the standard procedure [18]. The VH and CD were determined via a previously described procedure [19]. Images of the VH and CD were captured and enlarged at 40× magnification via a light microscope with Image-Pro Plus version 6.0. Software (Ellipse Ci-L, Nikon, Japan).

RNA extraction and real-time qPCR (RT-qPCR)

Total RNA ($n=9$/ group) was extracted from a homogenized ileum tissue sample using a TRIzol reagent kit (TaKaRa Biotechnology, Dalian, Liaoning, PR China) following the manufacturer's instructions. The total RNA purity and quantity were determined using a NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The optical density of all RNA samples ranged from 260 to 280. Afterward, the total RNA sample value was set to 1 µg to generate first-strand complementary DNA (cDNA) using the Prime Script RT reagent kit for removing genomic DNA by DNase (TaKaRa Biotechnology, Dalian, Liaoning, PR China). The qPCR mixture (20 µL) was set up as follows: 2 µL of diluted cDNA, 0.4 µL of each primer (10 µM), 10 µL of 2×SYBR Green Pro Taq HS Premix (Takara, Biotechnology Co., Ltd, Dalian, China), 0.4 µL of ROX dye, and 6.8 µL of double-distilled water (H_2O_2) on a 7,500 qPCR detection system (Bio-Rad, San Diego, CA, USA). The temperature-cycling program for the PCR was as follows: 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 30 s. The melting curve was recorded at 65 °C for 5 s. The mRNA expression levels in the ileum are shown in Table 2. The gene expression levels of the target genes were assessed by the $2^{-\Delta\Delta Ct}$ technique [20], with the reference gene GAPDH used as the housekeeping gene.

Table 2 The prime sequence used for quantitative real-time PCR analysis for gene expression

Gene[a]	Primer sequences(5′ − 3′)	Accession ID	Product size (bp)
GAPDH	F-GGTGAAAGTCGGAGTCAACGG	NM_204305.2	108
	R-TCGATGAAGGGATCATTGATGGC		
Claudin-1	F-GGTTGGTGTGTTTGTTGCTG	NM_001013611.2	199
	R-TCTGGTGTTAACGGGTGTGA		
Occludin	F-GTCTGTGGGTTCCTCATCGT	NM_205128.1	155
	R-TTCTTCACCCACTCCTCCAC		
ZO-1	F-ACCAGAGGTCAGAGCCTTCA	XM_046925214.1	193
	R-AGCGGTGGTGTTTGTTTTTC		
MUC-2	F-GCTACAGGATCTGCCTTTGC	XM_040673077.2	152
	R-AATGGGCCCTCTGAGTTTTT		
IL-10	F-GATGCTGCGCTTCTACACCG	NM_001004414.4	207
	R-TCCCGTTCTCATCCATCTTC		
TLR4	F-CACAGCTCTGGATTTCAGCA	NM_001030693.2	171
	R-TTCCGCAGTAGATCCTGCTT		
NF-kB	F-AGAGGATGCTTCGTTGTGCT	NM_001396396.1	186
	R-TCCTGGACAGCAGTGAGATG		
TGFβ	F-GGAGGAGGAGAAGGAGGAGA	NM_205454.2	206
	R-GGAACTCTGCTCGAAACAGG		
IFNγ	F-AGCCGCACATCAAACACATA	NM_205149.2	153
	R-TCCTTTTGAAACTCGGAGGA		
TNFα	F-CTGTTCTATGACCGCCCAGT	NM_204267.2	169
	R-TCAGAGCATCAACGCAAAAG		

[a] *IL-10* interleukin-10, *TNF-α* tumour necrosis factor- α, *IFN-γ* interferon-γ, *TLR-4* toll-like receptor-4, *TGF-β* transforming growth factor-β, *NF-κB* nuclear factor kappa B, *MUC-2* mucin-2, *ZO-1* zonula occludens-1, *GAPDH* glyceraldehyde 3 phosphate dehydrogenase

Statistical analysis

Data obtained from the current experimental study were statistically analyzed on a 2×3 factorial design based on the following statistical model: $Y_{ijk}=\mu+T_i+S_j+TS_{ij}+e_{ijk}$ where Y_{ijk} represents the observation, µ denotes the overall mean, T_i indicates the effect of the ambient temperature, S_j signifies the effect of the sodium butyrate level, TS_{ij} reflects the interaction between the ambient temperature and sodium butyrate level, and e_{ijk} represents random error via the GLM procedure in SPSS (version 20, SPSS, Inc., Armonk, New York, USA). The significant differences between treatment groups were investigated using Duncan's multiple-range tests. The threshold for significance was set at $P<0.05$, and the tendency toward significance was set at $0.05<P<0.10$. The data in the tables are presented as the means with their pooled SEM.

Results

Performance parameters

The effects of dietary sodium butyrate supplementation, temperature, and their interactions on the performance parameters of growing layer chickens are shown in Table 3. The two-way probabilities of the effects of dietary

SB supplementation level, temperature and their interaction (SB × T) on the FBW, ADFI ADG, and F/G ratio were no significantly different ($P < 0.05$). Exposure to heat stress significantly decreased ($P < 0.05$) the FBW, ADG, and ADFI but not the F/G ratio, and increased mortality during the entire experimental period. In addition, the SB × T interaction significantly ($P < 0.05$) decreased mortality in the HSB2 group compared with the other groups under the HS conditions.

Immune organ indices

The immune organ parameters are shown in Table 4. The results revealed that, compared to thermoneutral conditions, high ambient temperatures significantly decreased ($P < 0.05$) the thymus, liver, and heart weights. In addition, dietary supplementation with 0.5 SB g/kg resulted in increased ($P < 0.05$) spleen weight in growing layer chickens.

Relative length of the small intestinal segment

The lengths of the intestinal segments used the present study are presented in Table 5. Compared with thermoneutral conditions, high ambient temperature significantly decreased ($P < 0.05$) the length of the jejunum, ileum, and cecum length.

Ileum morphology

The effects of three levels of SB supplementation at ambient temperature and their interaction on the ileum morphology of growing layer chickens are summarized in Table 6 and Fig. 1. Compared with those under thermoneutral conditions, the VH and VH/CD ratio under high-temperature conditions significantly decreased ($P < 0.05$). In addition, dietary supplementation with 0.5 SB g/kg diet resulted in higher VH and VH/CD ratio than the other treatments did. The T × SB interaction ($P < 0.05$) affected the VH and VD/CD in the LSB2 and HSB2 groups, which were greater than those in the other groups and lower than those in the CD in the LSB2 and HSB3 groups.

Serum antioxidant indices

The serum antioxidant results are presented in Table 7. The results regarding the impact of dietary supplementation in the 0.5 SB g/kg diet group revealed a lower ($P < 0.05$) serum MDA concentration than that in the

Table 3 Effects of ambient temperature and different levels of sodium butyrate (SB) on growth performance, during the growing stage of laying chickens

Parameters	IBW (g)	FBW (g)	ADG (g)	ADFI (g)	F/G (g/g)	Mortality (%)
Ambient temperature (T)						
L, 25 °C	415.47	697.53[a]	9.40[a]	20.58[a]	2.26	8.33[b]
H, 35 °C	415.38	669.81[b]	8.48[b]	19.27[b]	2.31	17.50[a]
Sodium Butyrate (SB)						
SB1, 0 g/kg diet	415.28	670.72	8.51	19.85	2.38	15.00
SB2, 0.5 g/kg diet	415.56	700.25	8.82	20.29	2.19	10.00
SB3, 1 g/kg diet	415.44	680.05	8.49	19.61	2.27	13.75
Interaction T × SB						
LSB1	415.30	685.55	9.00	20.61	2.33	7.50[c]
LSB2	415.73	713.05	9.91	20.91	2.21	7.50[c]
LSB3	415.38	694.00	9.28	20.13	2.23	10.00[b]
HSB1	415.27	655.88	8.02	19.10	2.43	22.50[a]
HSB2	415.38	687.44	9.06	19.60	2.17	12.50[b]
HSB3	415.50	666.11	8.35	19.10	2.32	17.50[a]
SEM	4.15	13.29	0.48	0.30	0.12	0.33
Two ways probabilities*						
T	0.980	0.014	0.024	0.001	0.622	0.001
SB	0.998	0.087	0.130	0.094	0.234	0.073
T × SB	0.998	0998	0.989	0.633	0.840	0.025

IBW initial body weight, *FBW* final body weight, *ADG* average daily gain, *ADFI* average daily feed intake, *F/G* feed per gain ratio, *L* Low ambient temperature, *H* High ambient temperature, *SEM* standard error of the mean. LSB1: group fed without sodium butyrate (SB) supplement at low temperature, LSB2: group fed 0.5 SB g/kg diet at low temperature, LSB3: group fed 1.0 SB g/kg diet at low temperature, HSB1: group fed without SB supplement at high temperature, HSB2: group fed 0.5 SB g/kg diet at high temperature, HSB3: group fed 1.0 SB g/kg diet at high temperature

[a;b] Values in the same column with different letter superscripts mean significant differences ($P < 0.05$). Data represent treatment mean ± SEM from $n = 9$ chicks analyzed per treatment

* Overall treatment *P*-value

Table 4 Effects of ambient temperature and different levels of sodium butyrate (SB) on relative immune organ weight during the growing stage of laying chickens

Parameters	Spleen (g/kg BW)	Bursa (g/kg BW)	Thymus (g/kg BW)	Liver (g/kg BW)	Heart (g/kg BW)	Intestine (g/kg BW)	Ovary (g/kg BW)
Ambient temperature (T)							
L, 25 °C	1.61	3.11	3.42[a]	18.31[a]	5.02[a]	38.26	0.39
H, 35 °C	1.57	2.74	2.71[b]	16.67[b]	4.41[b]	36.35	0.37
Sodium Butyrate (SB)							
SB1, 0 g/kg diet	1.50[b]	2.89	2.83	17.20	4.43	36.93	0.37
SB2, 0.5 g/kg diet	1.79[a]	3.03	3.35	18.23	4.94	38.38	0.40
SB3, 1 g/kg diet	1.49[b]	2.86	3.01	17.04	4.78	36.61	0.38
Interaction T × SB							
LSB1	1.58	3.14	3.42	18.58	4.92	38.63	0.38
LSB2	1.74	3.17	3.66	19.20	5.16	39.14	0.42
LSB3	1.52	3.01	3.18	17.14	4.98	37.20	0.38
HSB1	1.41	2.63	2.25	15.81	3.95	35.22	0.36
HSB2	1.84	2.90	3.04	17.26	4.72	37.61	0.38
HSB3	1.46	2.71	2.84	16.95	4.58	36.20	0.37
SEM	0.12	0.27	0.29	0.80	0.30	1.62	0.03
Two ways probabilities*							
T	0.675	0.114	0.004	0.017	0.019	0.152	0.396
SB	0.025	0.794	0.204	0.288	0.249	0.512	0.593
T × SB	0.553	0.893	0.363	0.275	0.580	0.711	0.891

L, Low ambient temperature, *H* High ambient temperature, *BW* Body weight, *SEM* standard error of the mean. LSB1: group fed without sodium butyrate (SB) supplement at low temperature, LSB2: group fed 0.5 SB g/kg diet at low temperature, LSB3: group fed 1.0 SB g/kg diet at low temperature, HSB1: group fed without SB supplement at high temperature, HSB2: group fed 0.5 SB g/kg diet at high temperature, HSB3: group fed 1.0 SB g/kg diet at high temperature

[a,b] Values in the same column with different letter superscripts mean significant differences ($P < 0.05$). Data represent treatment mean ± SEM from $n = 9$ chicks analyzed per treatment

* Overall treatment *P*-value

other groups of growing layer chickens. On the other hand, the activities of the antioxidant enzymes CAT and GSH-Px significantly decreased ($P < 0.05$) at high ambient temperature compared with those under thermoneutral conditions, while that of T-AOC tended to decrease.

Gene expression in ileum tissue

The relative mRNA expression in ileum tissue is summarized in Table 8. Compared with that under thermoneutral conditions, the expression of the occluding, ZO-1, claudin-1, and IL-10 genes was significantly downregulated ($P < 0.05$) at high ambient temperatures, while the expression of TGFβ was upregulated. In addition, dietary supplementation with SB resulted in lower ($P < 0.05$) levels of NF-κB, TGFβ, and IFNγ than the other treatments did. However, there was an effect of the T × SB interaction ($P < 0.05$) on TGFβ gene expression in the LSB2 and HSB2 groups, being downregulated compared with that in the groups of growing layer chickens.

Discussion

Poultry are particularly vulnerable to HS due to lacking sweat glands to dissipate heat and having a naturally high body temperature. However, HS in poultry induced by high ambient temperature has a detrimental effect on production performance and intestinal capacity and results in significant financial losses to farmers [21, 22]. As previously reported [23], HS exposure in growing broiler chickens leads to behavioral and physiological changes that have negative consequences on their ADFI and ADG. The present study revealed that, compared with thermoneutral conditions, high ambient temperatures significantly decreased the ADFI, ADG, and FBW and increased mortality growing layer chickens. These results support the findings of previous studies reporting that high ambient temperature hinder the growth performance parameters of growing layer chickens by decreasing the ADFI and ADG [24, 25]. Moreover, our results indicate that the interactive effect of dietary SB supplementation with ambient temperature (T × SB) significantly improved mortality in the HSB2 group compared with the other groups. These findings imply that adding SB to the diet of growing layer chickens under high

Table 5 Effects of ambient temperature and different sodium butyrate (SB) levels on the relative length of intestinal segments during the growing stage of laying chickens

Parameters	Duodenum (cm/kg BW)	Jejunum (cm/kg BW)	Ileum (cm/kg BW)	Cecum (cm/kg BW)
Ambient temperature (T)				
L, 25 °C	10.36	45.95[a]	30.90[a]	11.93[a]
H, 35 °C	9.88	43.66[b]	29.20[b]	10.61[b]
Sodium Butyrate (SB)				
SB1, 0 g/kg diet	10.05	44.77	29.89	11.11
SB2, 0.5 g/kg diet	10.18	45.60	31.05	11.37
SB3, 1 g/kg diet	10.13	44.05	29.21	11.33
Interaction T × SB				
LSB1	10.37	46.54	32.35	11.91
LSB2	10.38	47.05	32.04	11.95
LSB3	10.33	44.25	29.32	11.92
HSB1	9.72	43.00	28.43	10.31
HSB2	9.97	44.15	30.06	10.80
HSB3	9.94	43.84	29.10	10.74
SEM	0.35	1.38	0.91	0.53
Two ways probabilities*				
T	0.097	0.049	0.027	0.006
SB	0.928	0.535	0.137	0.875
T × SB	0.915	0.494	0.335	0.903

L Low ambient temperature, *H* High ambient temperature, *BW* Body weight, *SEM* standard error of the mean. LSB1: group fed without sodium butyrate (SB) supplement at low temperature, LSB2: group fed 0.5 SB g/kg diet at low temperature, LSB3: group fed 1.0 SB g/kg diet at low temperature, HSB1: group fed without SB supplement at high temperature, HSB2: group fed 0.5 SB g/kg diet at high temperature, HSB3: group fed 1.0 SB g/kg diet at high temperature

[a;b] Values in the same column with different letter superscripts mean significant differences ($P < 0.05$). Data represent treatment mean ± SEM from $n = 9$ chicks analyzed per treatment

* Overall treatment *P*-value

ambient temperatures might alleviate HS-induced oxidative stress (OS) and balance electrolytes to reduce the effects of chronic HS during the growing phase [8].

Immune organ weights were used to assess a chicken's immunological health; the proper development of these organs is important for the humoral and cellular immune systems. The immune system serves as the cornerstone for attaining immunological function, and the spleen, bursa of Fabricius, thymus, and liver are frequently weighed due to their vital importance in the formation and operation of cellular and humoral immunity [25]. The present study revealed that high temperature significantly decreased the relative weights of the thymus, liver, and heart in growing layer chickens. These findings support those of previous studies indicating that lymphoid organ weight was reduced in broilers subjected to 6 h of HS per day [26] and reporting [27] that 15 d of thermal stress reduced the development of the thymus and liver in black-bone chickens. Moreover, the thymus is the organ in which T cells are activated and mature before move to the peripheral blood to develop immune protection. Similarly, the spleen is another key immune organ in the animal body and plays a vital role in the immunological system, in this study the relative weight of the spleen increased in chickens whose diet was supplemented with SB under HS conditions. The dietary addition of SB might improve the intestinal bacterial environment, which is able to strengthen the immune system along with the digestive system to improve immune organ weight, another explanation might be that antioxidant effects alleviate HS-induced OS [28]. The results of the present study revealed that the relative lengths of the jejunum, ileum, and cecum were significantly shorter in the high-ambient temperature groups than in the low-ambient temperature groups in the growing layer chickens. These results are consistent with earlier studies indicating that 20 d of HS (34 °C) in broilers decreased the relative lengths of the jejunum and ileum and relative weight of the jejunum [29]. These findings show that HS retards animal growth while decreasing the lengths and weights of related immune organs, ultimately affecting immune development in the chickens. However, the greater the relative weights of the immune organs and lengths of the intestinal segments are, the greater the performance

Table 6 Effects of ambient temperature and different sodium butyrate (SB) levels on intestinal morphology during the growing stage of laying chickens

Parameters	VH (μm)	CD (μm)	VH/CD (μm/μm)
Ambient temperature (T)			
L, 25 °C	846.18[a]	113.73	7.50[a]
H, 35 °C	771.18[b]	114.74	6.79[b]
Sodium Butyrate (SB)			
SB1, 0 g/kg diet	815.11[a]	117.67	7.01[a]
SB2, 0.5 g/kg diet	850.44[a]	111.86	7.65[a]
SB3, 1 g/kg diet	760.50[b]	113.17	6.78[b]
Interaction T × SB			
LSB1	955.44[a]	113.01[ab]	8.49[a]
LSB2	826.11[ab]	110.20[b]	7.51[b]
LSB3	757.00[b]	117.98[a]	6.50[bc]
HSB1	674.77[b]	122.33[a]	5.54[c]
HSB2	874.77[ab]	113.52[ab]	7.78[b]
HSB3	764.00[b]	108.38[b]	7.05[b]
SEM	23.26	3.36	0.28
Two ways probabilities*			
T	< 0.001	0.716	0.003
SB	< 0.001	0.205	0.009
T × SB	< 0.001	0.022	< 0.001

L Low ambient temperature, *H* High ambient temperature, *SEM* standard error of the mean. LSB1: group fed without sodium butyrate (SB) supplement at low temperature, LSB2: group fed 0.5 SB g/kg diet at low temperature, LSB3: group fed 1.0 SB g/kg diet at low temperature, HSB1: group fed without SB supplement at high temperature, HSB2: group fed 0.5 SB g/kg diet at high temperature, HSB3: group fed 1.0 SB g/kg diet at high temperature

[a;b] Values in the same column with different letter superscripts mean significant differences ($P < 0.05$). Data represent treatment mean ± SEM from $n = 9$ chicks analyzed per treatment

* Overall treatment *P*-value

parameters ADFI and ADG during the growing phase of layer chickens.

Gut health is vital for feed digestion and nutrient absorption; therefore, maintaining the proper morphological structure of the intestine for function, optimal growth performance, and overall health status is essential [30]. The key component of the digestive system is the small intestine, whose morphological structure and integrity are damaged by HS, leading to decreased feed intake and nutrient absorption function and ultimately affecting chicken growth performance [31]. In this study, the results revealed compromised intestinal integrity, indicated by the decreased VH and VH/CD ratio, and increasing CD in the high ambient temperature-induced HS group resulting in decreased absorptive epithelial cell and villus surface area, which are correlated with poor growth performance. These results agree with those of earlier similar studies indicating that HS (32 °C) at 10 h per day decreases the VH and VH/CD ratio in broilers, and increases the CD of the duodenum and ileum in the small intestine [30, 32]. However, dietary supplementation with SB in growing layer chickens increased the VH and VH/CD ratio while decreasing the CD in the ileum. Our findings are consistent with previous results indicating a significant improvement in yellow-feather broiler chickens small intestine morphometric structure with the addition of SB of their diet [33]. We observed that the T × SB interaction resulted in significant improvements in the LSB2 and HSB2 groups compared to the other groups. These findings imply that adding SB to the diet might alleviate HS-induced morphological damage to the small intestine by improving the OS and the inflammatory response. A previous study [34] confirmed that the inclusion of SB in diets of laying hens leads to a substantial increase in the VH of the small intestine segments (jejunum and ileum). Similar findings were reported [35] indicating that SB supplementation substantially increased the VH and VH/CD ratio in the small intestine of broiler chickens.

Heat stress is a factor that causes OS, which is characterized by the excessive formation of reactive oxygen species (ROS) and disruption of redox signaling between the oxidative and antioxidant systems [36]. The health and physiological status of the chickens were determined by evaluating their serum antioxidant activity and immune system. In this study, growing layer chickens in the high-ambient-temperature group induced HS and had a higher MDA content, with lower CAT and GSH-Px activities than those in thermoneutral group, which is consistent with previous results [37, 38]. In addition, dietary supplementation with SB significantly decreased the serum MDA content in response to decreased lipid peroxidation, a marker for disturbance of cellular homeostasis and immune status. Lipid peroxidation produces MDA, which functions as an OS by assembling ROS and reducing the activity of the antioxidant enzymes CAT, GSH-Px, and T-SOD, which are significant biomarkers of cellular antioxidant mechanisms [6]. These results indicate that high ambient temperature-induced HS reduced the ROS scavenging capacity by lowering the activity of antioxidant enzymes, leading to increased production of ROS-induced lipid peroxidation and MDA accumulation. However, dietary supplementation with SB under HS conditions during the growing phase of layer chickens has a beneficial effect by reducing the MDA content, which is a marker of OS. Overall, evidence indicates that maintaining a proper redox balance is crucial for the stability of cells and tissues in all biological systems, including processes such as cell proliferation, metabolism, autophagy, and oxidation [39].

Intestinal tight junction protein (TJP), which is composed of multiple protein complexes such as occluding, Zo-1, and claudin-1 is involved in the formation of a

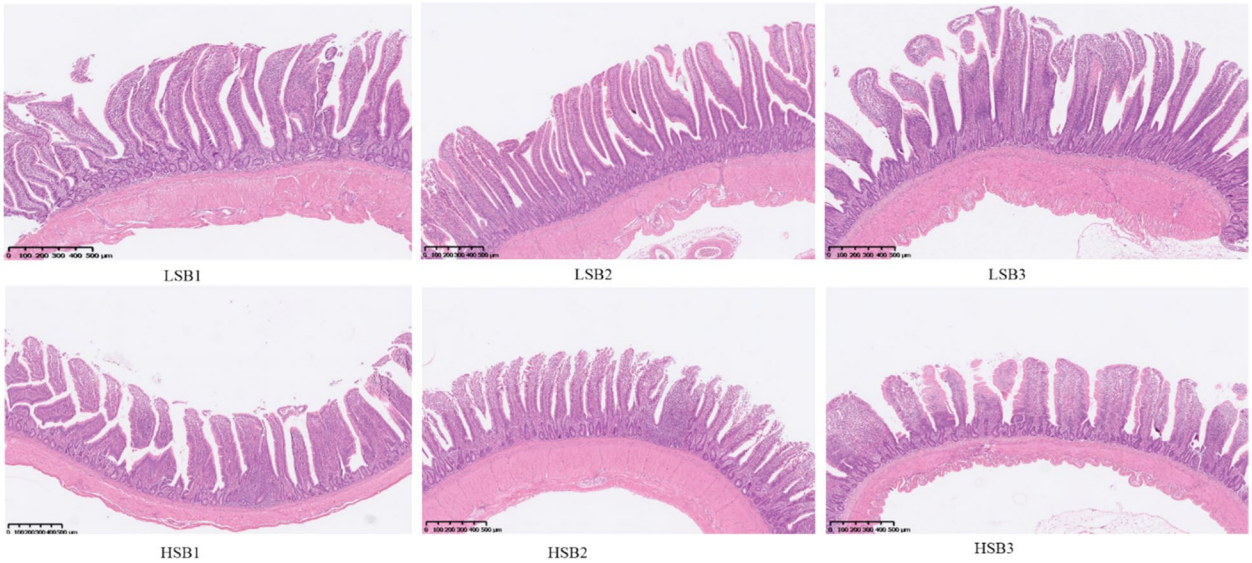

Fig. 1 Effects of two levels of temperatures and three levels of sodium butyrate (SB) on ileum morphology at the growing phase of layer chicken. Hematoxylin and Eosin stain, scale bar, 100 µm. L, low ambient temperature; H, high ambient temperature; SB1, supplementation of 0 g SB/kg diet; SB2, supplementation of 0.5 g SB/kg diet; SB3, supplementation of 1 g SB/kg diet

Table 7 Effects of ambient temperature and different levels of sodium butyrate (SB) on antioxidant enzyme activities during the growing stage of laying chickens

Parameters	MDA (nmol/ml)	H2O2 (µmol/ml)	T-AOC (U/mL)	CAT (U/mL)	T-SOD (U/mL)	GSH-Px (U/mL)
Ambient temperature (T)						
L, 25 °C	3.73	51.93	5.72	5.65[a]	163.18	322.40[a]
H, 35 °C	3.98	56.88	4.70	4.19[b]	159.73	273.00[b]
Sodium Butyrate (SB)						
SB1, 0 g/kg diet	4.89[a]	57.96	5.07	4.87	159.37	284.79
SB2, 0.5 g/kg diet	3.28[b]	51.34	5.37	5.13	165.29	313.75
SB3, 1 g/kg diet	3.40[b]	53.91	5.19	4.76	159.71	294.56
Interaction T × SB						
LSB1	4.19	54.27	5.57	5.75	161.74	313.76
LSB2	3.44	50.05	5.82	5.89	168.63	339.46
LSB3	3.57	51.46	5.76	5.33	159.16	313.97
HSB1	5.60	61.65	4.57	4.00	157.00	255.81
HSB2	3.11	52.63	4.91	4.37	161.94	288.04
HSB3	3.24	56.36	4.63	4.19	160.26	275.15
SEM	0.46	4.25	0.69	0.64	5.28	16.13
Two ways probabilities*						
T	0.511	0.160	0.081	0.008	0.428	< 0.001
SB	< 0.001	0.301	0.914	0.844	0.459	0.199
T × SB	0.106	0.853	0.987	0.890	0.746	0.835

MDA malondialdehyde, *H2O2* Hydrogen peroxide, *T-AOC* Total antioxidant capacity, *CAT* catalase, *T-SOD* Total superoxide dismutase, *GSH-Px* Glutathione peroxidase, *L* Low ambient temperature, *H* High ambient temperature, *SEM* standard error of the mean. LSB1: group fed without sodium butyrate (SB) supplement at low temperature, LSB2: group fed 0.5 SB g/kg diet at low temperature, LSB3: group fed 1.0 SB g/kg diet at low temperature, HSB1: group fed without SB supplement at high temperature, HSB2: group fed 0.5 SB g/kg diet at high temperature, HSB3: group fed 1.0 SB g/kg diet at high temperature

[a;b] Values in the same column with different letter superscripts mean significant differences ($P < 0.05$). Data represent treatment mean ± SEM from $n = 9$ chicks analyzed per treatment

* Overall treatment *P*-value

Table 8 Effects of ambient temperature and different levels of sodium butyrate (SB) on inflammation and tight junction-related mRNA expression during the growing stage of laying chickens

Parameters	MUC-2	Occludin	ZO-1	Claudin-1	TLR4	NF-κB	TGFβ	IFNγ	TNFα	IL-10
Ambient temperature (T)										
L, 25 °C	0.92	0.98[a]	1.07[a]	1.08[a]	1.09	0.89	1.11[b]	1.11	1.01	1.45[a]
H, 35 °C	0.80	0.79[b]	0.80[b]	0.77[b]	1.17	1.03	2.07[a]	1.56	1.12	0.82[b]
Sodium Butyrate (SB)										
SB1, 0 g/kg diet	0.89	0.90	0.92	0.92	1.31	1.20[a]	1.96[a]	1.92[a]	1.17	1.14
SB2, 0.5 g/kg diet	0.90	0.90	0.99	0.97	1.01	0.81[b]	1.10[b]	1.02[b]	0.99	1.27
SB3, 1 g/kg diet	0.79	0.86	0.91	0.88	1.07	0.87[b]	1.72[a]	1.06[b]	1.03	1.00
Interaction T × SB										
LSB1	1.04	1.04	1.04	1.11	1.17	1.14	1.13[b]	1.28	1.11	1.56
LSB2	0.96	0.98	1.15	1.17	1.01	0.70	1.08[b]	1.00	0.95	1.63
LSB3	0.78	0.92	1.02	0.98	1.07	0.82	1.14[b]	1.04	0.98	1.17
HSB1	0.75	0.76	0.79	0.73	1.45	1.27	2.79[a]	2.56	1.23	0.72
HSB2	0.85	0.82	0.82	0.78	1.01	0.92	1.12[b]	1.04	1.04	0.91
HSB3	0.83	0.80	0.80	0.78	1.07	0.92	2.30[a]	1.07	1.08	0.82
SEM	0.13	0.10	0.11	0.15	0.18	0.17	0.32	0.33	0.14	0.37
Two ways probabilities*										
T	0.261	0.043	0.006	0.015	0.559	0.291	0.001	0.099	0.376	0.043
SB	0.620	0.903	0.757	0.828	0.232	0.051	0.029	0.013	0.442	0.763
T × SB	0.509	0.743	0.898	0.817	0.670	0.942	0.046	0.102	0.990	0.792

MUC-2 mucin-2, *ZO-1* zonula occludens-1, *TLR-4* toll-like receptor-4, *NF-κB* nuclear factor kappa B, *TFG-β* transforming growth factor –β, *TNF-α* tumour necrosis factor-α, *IFN- γ* interferon- γ, *IL-10* interleukin-10, *L* Low ambient temperature, *H* High ambient temperature, *SEM* standard error of the mean. LSB1: group fed without sodium butyrate (SB) supplement at low temperature, LSB2: group fed 0.5 SB g/kg diet at low temperature, LSB3: group fed 1.0 SB g/kg diet at low temperature, HSB1: group fed without SB supplement at high temperature, HSB2: group fed 0.5 SB g/kg diet at high temperature, HSB3: group fed 1.0 SB g/kg diet at high temperature

[a;b] Values in the same column with different letter superscripts mean significant differences ($P < 0.05$ Data represent treatment mean ± SEM from $n = 9$ chicks analyzed per treatment

* Overall treatment *P*-value

supportive structural mucosal barrier to protect intestinal permeability [40, 41]. In the present study, the expression of the TJP genes occluding, Zo-1 and claudin-1, and the anti-inflammatory cytokine gene IL-10 was significantly downregulated at high (H) ambient temperature compared with low (L) ambient temperature, while that of TGF-β was upregulated. These results agree with earlier findings that showed that high ambient temperatures downregulated the mRNA expression of the TJP genes occluding, Zo-1, and claudin-1 in the small intestine [42, 43]. Consequently, the disruption of the function of the animal gut mucosal barrier induced by downregulated mRNA expression of TJPs (occluding, ZO-1 and claudin-1) eventually leads to increased gut permeability. These results indicate that high ambient temperature-induced HS increases OS disruption of the intestinal morphology and destruction of the gut structure, ultimately increasing pathogen permeability. Previous studies reported that dietary SB has a beneficial effect on alleviating intestinal barrier dysfunction by increasing the relative mRNA expression of TJPs (occluding, ZO-1 and claudin-1) in the small intestine segments (jejunum and ileum) of laying chickens [18, 44]. The elevated levels of proinflammatory cytokines and decreased levels of anti-inflammatory cytokines in high ambient temperature-induced HS growing layer chickens indicated that high temperature modulated the initiation of an inflammatory response. Notably, high ambient temperatures can trigger the NF-κB signaling pathway, which is a prominent transcription factor implicated in inflammatory disorders, by leading to tissue damage in broilers [45, 46]. A previous study [47] reported that acute HS intensifies the NF-κB pathway and promotes the development of intestinal inflammation in broilers. However, dietary supplementation with SB significantly downregulated the expression of the proinflammatory cytokine genes NF-κB, TGF-β, and IFN-γ compared with that in the other groups. Our results were consistent with those of a previous study in while SB supplementation inhibited the NF-κB signaling pathway by downregulating TGF-β and IFN-γ gene expression in the ileum of HS-induced growing layer chickens [48]. These findings indicate that dietary SB supplementation ameliorates the inflammatory response in the ileum of HS-induced growing layer chickens, which might have a beneficial effect on intestinal morphology and junction protein structure.

Conclusion

In conclusion, HS-induced morphological damage and oxidative stress and disrupted the TJPs (occluding, ZO-1 and claudin-1) structure in the ileum of growing layer chickens reared at high ambient temperatures. Supplementation with 0.5 SB g/kg diet is potentially a suitable solution for alleviating HS-induced TJPs structural dysfunction, oxidative stress, and the inflammatory response (NF-κB, TGFβ, and IFNγ). The findings of this study indicate that SB supplementation attenuates intestinal damage under HS conditions in the growing phase of layer chickens.

Abbreviations

SB	Sodium butyrate
HS	Heat stress
ROS	Reactive oxygen species
VH	Villus height
CD	Crypt depth
IBW	Initial body weight
FBW	Final body weight
ADG	Average daily gain
ADFI	Average daily feed intake
F/G	Feed per gain ratio
L	Low ambient temperature
H	High ambient temperature
SEM	Standard error of the mean
MDA	Malondialdehyde
H2O2	Hydrogen peroxide
T-AOC	Total antioxidant capacity
CAT	Catalase
T-SOD	Total superoxide dismutase
GSH-Px	Glutathione peroxidase
MUC-2	Mucin-2
ZO-1	Zonula occludens-1
TLR-4	Toll-like receptor-4
NF-κB	Nuclear factor kappa B
TFG-β	Transforming growth factor –β
TNF-α	Tumour necrosis factor- α
IFN- γ	Interferon- γ
IL-10	Interleukin-10
GAPDH	Glyceraldehyde 3 phosphate dehydrogenase
TJP	Intestinal tight junction protein

Acknowledgements

The assistance of my teachers and lab mates in this work is greatly acknowledged.

Authors' contributions

M.T.S.; Conceptualization, Data curation, Formal analysis, Software, Writing-original draft. S.W, W. X, A. E.; Investigation, Methodology, Resources Validation, Writing- reviewing and editing. Y.Z, C. J, X. H, K. L, Y. L.; Data curation Formal analysis, Software. C. Z.; Funding acquisition, Project administration, W.C.; Funding acquisition, Project administration, Supervision. All authors reviewed the article and approved the final version.

Funding

This work was supported by the National Key R&D Program of China (Grant No. 2018YFE0128200), Guangdong Feed Industry Technology System (Grant No. 2024CXTD14), State Key Laboratory of Swine and Poultry Breeding Industry (Grant No. GDNKY-Z00Z-K3, GDNKY-Z00Z-K22), the Project of Science and Technology innovation strategy (Grant No. ZX202401-06), the Foreign Expert Project (Grant No. H20240494). Talented Young Scientists Program TYSP (Grant No. P19U42006), the Opening Project of Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition (Grant No. 2022SZ01). The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Data availability

All datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The procedure and protocol used in this research were authorized by the Committee of Institutional Animal Care of the Guangdong Academy of Agricultural Sciences, China, with approval number ACUCGAAC2022. The sample analysis was performed at the Animal Nutrition and Feed Science, the Key Laboratory Department of the Ministry of Agriculture and Rural Affairs, and the Laboratory of Molecular Biology, Institute of Animal Science. This experiment was conducted under the ARRIVE guidelines to minimize animal suffering.

Consent of publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China. [2]State Key Laboratory of Livestock and Poultry Breeding, Guangzhou 510640, China. [3]Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture and Rural Affairs, Guangzhou 510640, China. [4]Guangdong Public Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China. [5]Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China. [6]Department of Animal Production, Faculty of Agriculture, Benha University, Moshtohor 13736, Egypt. [7]College of Animal Science and Technology, Innovative Institute of Animal Healthy Breeding, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China.

Received: 15 November 2024 Accepted: 10 February 2025
Published online: 03 March 2025

References

1. Salem HM, Alqhtani AH, Swelum AA, Babalghith AO, Melebary SJ, Soliman SM, Khafaga AF, Selim S, El-Saadony MT, El-Tarabily KA. Heat stress in poultry with particular reference to the role of probiotics in its amelioration: An updated review. J Therm Biol. 2022;108:103302.
2. Lacetera N. Impact of climate change on animal health and welfare. Anim Front. 2019;9(1):26–31.
3. Ayo J, Ogbuagu N. Heat stress, haematology and small intestinal morphology in broiler chickens: insight into impact and antioxidant-induced amelioration. Worlds Poult Sci J. 2021;77(4):949–68.
4. Teyssier J-R, Brugaletta G, Sirri F, Dridi S, Rochell SJ. A review of heat stress in chickens. Part II: Insights into protein and energy utilization and feeding. Front Physiol. 2022;13:943612.
5. Deng C, Zheng J, Zhou H, You J, Li G. Dietary glycine supplementation prevents heat stress-induced impairment of antioxidant status and intestinal barrier function in broilers. Poult Sci. 2023;102(3): 102408.
6. Chen S, Xue Y, Shen Y, Ju H, Zhang X, Liu J, Wang Y. Effects of different selenium sources on duodenum and jejunum tight junction network and growth performance of broilers in a model of fluorine-induced chronic oxidative stress. Poult Sci. 2022;101(3): 101664.
7. Song J, Xiao K, Ke Y, Jiao L, Hu C, Diao Q, Shi B, Zou X. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult Sci. 2014;93(3):581–8.
8. Lan R, Zhao Z, Li S, An L. Sodium butyrate as an effective feed additive to improve performance, liver function, and meat quality in broilers under hot climatic conditions. Poult Sci. 2020;99(11):5491–500.
9. El-Sawy AE-SF, El-Maddawy ZK, Ibrahiem HS, Bo-Ghazel E-S. The growth Promoting Effect of Beta-glucan in Comparison with Sodium Butyrate on Broiler Chicks. Alex J Vet Sci. 2015;44(1):23–37.
10. Jesus GF, Pereira SA, Owatari MS, Addam K, Silva BC, Sterzelecki FC, Sugai JK, Cardoso L, Jatobá A, Mouriño JL. Use of protected forms of sodium

butyrate benefit the development and intestinal health of Nile tilapia during the sexual reversion period. Aquaculture. 2019;504:326–33.
11. Elnesr SS, Alagawany M, Elwan HA, Fathi MA, Farag MR. Effect of sodium butyrate on intestinal health of poultry–a review. Annals of Animal Science. 2020;20(1):29–41.
12. El-Saadony MT, Umar M. Hassan F-u, Alagawany M, Arif M, Taha AE, Elnesr SS, El-Tarabily KA, Abd El-Hack ME: Applications of butyric acid in poultry production: the dynamics of gut health, performance, nutrient utilization, egg quality, and osteoporosis. Anim Health Res Rev. 2022;23(2):136–46.
13. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. Cell Res. 2021;31(2):107–25.
14. Zhang W, Gao F, Zhu Q, Li C, Jiang Y, Dai S, Zhou G. Dietary sodium butyrate alleviates the oxidative stress induced by corticosterone exposure and improves meat quality in broiler chickens. Poult Sci. 2011;90(11):2592–9.
15. Chen W, Wang S, Zhang H, Ruan D, Xia W, Cui Y, Zheng C, Lin Y. Optimization of dietary zinc for egg production and antioxidant capacity in Chinese egg-laying ducks fed a diet based on corn-wheat bran and soybean meal. Poult Sci. 2017;96(7):2336–43.
16. Wu Q, Liu N, Wu X, Wang G, Lin L. Glutamine alleviates heat stress-induced impairment of intestinal morphology, intestinal inflammatory response, and barrier integrity in broilers. Poult Sci. 2018;97(8):2675–83.
17. Ling J, Gao Y-Y, Hui Y, Wang W-C, Lin Z-P, Yang H-Y, Huang S-B, Lin Y. Effects of dietary fiber and grit on performance, gastrointestinal tract development, lipometabolism, and grit retention of goslings. J Integr Agric. 2014;13(12):2731–40.
18. Luo D, Li J, Xing T, Zhang L, Gao F. Combined effects of xylo-oligosaccharides and coated sodium butyrate on growth performance, immune function, and intestinal physical barrier function of broilers. Anim Sci J. 2021;92(1): e13545.
19. Choe D, Loh T, Foo H, Hair-Bejo M, Awis Q. Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by Lactobacillus plantarum strains. Br Poult Sci. 2012;53(1):106–15.
20. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001;25(4):402–8.
21. Chaudhary A, Mishra P, Al Amaz S, Mahato PL, Das R, Jha R, Mishra B. Dietary supplementation of microalgae mitigates the negative effects of heat stress in broilers. Poult Sci. 2023;102(10): 102958.
22. Khan RU, Naz S, Ullah H, Ullah Q, Laudadio V. Qudratullah, Bozzo G, Tufarelli V: Physiological dynamics in broiler chickens under heat stress and possible mitigation strategies. Anim Biotechnol. 2023;34(2):438–47.
23. Sahin K, Orhan C, Tuzcu M, Ali S, Sahin N, Hayirli A. Epigallocatechin-3-gallate prevents lipid peroxidation and enhances antioxidant defense system via modulating hepatic nuclear transcription factors in heat-stressed quails. Poult Sci. 2010;89(10):2251–8.
24. Alhenaky A, Abdelqader A, Abuajamieh M, Al-Fataftah A-R. The effect of heat stress on intestinal integrity and Salmonella invasion in broiler birds. J Therm Biol. 2017;70:9–14.
25. Roushdy EM, Zaglool AW, El-Tarabany MS. Effects of chronic thermal stress on growth performance, carcass traits, antioxidant indices and the expression of HSP70, growth hormone and superoxide dismutase genes in two broiler strains. J Therm Biol. 2018;74:337–43.
26. Safdari-Rostamabad M, Hosseini-Vashan SJ, Perai AH, Sarir H. Nanoselenium supplementation of heat-stressed broilers: effects on performance, carcass characteristics, blood metabolites, immune response, antioxidant status, and jejunal morphology. Biol Trace Elem Res. 2017;178:105–16.
27. Liu L, He J, Xie H, Yang Y, Li J, Zou Y. Resveratrol induces antioxidant and heat shock protein mRNA expression in response to heat stress in black-boned chickens. Poult Sci. 2014;93(1):54–62.
28. Zhao F, Pang W, Zhang Z, Zhao J, Wang X, Liu Y, Wang X, Feng Z, Zhang Y, Sun W. Pomegranate extract and exercise provide additive benefits on improvement of immune function by inhibiting inflammation and oxidative stress in high-fat-diet-induced obesity in rats. J Nutr Biochem. 2016;32:20–8.
29. Wang C, Zhao F, Li Z, Jin X, Chen X, Geng Z, Hu H, Zhang C. Effects of resveratrol on growth performance, intestinal development, and antioxidant status of broilers under heat stress. Animals. 2021;11(5):1427.
30. Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, Pinheiro M, Sakai M. Sá LRMd, Ferreira AJP, Palermo-Neto J: Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult Sci. 2010;89(9):1905–14.
31. Khonyoung D, Yamauchi K-E. Yamauchi K-e: Improved growth performance due to hypertrophied intestinal absorptive epithelial cells by heat-killed Lactobacillus sakei HS-1 in broiler chickens. J Anim Sci. 2019;97(5):2066–75.
32. Liu G, Zhu H, Ma T, Yan Z, Zhang Y, Geng Y, Zhu Y, Shi Y. Effect of chronic cyclic heat stress on the intestinal morphology, oxidative status and cecal bacterial communities in broilers. J Therm Biol. 2020;91: 102619.
33. Ruixia L, Wu F, Wang Y, Yin F. Sodium butyrate alleviates heat stress-induced damage to morphology, anti-oxidant status, inflammatory response and barrier integrity in jejunum of broilers. Ital J Anim Sci. 2024;23(1):215–26.
34. Zhang Q, Zhang K, Wang J, Bai S, Zeng Q, Peng H, Zhang B, Xuan Y, Ding X. Effects of coated sodium butyrate on performance, egg quality, nutrient digestibility, and intestinal health of laying hens. Poult Sci. 2022;101(9):102020.
35. Liu J, Bayir H, Cosby D, Cox N, Williams S, Fowler J. Evaluation of encapsulated sodium butyrate on growth performance, energy digestibility, gut development, and Salmonella colonization in broilers. Poult Sci. 2017;96(10):3638–44.
36. Chang Q, Cai H, Wei L, Lan R. Chitosan oligosaccharides alleviate acute heat stress-induced oxidative damage by activating ERK1/2-mediated HO-1 and GSH-Px gene expression in breast muscle of broilers. Poult Sci. 2022;101(1): 101515.
37. Baker A, Lin C-C, Lett C, Karpinska B, Wright MH, Foyer CH. Catalase: A critical node in the regulation of cell fate. Free Radical Biol Med. 2023;199:56–66.
38. Yousefi J, Taherpour K, Ghasemi HA, Akbari Gharaei M, Mohammadi Y, Rostami F. RETRACTED ARTICLE: Effects of emulsifier, betaine and l-carnitine on growth performance, immune response, gut morphology and nutrient digestibility in broiler chickens exposed to cyclic heat stress. Br Poult Sci. 2023;64(4):iii–xvi.
39. Chouchene L, Kessabi K, Gueguen M-M, Kah O, Pakdel F, Messaoudi I. Interference with zinc homeostasis and oxidative stress induction as probable mechanisms for cadmium-induced embryo-toxicity in zebrafish. Environ Sci Pollut Res. 2022;29(26):39578–92.
40. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci. 2013;70:631–59.
41. Otani T, Furuse M. Tight junction structure and function revisited. Trends Cell Biol. 2020;30(10):805–17.
42. Alhotan RA, Al Sulaiman AR, Alharthi AS, Abudabos AM. Protective influence of betaine on intestinal health by regulating inflammation and improving barrier function in broilers under heat stress. Poult Sci. 2021;100(9):101337.
43. Song Z, Cheng K, Zhang L, Wang T. Dietary supplementation of enzymatically treated Artemisia annua could alleviate the intestinal inflammatory response in heat-stressed broilers. J Therm Biol. 2017;69:184–90.
44. Miao S, Zhou W, Li H, Zhu M, Dong X, Zou X. Effects of coated sodium butyrate on production performance, egg quality, serum biochemistry, digestive enzyme activity, and intestinal health of laying hens. Ital J Anim Sci. 2021;20(1):1452–61.
45. Xu P, Lin H, Jiao H, Zhao J, Wang X. Chicken embryo thermal manipulation alleviates postnatal heat stress-induced jejunal inflammation by inhibiting Transient Receptor Potential V4. Ecotoxicol Environ Saf. 2023;256:114851.
46. Liu Y-R, Yang N-J, Zhao M-L, Tang Z-S, Duan J-A, Zhou R, Chen L, Sun J, Song Z-X, Hu J-H. Hypericum perforatum L. regulates glutathione redox stress and normalizes Ggt1/Anpep Signaling to alleviate OVX-induced kidney dysfunction. Front Pharmacol. 2021;12:628651.
47. Liu Y-L, Ding K-N, Shen X-L, Liu H-X, Zhang Y-A, Liu Y-Q, He Y-M, Tang L-P. Chronic heat stress promotes liver inflammation in broilers via enhancing NF-κB and NLRP3 signaling pathway. BMC Vet Res. 2022;18(1):289.
48. Bedford A, Gong J. Implications of butyrate and its derivatives for gut health and animal production. Animal Nutrition. 2018;4(2):151–9.

Publisher's Note