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Original Article

Effect of β-glucan Dietary Supplementation on Performance, Nutrient Digestibility, Immune Response and Heat Shock Proteins in Broiler Chickens under Heat-Stress by Early Heat Acclimation

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ABSTRACT

The study examined the impact of early heat acclimation, heat stress, and β-glucan supplementation on broiler chickens' productivity, immune parameters, thyroid hormones, gut microbiota, and nutrient digestibility. A total number of 1080 male broiler chicks (Cobb 500) were randomly assigned to nine treatments (3 × 3 factorial arrangement), including heat exposure (control, heat stress, heat shock at 3 days) and three β-glucan levels (0, 100, and 200 mg β-glucan /kg diet). Compared to the thermo-neutral control group, the results showed that in broilers exposed to heat stress or early heat shock, body weight gain and feed intake decreased significantly ($p<0.05$). Conversely, the mortality rate, antibody titers against the infectious bronchitis (IBV) and Newcastle disease viruses (NDV), immunoglobulin M, immunoglobulin G, liver heat shock protein 70 (HSP70), H/L ratio, and toe web swelling were significantly ($p<0.05$) increased. Supplementing with βG increased *lactobacillus* counts while decreasing those of *E. coli* and total aerobic bacteria. Significant reductions in nutrient digestibility were observed in heat stress or early heat shock exposure, specifically for nitrogen, ether extract, and organic matter.

INTRODUCTION

Large amounts of reactive oxygen can be produced by heat stress (HS), which can also increase the types of harmful bacteria in poultry microbiota, decrease the capacity of the intestinal tract to absorb nutrients and antioxidants, damage the immune system and the health of the intestines, and lower growth performance indicators (Liu *et al*., 2023). Furthermore, according to Xie *et al*. (2015), tissue damage can result from prolonged heat stress. When an animal experiences heat stress (HS), its body temperature rises because it cannot release extra heat into the surrounding air (Sugiharto, 2020). Studies have demonstrated that although chronic heat stress can result in more extensive alterations, acute heat stress can trigger a heat shock response that quickly starts the synthesis of heat shock proteins and alters gene expression (Xie *et al*., 2014), making it similar to characteristics observed in summertime and external conditions (Al Qaisi *et al*., 2022). In livestock and poultry, heat stress results in decreased food intake, delayed growth, intestinal disequilibrium, decreased reproductive performance, immunity, and endocrine disorders (Chen & Yong, 2021). According to a review by Vandana *et al*. (2021), HS in poultry can vary from 27°C to 38°C for 1 to 24 hours (acute), 7 days (moderate), or longer than 7 days (chronic). Heat shock protein families—HSP70, and HSP90 in chickens are highly-conserved proteins essential for cell survival and cytoprotection against various stresses, including HS. According to Gouda *et al*. (2024), the best reaction for a cell to withstand heat shock is to increase HSP levels, which protects cellular proteins from heat shock-induced damage. Numerous researchers have documented several intervention techniques to lessen the harmful effects of HS.

Reducing heat stress through early heat conditioning is a promising management strategy. Positive results have been reported, and this approach is affordable and suitable for immediate implementation on various farms. In the first week of early heat exposure (3–6-d-old), Arjona *et al*. (1988) reported significantly less weight gain; however, the growth accelerated to achieve higher marketing weights than in the control. According to Lin *et al*. (2006a), early heat control is a promising management strategy to increase broiler heat resistance. Furthermore, early-age thermal conditioning (TC) lowers body temperature and improves broiler resistance to heat stress over time, according to research by De Basilio *et al*. (2003).

Furthermore, Hassan & Reddy (2012) found that chicks that had received early thermal conditioning responded to acute heat stress at the marketing age very similarly to birds that had received chronic heat stress adaptation, suggesting that early thermal conditioning had a protective effect. Early heat conditioning lessens the adverse effects of acute HS on broiler performance, according to Kang & Shim (2021). Blood triiodothyronine concentrations improved body weight and feed efficiency with early heat exposure (Yahav & McMurtry, 2001). According to Zaboli *et al*. (2017), early heat conditions after hatching positively inhibited the growth decrease from late chronic HS. According to Yeğenoğlu *et al*. (2009), heat stress effects may be mitigated by groups exposed to early heat treatment following chronic hypoxia, as these groups expressed higher levels of HSP70.

According to Sadeghi *et al*. (2013), beta-glucans are glucose polymers obtained from the cell walls of bacteria, fungi, yeast, and cereals. Jacob & Pescatore (2017) observed that beta-glucans are carried to the small intestine, passing through Peyer's patches in the gut-associated lymphoid tissue (GALT), and then distributed throughout the body. Beta-glucans bind to particular receptors to mediate their effects, triggering the release of antibodies, activating natural killer cells, and stimulating macrophages. Bird macrophages produce cytokines and chemokines like interferon-gamma (IFN-γ), interleukin 4 (IL4), interleukin 6 (IL6), interleukin 8 (IL8), and interleukin 10 (IL10), just like their mammalian counterparts (Cox *et al*., 2010; Han *et al*., 2017). Beta-glucans can directly stimulate specific and non-specific immunity and affect the expression of genes and proteins linked to immunity. According to Jacob & Pescatore (2017), immune function is stimulated to counteract the adverse effects of enteric infection or immune suppression brought on by high-stress rearing conditions. In monogastric animals, adding β-glucan to the diet improves immune function and has no adverse effects on growth performance (Horst *et al*., 2019; Divya *et al*., 2020). To address the problem of antibiotic resistance in humans resulting from eating broiler meat and feed, β-glucan was added to the broiler diet without affecting the overall performance of the birds. This improved the immune system and the beneficial gut flora, especially *Lactobacilli* (Hashaam *et al*., 2024). There are numerous studies on the effects of heat stress and b-glucans as feed additives combined with early heat exposure, but none investigated how these factors interact to affect broiler chick performance. Thus, this study aims to describe how early heat acclimation affects the immune system, gut microbiota, and digestibility of nutrients (%) of broiler chicks under heat stress with and without the the inclusion of β-glucan in the diet.

MATERIALS AND METHODS

Experimental Site and Time

The experiment was designed at the Animal Production Research Institute and Agriculture Research Center in Giza, Egypt, from February to March 2023.

Birds, Diet, and Treatments

One thousand eighty one-day-old male broiler chicks (Cobb 500) with an average initial BW of 41.48 ± 0.18 g were used in the study. Each chick was housed in a separate floor pen and individually weighed before being randomly distributed into one of 9 treatments, with 4 replicates (30 chicks each) per pen, in which they were reared between the ages of 1 and 35 days of age. The study was conducted with a 3 × 3 factorial arrangement, including varying heat exposure (control, heat stress, and heat shock for 3 days) and three β-glucan levels (0, 100, and 200 mg / kg diet). In the control groups (thermo-neutral groups), birds were kept at 33 ± 1°C for the initial 3 days and the temperature was slowly decreased to 25°C until the end of the experiment. In the heat stress groups, birds were daily exposed to a hot ambient temperature (33 ± 1°C using gas heaters) and 65% relative humidity during the experimental period. In the heat shock groups, birds were exposed to heat stress (33

± 1°C using gas heaters) and early heat (40°C for 4 h using gas heaters from 12.00 to 4.00 pm) on consecutive days (days 3 to 5). The β-glucan used was obtained from *Saccharomyces cerevisiae*, local bakery yeast, using the procedures described by Williams *et al*. (1991) and modified according to Chaung *et al*. (2009). The lighting program was adjusted to 24 L: 0 D for the first 3 days of life and then to 23 L: 1 D for the remainder of the experiment. Corn-soybean meal basal diets were used for the experimental treatments to meet the strain requirements (NRC, 1994). Table 1 shows the composition and analytical calculations of the basal diets during the experimental periods (starter and grower). The broiler chicks were given a starter diet *ad libitum* for the first 21 days, followed by a grower diet from 21 to 35 days.

Table 1 – The proximate chemical composition of the basal diet as fed basis (%).

Composition (per 100 Kg)	Starter (1-21 d)	Finisher (21-35 d)
Yellow corn (7.5%CP)	56.34	60.73
Soybean meal (47% CP)	33.00	29.00
Corn gluten meal (60% CP)	3.63	0.87
Dicalcium phosphate	1.90	1.75
Vegetable oil	2.72	5.54
Salt	0.30	0.32
Limestone	0.80	0.81
Vit. and Min. Premix*	0.30	0.30
DL-Methionine	0.35	0.31
L-Lysine-HCl	0.33	0.29
Total	**100**	**100**
Calculated analysis: (NRC, 1994)**		
Crude protein (CP), %	22.81	20.00
ME, kcal/kg	3023	3210
Ether extract	5.42	8.25
Crude fiber	3.72	3.47
Calcium	0.86	0.82
Av. Phosphorus	0.42	0.39
Lysine	1.39	1.22
Methionine	0.71	0.61
Methionine + cysteine	1.10	0.94

*Vitamin and mineral premix: each 3 Kg of vitamin and mineral premix (Special component from commercial source AGRIVET Co.) Contains: Vit. A., 12000000 IU; Vit. D3, 2000000 IU; Vit. K3, 2000 mg; Vit. E, 10000 mg; Vit. B1, 100 mg; Vit. B2, 5000 mg; Vit. B6, 1500 mg; Vit. B12, 10 mg; Biotin, 50 mg; Choline chloride, 250000 mg; Pantothenic acid, 10000 mg; Nicotenic acid, 3000 mg; Folic acid, 1000 mg; Manganese, 60000 mg; Iron, 30000 mg; Selenium, 100 mg; Copper, 10000 mg; Iodine, 1000 mg; Cobalt, 100 mg; Carrier (Ca Co3) adds to 3kg.
** Calculated according to NRC (1994) requirements.

Growth Performance Parameters

FI and BW of birds were calculated by weighing them on days 1, 21, and 35. Each pen was used to calculate BWG and FI, corrected by mortality for each period; and FCR was calculated using FI corrected/ BWG. The mortality rate at the end of the experiment was calculated as a percentage of the total number of birds.

Blood Profile

Blood samples from birds from all treatment groups were taken and analyzed for stress indicators, biochemical measurements, immunological parameters, and antioxidant markers after the end of the experimental period (35 days of age). The analysis of blood samples (5 mL per bird) was divided into three parts: the initial component was put into a tube with EDTA to obtain fresh blood for measuring heterophils (H%), lymphocytes (L%), and the H/L ratio calculated using the Powers (2011) method. The second part of the blood sample was placed in a tube that contained heparin, and then centrifuged for 15 minutes at 3,000 rpm to obtain plasma that was stored at 20°C to assess plasma metabolites. The third part of the blood sample was inserted into a tube not covered with heparin to obtain serum and estimate serum cortisol, T3, and T4 levels using a radioimmunoassay kit. The determination of antioxidant components and antioxidant enzymes (superoxide dismutase SOD, glutathione peroxidase GPX, and total antioxidant capacity TAC) was made using commercial kits produced by Bio-diagnostic in Egypt. A hemagglutination inhibition test was utilized to determine antibody levels against Avian Influenza virus (H9N2), Newcastle disease virus (NDV), and Infectious Bronchitis virus (IBV), using the HI (hemagglutination inhibition test) and expressing it as log2. The liver levels of HSP70 (HSP70) were determined via ELISA kits (Uscn Life Science Inc., Wuhan, China).

Immune response evaluation

Antibody Titers against Sheep Red Blood Cells (SRBCs)

At 28 days of age, six broiler chicks were measured for antibody response against SRBCs in each treatment. Chicks were injected with 1 mL of a saline suspension of 5% SRBCs. Serum tests were collected on the 7th day of every infusion to determine the primary antibody titers of SRBCs separately. Agglutination tests using the microtiter strategy were employed to measure the creation of immune responses (Loa *et al*., 2001).

Toe web swelling

At 28 days of age, eight broiler chicks from each experimental group's cell-mediated immune response were assessed using the magnitude of toe web swelling induced by intradermal phytohemagglutinin (PHA-P) mitogen injection with 100 µg of PHA-P dissolved in 0.1 mL of sterile PBS buffer into the toe web between the third and fourth digits. The thickness of the toe web was measured before injection and 24 hours after injection. The swelling response of the toe web was measured in decimeters by subtracting the thickness before and after injection (Loa *et al*., 2001).

Immunoglobulin

IgG and IgM in blood were determined using a commercial ELISA kit from Bethyl Laboratories (Montgomery, AL, USA) (Gao *et al*., 2008).

Gut Microbial Count

Four broiler chicks were randomly chosen from each treatment, weighed, slaughtered, and defeathered at 35 days of age. Aseptic conditions were used to collect the gut microbes of the slaughtered birds for microbiological examination. The total counts of anaerobic, *Lactobacilli*, and *Escherichia coli* were assessed using selective media (Günal *et al*., 2006).

Nutrient Digestibility

The nutrient digestibility was assessed by allocating 10 birds from each treatment into individual battery cages during the last 7 days of the experiment (35 days). The digestibility of the nutrients was evaluated through the simultaneous collection of fecal matter daily. Horwitz *et al*. (1971) stated that the samples were ground after being oven-dried for 24 hours at 60°C. The Kjeldahl method required the calculation of crude protein multiplied by a factor of 6.25. Ash was confirmed using a muffle furnace for 24 hours at 550°C for the studied samples. Sample digestion with NaOH and H2SO4 was used to identify the crude fiber content. A mixture of methanol and chloroform was employed to extract lipids from samples (Bligh & Dyer, 1959). Drying in an oven at 105°C for 24 hours was used to investigate moisture.

Statistical Analysis

The significance of using GLM with the SAS procedure (Version 9.2, 2009) was determined by analyzing the data collected from this study.

The GLM model was used:

$$Y_{ijk} = \mu + H_i + G_j + H * G_{ij} + E_{ijk}$$

Where:

Y_{ijk} is the value of any observation.

µ = the overall population mean.

H_i: is the ith effect of heat exposure (i= thermo-neutral, heat stress, heat shock at 3 days of age).

G_j: is the jth effect of β-glucan (j = 0, 100, and 200 mg/kg).

$H*G_{ij}$ = Interaction of heat exposure* β-glucan levels (ij- 1, 2,…………9).

E_{ijk}= experimental random error.

The differences between the means of different groups were detected using Duncan's multiple-range test (Duncan, 1955). The Chi-square test was used to analyze mortality percentages.

RESULTS

Growth Performance

Tables 2 and 3 display the growth performance of broiler chickens treated thermoneutrally, only under heat stress, or exposed to early heat shock, with or without βG supplementation. Body weight (BW), body weight gain (BWG), and feed intake (FI) all significantly decreased (p<0.05 and 0.01) after the period between days 21–35 and the whole trial period. The feed conversion ratio (FCR) and mortality rate (%) of broilers exposed to heat stress or early heat shock were significantly (p<0.01) higher than those in the thermo-neutral control group. The mortality rate (MR) dropped dramatically in chicks exposed to early heat shock, with a significant relative decrease of 6.67% compared to the heat stress group (12.22%). Furthermore, throughout the trial, the MR of chickens fed a diet containing 200 mg of βG (5.93%) decreased significantly (p<0.05) in comparison to the control group (12.22%). Furthermore, there were no notable interactions between the βG and heat exposure groups.

Table 2 – Body weight, body gain (g) and mortality rate (%) of chickens fed diets without or with β-glucan under heat exposure at different ages.

Items	Live body weight			Body weight gain			Mortality rate (%)		
	1d	21d	35d	1-21d	22-35d	1-35d	1-21d	22-35d	1-35d
Effects of heat exposure									
Thermo-neutral	41.67	725[a]	2050[a]	683[a]	1325[a]	2008[a]	4.81	2.22	7.03[a]
Heat stress	41.29	621[c]	1547[c]	580[c]	926[c]	1506[c]	8.15	4.07	12.22[b]
Heat stress+Heat shock at 3d	41.49	649[b]	1696[b]	607[b]	1047[b]	1654[b]	4.81	2.22	6.67[a]
SEM	0.25	9.46	15.52	9.43	12.20	15.47	-	-	-
Sig.	NS	**	**	**	**	**	NS	NS	*
Effect of β-glucan (mg/kg diet)									
0	41.33	646[b]	1729[b]	605[b]	1083	1688[b]	8.52	4.07	12.22[b]
100	41.31	668[ab]	1776[a]	627[ab]	1108	1735[a]	4.81	2.96	7.78[a]
200	41.81	681[a]	1788[a]	639[a]	1107	1746[a]	4.44	1.48	5.93[a]
SEM	0.24	17.46	75.64	17.42	60.14	75.58			
Sig.	NS	*	*	*	NS	*	NS	NS	*
Interaction effect	NS	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviation: NS, not significant. [abc]Means within the same column bearing different letters are significantly differ at (p<0.05). * p<0.05.

Table 3 – Feed intake (g) and Feed conversion (feed/gain; g/g) of chickens fed diets without or with β-glucan under heat exposure at different ages.

Items	Feed intake (g)			Feed conversion (feed/gain; g/g)		
	1-21d	22-35d	1-35d	1-21d	22-35d	1-35d
Effects of heat exposure						
Thermo-neutral	1117.39[a]	1955.16[a]	3073.17[a]	1.64[b]	1.48[c]	1.53[c]
Heat stress	1067.04[b]	1868.53[b]	2935.57[b]	1.85[a]	2.02[a]	1.95[a]
Heat stress+Heat shock at 3d	1099.53[a]	1919.56[ab]	3019.09[a]	1.81[a]	1.83[b]	1.83[b]
SEM	9.68	20.22	19.14	0.03	0.02	0.02
Sig.	**	*	**	**	**	**
Effect of β-glucan (mg/kg diet)						
0	1095.17	1900.17	3002.06	1.82[a]	1.80	1.80[a]
100	1100.00	1908.53	3008.53	1.76[ab]	1.76	1.76[b]
200	1088.80	1928.44	3017.24	1.71[b]	1.78	1.75[b]
SEM	12.12	23.47	27.80	0.04	0.09	0.06
Sig.	NS	NS	NS	*	NS	*
Interaction effect	NS	NS	NS	NS	NS	NS

Abbreviation: NS, not significant. [abc]Means within the same column bearing different letters are significantly differ at (p<0.05). * p<0.05.

Immune Parameters

Table 4 shows the immune parameters as a function of thermoneutrality, heat stress alone, early heat shock exposure, and βG supplementation. When compared to the thermal neutral control chickens, broilers that were only exposed to heat stress or early heat shock exposure showed significantly (p<0.05) higher antibody titers of 3.49 and 5.80% in cases of infection with the bronchitis virus (IBV), 3.62 and 5.31% Newcastle disease virus (NDV), 13.25 and 19.49% immunoglobulin M (IgM), 9.81 and 17.52% immunoglobulin G (IgG), 81.04 and 60.66% H/L, and 9.68 and 12.46% HSP70 of Liver, and significantly (p<0.05) decreased antibody titers against sheep red blood cells (SRBCs). Comparing the βG-supplemented groups to the control group, there was a significant increase in IBV, NDV, IgG, IgM, HSP70, toe web swelling, and SRBCs (p<0.05 and 0.01). After the experimental phase, the values of certain immune parameter were not significantly affected by the interaction between heat exposure and βG.

Table 5 shows that, compared to the control group, broilers exposed to either heat stress alone or early heat shock exposure had significantly (p<0.05) increased cortisol, decreased T3, and insignificantly decreased T4. Triiodothyronine (T3) and thyroxine (T4), released by the thyroid gland, are essential for sustaining the metabolic rate. Table 5 shows that, when compared to the control group, the βG dietary treatment combination had no significant effect on T3 or T4, although the level of βG did significantly (p<0.05) lower cortisol levels. By the time the experiment ended, cortisol, T3, and T4 values were not significantly impacted by the level of βG.

Table 4 – Some immunological parameters and heat shock protein of the liver of chickens fed diets without or with β-glucan under heat exposure at the end of experimental period.

Items	Infectious bronchitis virus (IBV)	Newcastle disease virus (NDV)	IgG (mg/mL)	IgM (mg/mL)	H/L	Heat shock protein (HSP70) of Liver	Toe web swelling (mm)	SRBC, log[2]
Effects of heat exposure								
Thermo-neutral	6.49[b]	6.32[b]	1.44	1.11	0.29[c]	4.51[b]	0.347[a]	6.26[a]
Heat stress	6.72[ab]	6.55[ab]	1.63	1.22	0.53[a]	4.95[a]	0.289[b]	4.85[c]
Heat stress+Heat shock at 3d	6.87[a]	6.66[a]	1.72	1.30	0.47[b]	5.08[a]	0.324[ab]	5.68[b]
SEM	0.11	0.09	0.15	0.12	0.01	0.09	0.017	0.17
Sig.	*	*	NS	NS	**	**	*	**
Effect of β-glucan (mg/kg diet)								
0	6.48[b]	6.32[b]	1.16[b]	0.92[b]	0.43	4.65[b]	0.280[b]	5.24[b]
100	6.89[a]	6.59[a]	1.70[a]	1.31[a]	0.44	4.87[ab]	0.327[a]	5.66[ab]
200	6.72[ab]	6.63[a]	1.94[a]	1.40[a]	0.43	5.02[a]	0.353[a]	5.88[a]
SEM	0.11	0.09	0.11	0.10	0.04	0.12	0.016	0.25
Sig.	*	*	**	*	NS	*	**	*
Interaction effect	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviation: NS, not significant. [abc]Means within the same column bearing different letters are significantly differ at (p<0.05). * p<0.05.

Bacteria Count

When compared to the control group, the gut microbiota of broiler chicks exposed to heat stress or early heat shock showed a significant (p<0.05) decrease in *lactobacillus*, but no significant reduction in the counts of *E. coli* or total aerobic bacteria (Table 5). The counts of *E. coli* and total aerobic bacteria were significantly (p<0.05) lower when BG was used in conjunction with the control group, whereas the counts of *Lactobacillus* were significantly (p<0.05) higher. The results of certain bacteria counts were not significantly impacted by the interaction between heat exposure and the level of BG at the end of the experimental phase (Table 5).

Table 5 – Hormonal profile and bacteria count of chickens fed diets without or with β-glucan under heat exposure at the end of experimental period.

Items	Kidney hormone Cortisol (μg/dL)	Thyroid hormone		Bacteria count (log10 cfu/g count)		
		T3 (ng\mL)	T4 (mg/dL)	Total aerobic bacteria (log_{10} cfu/g)	*Lactobacilli* (log_{10} cfu/g)	*Escherichia coli* (log_{10} cfu/g)
Effects of heat exposure						
Thermo-neutral	1.42[b]	2.17[a]	8.92	8.35	5.33[a]	5.52
Heat stress	1.81[a]	2.05[ab]	8.61	8.73	4.81[b]	5.81
Heat stress+Heat shock at 3d	1.73[a]	1.94[b]	8.42	8.63	5.04[ab]	5.70
SEM	0.12	0.05	0.15	0.15	0.14	0.11
Sig.	*	*	NS	NS	*	NS
Effect of β-glucan (mg/kg diet)						
0	1.98[a]	2.14	8.53	8.83[a]	4.77[b]	5.89[a]
100	1.55[b]	2.02	8.67	8.59[ab]	5.16[a]	5.70[ab]
200	1.42[b]	2.01	8.74	8.28[b]	5.25[a]	5.45[b]
SEM	0.10	0.06	0.17	0.16	0.15	0.12
Sig.	**	NS	NS	*	*	*
Interaction effect	NS	NS	NS	NS	NS	NS

Abbreviation: NS, not significant. [ab]Means within the same column bearing different letters are significantly differ at (p<0.05). * p<0.05.

Nutrient digestibility

Table 6 displays the nutrient digestibility results for broiler chickens treated thermoneutrally, only under heat stress, or exposed to early heat shock, with or without βG supplementation. Compared to the control group, broilers exposed to heat stress alone or early heat shock exposure had significantly (p<0.05 and 0.01) reduced N digestibility, EE, and OM digestibility, but not DM, CF, or TDN digestibility. Additionally, broiler chicks' DM, N, OM, and TDN digestibility may increase with BG supplementation (p<0.05). After the experimental phase, the interaction between heat exposure and the level of BG did not significantly affect digestibility values (except for crude fiber).

Table 6 – Nutrient digestibility (%) of chickens fed diets without or with β-glucan under heat exposure at the end of the experimental period.

Items	Dry matter	Nitrogen	Crude fiber	Ether extract	Organic matter	TDN
Effects of heat exposure						
Thermo-neutral	70.92	70.67[a]	21.73	88.52[a]	76.56[a]	72.33
Heat stress	70.15	68.31[b]	19.42	87.46[b]	75.35[ab]	71.34
Heat stress+Heat shock at 3d	70.52	67.64[b]	19.65	86.77[b]	75.09[b]	71.09
SEM	0.58	0.68	0.91	0.25	0.46	0.45
Sig.	NS	**	NS	**	*	NS
Effect of β-glucan (mg/kg diet)						
0	69.36[b]	67.65[b]	20.37	87.33	74.72[b]	70.46[b]
100	70.72[ab]	68.77[ab]	20.02	87.54	75.88[ab]	71.76[a]
200	71.51[a]	70.20[a]	20.42	87.87	76.39[a]	72.53[a]
SEM	0.51	0.74	0.95	0.35	0.45	0.39
Sig.	*	*	NS	NS	*	**
Interaction effect	NS	NS	NS	NS	NS	NS

Abbreviation: NS, not significant. [ab]Means within the same column bearing different letters are significantly differ at (p<0.05). * p<0.05.

DISCUSSION

The severe adverse effects of heat stress on poultry species' physiology, productivity, and health can result in significant economic losses (Oke *et al*., 2021; Madkour *et al*., 2022; Sun *et al*., 2023). Research has shown that HS exposure results in the following effects in chickens: reduced meat quality (Davis *et al*., 2022), increased feed conversion ratios (Zhang *et al*., 2017; Gebreyohans *et al*., 2023), immunity loss (Hirakawa *et al*., 2020), and body weight loss (Goo *et al*., 2019). The decreased feed intake seen during heat stress may be the result of lesions in the appetite center of the hypothalamic lateral nucleus.

Furthermore, intestinal motility and blood flow were reduced, which may have increased the time food takes to enter the body and postponed the thermogenic effect of eating (Van Handel-Hruska *et al*., 1977).

Nonetheless, poor appetite and reduced feed intake may be connected to the deteriorating performance of heat-stressed broilers as a defense mechanism to lessen heat stress in the body. Broilers raised in different thermal environments demonstrated a significant 14% reduction in feed intake due to high temperatures, according to Laganá *et al*. (2007). Reduced food intake is linked to the decline in growth performance brought on by heat stress (Peng *et al*., 2022). According to Li *et al*. (2023), heat stress dramatically decreased the broiler chicks' average daily and overall weight gain (p<0.05). When Sun *et al*. (2023) treated 28-day-old broilers with heat stress, they discovered that 42-day-old broilers had significantly lower FI, DG, and FCR.

Furthermore, Yilmaz & Gul (2023) discovered that heat stress decreased the DG in broilers that were 42 days old but had no appreciable impact on the FCR. Heat stress can hinder growth by reducing the digestibility of proteins and nutrients. As the primary regulator of muscle metabolism in the endocrine system, insulin growth factor is involved in all phases of muscle formation and regeneration, which can boost differentiation and increase protein synthesis, whereas heat stress has an impact on insulin growth factor secretion, which decreases protein synthesis. (Nawaz *et al*., 2021). On the other hand, early heat shock exposures were linked to a lower death rate (Nagwa *et al*., 2012; Morsy *et al*., 2018). This finding may be explained by improved immunity and thermotolerance. Kang & Shim (2021) demonstrated that the early heat exposure group showed lower body weight gain, and FCR was reduced (p<0.05), indicating a positive effect on feed efficiency.

Supplementing broiler chicks with β-glucan may improve their BWG, FCR, and mortality rate. Chae *et al*. (2006) found higher BWG in chickens fed finisher diets containing 200 and 400 mg kg of β-glucan (derived from *Saccharomyces cerevisiae*). Similarly, Zhang *et al*. (2008) claimed that β-glucan supplementation ranging from 25 to 200 mg/kg can maximize broiler performance and improve average daily gain and FCR. Increasing the β-1,3-glucan content in the diet to 100–200 g/T can improve the BWG of birds, according to Zhang *et al*. (2020). Other researchers have also shown that β-glucan improves broiler performance (Tokunaka *et al*., 2000; Thanardkit *et al*., 2002). According to the current research, adding βG along with heat exposure—either heat stress or an early heat shock exposure at age three—significantly improved the FCR, HSP70, and immunity responses—namely, toe web swelling and SRBC, log2. Furthermore, increasing the digestibility coefficients of CP, DM, ME, and cortisol significantly improved growth performance. Consequently, early heat shock exposure at 3 days of age and βG supplementation at 200 mg/kg diet during heat stress experimental periods may improve immunity responses and physiological stats, performance, and productivity. Early heat exposure (5 days old, 36 °C, 24 hours, followed by 28–42 days, 32–35 oC for 7 h/d) dramatically increased body weight gain (Günal, 2012). When comparing the broiler exposed to early heat shock exposure and heat stress alone to the thermoneutral control group, BW and BWG were significantly (p<0.01) lower (Ezzat *et al*., 2017). According to Lin *et al*. (2006b), this effect is most likely caused by a decrease in FI and/or an increase in blood levels of corticosterone, which exchanges power expenditure for the choice between protein catabolism and fat deposition. These results align with those observed by Sayed *et al*. (2023), who demonstrated that HS (32–35 °C) negatively impacted broiler body weight (BW), decreased appetite, decreased feed intake, and decreased final body weight. The reduced growth of HS-bred chickens may be because the higher outside temperature stimulated peripheral thermal sensors. These sensors then sent inhibitory nerve impulses to the hypothalamic hunger center, which caused the birds to reduce their feed and water intake to offset their increased needs due to evaporative heat loss (Sritharet *et al*., 2002; Ahmed *et al*., 2019). When broilers aged 21 to 35 days were supplemented with the addition β-1,3-glucan, the effect on FCR was more significant in heat-stressed birds than in nonchallenged broilers (Zhang *et al*., 2020). The findings from Mathlouthi *et al*. (2011) verified the feed conversion rate (FCR) enhancement of broilers fed β-glucan.

Concerning the humeral immune response, the groups that were only exposed to heat stress or early heat shock exhibited a significant (p<0.05) rise in antibody titers of IBV, NDV, H/L, and HSP70, while the IgG and IgM groups showed an insignificant increase. According to previous reports, heat stress immunosuppression reduces immunity to viral infection by compromising the effectiveness of vaccines like the AIV (Eladl *et al*., 2019; Tian *et al*., 2022) and NDV vaccines (Rauf *et al*., 2019). Since the response to a stressor's intensity and duration remain constant, H/L has been suggested as a more trustworthy index for acute stress (Kim *et al*., 2021; Lee *et al*., 2022). When utilizing the H/L ratio as a stress biomarker, the suggested reference values of roughly 0.20, 0.50, and 0.80 denote low, optimal, and high stress levels, respectively, (Gross & Siegel, 1993). According to the findings of Zahraa and Al-Ghamdi (2008), heat stress significantly increased the heterophil/lymphocyte ratio. Under both acute and chronic HS, increased HSP70 was seen in various tissues (Baxter *et al*., 2020; Emami *et al*., 2021). Notably, chicken breeds naturally exposed to high temperatures and resistant to HS exhibit widespread expression of HSP 70 (Cedraz *et al*., 2017). Additionally, several studies show that HSP70 is crucial in limiting the harmful effects of oxidative stress (Guo *et al*., 2007; Hao *et al*., 2012). According to Hosseini-Vashan *et al*. (2015), heat stress raised the H/L ratio and lowered the titers of total and IgG antibodies in the secondary response to SRBCs and antibody production against NDV (p<0.05). According to Nanto-HARA *et al*. (2021), immunoglobulin (IgY and IgM) plasma levels increased after 14 days at 33°C, stimulating immune responses. These results corroborated the findings of Şahin *et al*. (2009), who found that heat-stressed poultry had higher serum HSP-70 levels. Multiple authors have noted that different HS exposures increase the levels of HSP70 (Nagwa *et al*., 2012; Rizk *et al*., 2018). Numerous studies on intense heat exposure resulted in the expression of HSP70, which inhibits the release of nitric oxide, cytokines, and oxygen-free radicals, thereby enhancing immune function (Polla & Cossarizza, 1996; Morsy, 2013). The increased expression of HSP70 may also suggest that the stress chaperone induced by high chicken exposure has compromised the proteins. Reduced hyperthermia is the goal of mild heat stress; this can occur in the middle because tissues are protected from severe heat stress by prior experiences (Rajkumar *et al*., 2015). According to Nagwa *et al*. (2012), Morsy (2013), and Ezzat *et al*. (2017), heat stress or early heat exposure programs may increase HSP70 expression in the broiler chicks. As a molecular chaperone that binds to other cellular proteins, facilitates intracellular transport, folds into the appropriate secondary structures, and prevents protein aggregation, HSP70 release during heat stress conditions may be crucial in protecting stressed cells and reversing disorders brought on by stress. When comparing the heat-stressed group to the thermo-neutral control group, an overexpression of HSP-70 was observed (Alzarah *et al*., 2021). Immune response assay results showed that early heat shock exposure or only heat stress had a significant (p<0.05) immunosuppressive effect on humoral and cellular levels. Furthermore, only heat stress or early heat shock exposure were linked to a decrease (p<0.05) in the humoral (Anti-SRBCs), as well as cell-mediated (toe web swelling) immune response. The antibody titer response to SRBCs with lower lymphoid organ weight was significantly reduced in broilers raised under chronic heat stress as compared to those raised under thermoneutral conditions, according to Habibian *et al*. (2013) and Akhavan-Salamat *et al*. (2015).

The immune function as shown by the H/L ratio was not significantly affected by the BG level; however, there were significant changes (p<0.05 and p<0.01) between treatments, and these were more significant for IBV, NDV, IgG, IgM, HSP70 of the liver, toe web swelling, and SRBC. BG broiler supplementation, however, showed beneficial effects on all humoral (anti-SRBCs) and cell-mediated (toe web swelling) conditions. These outcomes align with the findings of Schwartz & Vetvicka (2021), who discovered that β-glucan plays a critical role in triggering innate and acquired immunity. According to Abd Al-Tawab (2019) and Zhen *et al*. (2020), broiler diets supplemented with 200–400 mg of pure β-glucan/kg diet improved the innate immunity of the birds by increasing lysozyme and phagocytic activity. It also improved the humoral immune response by enhancing the birds' antibody response to AIV and NDV vaccines.

Furthermore, according to Novak & Vetvicka (2008), b-glucans can be utilized as growth boosters for poultry and as an alternative to antibiotics. In their experiment, Kadhem and Mahdi (2015) demonstrated a noteworthy rise in antibody titer against IBV, mainly when β-glucan was obtained from drinking water. Additionally, it has been documented that adding β-glucan to the diet raised the titer of serum NDV HI, the NDV stimulation index of lymphocytes in the intestinal tract and peripheral blood, and the rate at which T lymphocytes differentiated into CD4+ T cells (Cao *et al*., 2023). Cheng *et al*. (2004) proposed that by regulating macrophage activity, β-glucan feeding boosted specific cell-mediated immune responses in broiler chickens. It has been shown that yeast β-glucans and oligo-mannans stimulate the production of mucin-2 and increase the number of goblet cells and their circulation, enhance macrophage activity, encourage phagocytosis, induce increased expression of intestinal tight junctions, and function as anti-inflammatory agents in poultry (Schwartz & Vetvicka, 2021; Bi *et al*., 2022; Cao *et al*., 2023). Omara *et al*. (2021) state that β-glucans can promote adaptive and innate immunity. By increasing the synthesis of cytokines and stimulating other cells like neutrophils, natural killer cells, and macrophages, β-glucan can influence the immune system (Moon *et al*., 2016). Furthermore, there may be an increase in IgG and IgA plasma levels, which can cause a humoral reaction (Vlassopoulou *et al*., 2021). The interaction between heat exposure and the level of BG at the end of the experimental period did not significantly affect the values of immune functions studied.

Conversely, broilers exposed to either heat stress alone or early heat shock showed a significant (p<0.05) increase in cortisol and a significant decrease in T3, but not a significant decrease in T4 compared to the control group. These results corroborated the findings of Ezzat *et al*. (2024), who found that, in comparison to the thermo-neutral control, heat stress was associated with a decrease in cortisol, T3, and the H/L ratio.

According to Nori-Sarma *et al*. (2022), high ambient temperatures and the ensuing thermal stress on birds during the monsoon season (June to September) were found to be the cause of higher blood levels of HSP-70 and cortisol in adult broiler (42-days-old) birds. These results supported the findings of Sohail *et al*. (2010), who suggested that, in comparison to broilers housed in a thermo-neutral zone, heat stress markedly increased ($p<0.05$) the concentration of cortisol. Nonetheless, the release of HSP70, which may be involved in sustaining metabolic rate and/or mitigating the adverse effects of stress, may account for the rise in T3 hormone in the chicks exposed to heat shock programs.

Additionally, the outcomes also showed strong agreement with the research conducted by Bahrami *et al*. (2012), who observed elevated cortisol levels (ng/mL) on days 28 and 42 (2.58) and 35 and 38 (2.58) of age in broiler chickens housed at a heat stress temperature of 33 ± 3 °C. The organs must perform additional tasks during heat stress, which requires more energy. In addition to using blood glucose, cortisol also contributes to the body's production of additional glucose from non-carbohydrate sources, primarily through the breakdown of muscle proteins, or gluconeogenesis. The hypothalamus-cortex system activates in response to the increased energy demand, as evidenced by the release of ACTHRH and the ensuing blood cortisol (Nori-Sarma *et al*., 2022). However, broilers exposed to thermal stress under a variety of conditions, including 36°C for 4 to 6 h daily from days 22 to 42 (Zaglool *et al*., 2019) and 38°C for 3 h daily from days 35 to 40 (Tollba & Hassan, 2003), have been shown to have significantly lower blood T3 concentrations. Furthermore, heat stress lowers circulating thyroid hormones like thyroxine (T4) and triiodothyronine (T3). According to Decuypere and Kuhn (1988), a decrease in peripheral T4 to T3 deiodination is the cause of the drop in T3 concentration during heat stress.

Conversely, Amer *et al*. (2022) discovered that groups supplemented with 1,3-β-glucans (0, 50, 100, and 150 mg 1,3-βG kg^{-1}) experienced a level-dependent linear increase in serum levels of T3 and T4 ($p<0.01$). Furthermore, Ezzat *et al*. (2024) demonstrated that T3 and T4 were not significantly affected by the combination of BG dietary treatment. On the other hand, when compared to the control group, there was a significant ($p<0.05$) decrease in cortisol with increasing levels of BG. After the experimental period, there was no significant difference in the levels of BG and heat exposure with respect to cortisol, T3, and T4 values.

Heat stress can have a direct effect on the composition of the gut microbiota through temperature changes, or it can have an indirect impact due to changes in the intestinal integrity, physiological status, behavior, and immune system activity of the birds, which can occur gradually or suddenly (Cao *et al*., 2021). Furthermore, Meng *et al*. (2020) showed that exposure to high ambient temperatures and heat stress significantly alters the gut microbiota of poultry. Heat stress can disrupt homeostasis processes, affecting the gut microbiota's composition by triggering stress hormones and neurotransmitters that alter the gut's physiological processes (Patra & Kar, 2021). This indicates that HS increases corticosterone concentrations and stimulates the hypothalamic-pituitary axis (HPA axis), which may modify the microbiota's composition and lower the immune system's activity in the intestine. Additionally, HS increased the number of pathogenic bacteria and decreased the number of beneficial microbiota in the gut (Elnesr & Abdelazim, 2023). In the cecum and ileum of laying hens, *Lactobacillus* counts were reduced due to HS (26°C vs. 33°C/20 days), but the amount of *E. coli* was also decreased (Tajima *et al*., 2007). Another study by Zhang *et al*. (2017) showed that, in comparison to age-matched broilers housed under thermoneutral conditions (22 °C), broilers subjected to cyclic heat stress (33 °C for 10 h/d) from 21 to 42 days of age had lower abundances of *Lactobacillus* and *Bifidobacterium* and higher abundances of *Salmonella*, *E. coli*, and *Clostridium*. According to Song *et al*. (2013), heat stress in Ross 308 broilers exposed to cyclic heat stress (33 °C for 10 h/d) from 21 to 42 days of age increased viable counts of the opportunistic pathogen *Escherichia coli* and decreased viable counts of *Lactobacillus* in the cecal contents. Similarly, in a different study using Ross 308 broilers, cyclic heat stress (33 °C for 10 h/d) from 22 to 42 days of age resulted in higher viable counts of opportunistic pathogens like Coliforms and *Clostridium* and lower viable counts of beneficial bacterial genera like *Lactobacillus* and *Bifidobacterium* in the small intestinal digesta when compared to broilers raised at thermoneutral temperature (22 °C) (Song *et al*., 2014).

In comparison to the control group, the total aerobic and *E. coli* counts of bacteria were significantly decreased ($p<0.05$), while the *Lactobacillus* count was expanded considerably with the help of BG ($p<0.05$). It has been shown that feeding β-glucans as dietary supplements to chickens increases the intestinal clearance of several significant pathogens, including

Salmonella spp. and *E. coli* (Lowry *et al*., 2005; Huff *et al*., 2010). β-glucans can diminish pathogenic bacterial colonization in the gut, enhancing intestinal health and maintaining mucosal integrity. Additionally, they can lessen the rivalry for nutrients between the microflora and the host, resulting in increased or more accessible ileal protein digestibility (Ferket *et al*., 2002; Gomez *et al*., 2012). According to research conducted by Horst *et al*. (2019) and Shao *et al*. (2016), 1,3/1,6-glucan has the potential to decrease the colonization of *Salmonella* in the intestinal tract, alleviate the degree of damage caused by the infection of the intestinal and visceral organs, increase the number of beneficial bacteria like *Bifidobacterium* and *Lactobacillus*, and have a significant immune effect against viral and parasitic diseases. Hashaam *et al*. (2024) demonstrated that supplementing with β-glucan positively increased the number of *Lactobacilli*. Supplementing with β-glucans enhances gut health and boosts resistance to disease while providing protection against several economically significant pathogens like *Salmonella enterica*, *Escherichia coli*, and *Eimeria* species (Anwar *et al*., 2017; Omara *et al*., 2021). According to Purnamasari *et al*. (2022), β-glucans can also be used as an antibiotic substitute, particularly when treating enteric pathogens like *E. coli* and *Salmonella* spp. The interaction between heat exposure and the level of BG at the end of the experimental period did not significantly affect the values of some of the studied bacteria counts.

It is well recognized that heat stress reduces enzymatic activity, nutrient absorption and intestinal barrier function, lowers the number of blood chicks in the upper gastrointestinal tract, and decreases the expression and activity of several macronutrient transporters as well as digestive enzymes (Belay *et al*., 1993; Brugaletta *et al*., 2022; Teyssier *et al*., 2022). Reductions in ileal or fecal N digestibility ranging from 1.5 to 10% have been reported in broilers raised in hot climates (Farjam *et al*., 2010; Attia *et al*., 2017; Attia *et al*., 2022). As with De Souza *et al*. (2016), this study found that the ileal digestibility of N decreased under constant HS, whereas this was not the case under cyclic HS. This suggests that the length or intensity of HS can affect N digestibility. In examining the variables influencing fat digestibility, it has been noted that birds subjected to heat stress exhibit reduced fat digestibility (Leeson & Summer, 2001). According to Zhang *et al*. (2020), heat stress dramatically decreased DM and energy digestibility ($p<0.05$). However, there were no appreciable changes in energy (De Souza *et al*., 2016) or DM digestibility (Attia *et al*., 2017; Kim *et al*., 2020), consistent with other studies' findings. According to Zhang *et al*. (2020), feeding diets containing β-1,3-glucan at doses of 100 to 200 g/T can improve energy digestibility under HS conditions compared to feeding diets without β-1,3-glucan ($p<0.05$).

The addition of BG to the diet affected the CF and EE digestion coefficients. However, compared to the control group, the digestibility coefficients of DM, N, OM, and TDN were significantly ($p<0.05$) higher for higher levels of BG supplementation. The BG may be responsible for poultry's increased utilization and nutritional digestibility. These molecules may also serve as valuable agents for promoting microbial growth or may enhance activity at the intestinal mucosa, leading to increased villus height. (Zhang *et al*., 2005). Our results are consistent with those of Sun *et al*. (2019), who found that the levels of β-glucanase supplementation (600, 1200, and 1800 β-glucanase units kg^{-1}) significantly ($p<0.05$) increased the digestibility of crude protein and gross energy linearly. According to Zhang *et al*. (2020), feeding diets containing β-1,3-glucan at doses of 100 to 200 g/T can improve energy digestibility under HS conditions compared to feeding diets without it ($p<0.05$). Hens fed a diet containing 200 mg of BG significantly ($p<0.05$) increased the digestibility coefficients of CP, DM, and ME, according to Ezzat *et al*. (2024). After the experiment, the interaction between heat exposure and the level of BG did not significantly affect the values of some of the nutrient digestibility coefficients studied.

CONCLUSION

It is possible to conclude that heat stress and early heat shock exposure significantly increased some antibody titers and stress levels in the chickens, such as cortisol and H/L ratio, while decreasing immune responses such as toe web swelling and antibodies against sheep red blood cells. Compared to the control group, supplementation with βG resulted in increased stress protein levels, immune responses, nutritional digestibility, and antibody titers. βG and heat exposure did not significantly affect thyroid hormone levels.

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Conceptualization, EW, RAM, OMMM, MHS, FIA, E-SIMM, A-KDE, and GAM; methodology, EW, RAM, OMMM, MHS, FIA, E-SIMM, A-KDE, and GAM; software, EW, RAM, OMMM, MHS, FIA, E-SIMM, A-KDE, and GAM; validation, EW, RAM, OMMM, MHS, FIA, E-SIMM, A-KDE, and GAM; formal analysis, EW, RAM, OMMM, MHS, FIA, E-SIMM, A-KDE, and GAM; investigation, EW, RAM, OMMM, MHS, FIA, E-SIMM, A-KDE, and GAM; resources, EW, RAM, OMMM, MHS, FIA, E-SIMM, A-KDE, and GAM; writing—original draft preparation, DCA, AMM, A-KDE, and AM; writing—review and editing, DCA, AMM, A-KDE, AL and AM; visualization, DCA, AMM, A-KDE, and AM; project administration, AA; funding acquisition, DCA, AMM, A-KDE, AL and AM. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data will be available upon request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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