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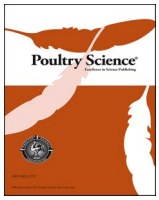

Effects of cold-pressed wheat germ oil and *Bacillus subtilis* on growth performance, digestibility, immune status, intestinal microbial enumeration, and gene expression of broilers under heat stress

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ABSTRACT

This study evaluated the effect of wheat germ oil (WGO), *Bacillus subtilis*, and their combination on growth performance, immune response, nutrient digestibility, intestinal microbial, oxidative status, and gene expression in heat-stressed broilers. Four hundred one-day-old male Ross 308 broilers were distributed into five pens (20 birds/pen) in four experimental groups: a control (CON) without additives, WGO group fed diet with WGO at 200 $mg.kg^{-1}$, BS group fed diet with *B. subtilis* at 500 $mg.kg^{-1}$ containing 5×10^8 $CFU.g^{-1}$, and CWB group received both WGO and *B. subtilis*. Heat stress exposure adversely affected broiler growth performance, carcass traits, immune response, and insulin-like growth factor 1 (IGF-1) and mucin2 (MUC2) mRNA expression. However, the CWB group showed a lower FCR, reduced mortality rate, and increased BWG compared to the other groups. Nutrient digestion was also improved, with a higher digestibility of ether extract, dry matter, and crude protein. By day 35, stress biomarkers like corticosterone and glucose levels were reduced, while triiodothyronine levels increased in the BS and CWB groups. The CWB group also showed lower malondialdehyde and interleukin-6 levels, with higher superoxide dismutase activity, and increased levels of IgA, IgG, and interleukin-10. Additionally, the CWB group had higher HDL levels and lower cholesterol and LDL levels ($P < 0.05$). Notably, CWB supplements modified the structure of the cecal microbial community by increasing *Lactobacillus* counts and decreasing *E. coli* and *C. perfringens* counts. Furthermore, the expressions of intestinal MUC2 and hepatic IGF-1 were up-regulated ($P < 0.05$) in the CWB group. This study provides evidence that supplementing heat-stressed broiler diets with a mixture of WGO and *B. subtilis* enhances antioxidant capacity, immune response, growth performance, and gut integrity via modulating the microbial community and regulating gene expression.

Introduction

Rising environmental temperatures have detrimental impacts on animals raised for meat, particularly in the poultry industry, where heat stress leads to substantial economic losses and threatens sustainability. Broiler chickens, a major part of global livestock, are highly vulnerable to heat stress, which hampers growth, weakens immunity, and increases mortality and morbidity (Abdel-Moneim et al., 2021). This stress also compromises the quality and safety of the final product. Heat stress causes changes in the structure, function, morphology, and mucosal damage of the chicken intestine, reducing blood flow, oxygenation, nutrient availability, and feed intake (Quinteiro-Filho et al., 2017). This

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results in increased gut permeability, inflammation, and impaired nutrient absorption, compromising overall gut health (Wu et al., 2018). On a cellular level, heat stress triggers reactive oxygen species (ROS) production, leading to oxidative stress and immune fatigue. To combat these effects, nutritionists have explored dietary interventions. Numerous studies highlight the positive impact of feed additives in mitigating heat stress and improving feed efficiency, such as prebiotics (Abd El-Hack et al., 2021; Shehata et al., 2022; Yang et al., 2023), essential oils (Elbaz et al., 2022b), probiotics (Abd El-Hack et al., 2020; Elbaz et al., 2023b), herbal extracts (Dosoky et al., 2021; Elbaz et al., 2021; Mesalam et al., 2021; Ebeid et al., 2023), and enhanced management and nutrition practices (Abdel-Moneim et al., 2021; Elbaz et al., 2022a; Siddiqui et al., 2022; Abdel-Moneim et al., 2023). These approaches are essential for maintaining poultry viability and profitability under heat stress conditions.

Probiotics are gaining increasing attention in poultry production due to their multiple properties, the most important of which are antimicrobial and immunomodulatory, which provide health benefits to the host. Numerous previous studies provide abundant evidence of their potential to improve poultry growth performance (Abdel-Moneim et al., 2020b; Elbaz et al., 2021), enhance nutrient digestion, and gut health (Jeong and Kim, 2014), positively modify gut microbiota, and boost immunity (Mountzouris et al., 2010). Common probiotic strains used in poultry diets include *Bifidobacterium, Lactobacillus*, and *Bacillus* (Abd El-Moneim et al., 2020). *B. subtilis* is particularly noted for its resilience to environmental stresses and its role in enhancing gut health, immunity, feed efficiency, and growth of broilers (Abdel-Moneim et al., 2020a), through mechanisms like competitive exclusion and the production of beneficial enzymes, vitamins, fatty acids, and antimicrobial peptides (Gelinas et al., 2024).

Wheat germ oil (WGO), extracted from wheat germ and comprising 8-14 % of whole wheat's weight (Arshad et al., 2013), is rich in linoleic and oleic acids and vitamins D, E, and A (Barakat et al., 2011). It boasts antioxidant properties due to its high content of biologically active compounds, such as phenolics, tocotrienols, tocopherols, and carotenoids, which offer nutritional and health benefits. WGO contains high levels of alpha-lipoic acid, known for its ability to neutralize ROS and chelate minerals (Gumus et al., 2015), thereby reducing oxidative stress (Barakat et al., 2011). It also enhances oxidative stability by regenerating self-antioxidants like vitamins E and C (Anwar and Mohamed, 2015). Additionally, WGO has been shown to improve the oxidative stability of fats in poultry meat (Arshad et al., 2013) and has demonstrated antimicrobial and anti-inflammatory effects (Zargar et al., 2023).

Given its beneficial properties, WGO can serve as an effective nutritional supplement to alleviate the deleterious impacts of heat stress in broilers. We hypothesized that supplementing broiler diets with WGO and *B. subtilis* would improve their growth, gut health, immune response, and oxidative status under heat stress. Therefore, this study evaluated the impact of WGO and *B. subtilis* on the aforementioned parameters, as well as on cecal microbiota and gene expression in broilers under heat stress conditions.

Methods and materials

Ethics statement

The animal utilization protocol (000-23-141) for this experiment was approved by the Desert Research Center Animal Care Committee, and all birds were cared for per the Ain Shams on Animal Care Guidelines.

Diets, and bird management

A total of 400 day-old male Ross 308 broilers were obtained from a commercial hatchery and distributed into five pens (20 birds/pen) across four experimental groups. The groups were as follows: chicks fed a diet with no feed supplements (CON), chicks fed a diet containing WGO at 200 $mg.kg^{-1}$, chicks fed a diet with *B. subtilis* ATCC19659 (BS) at 500 $mg.kg^{-1}$ containing 5×10^8 $CFU.g^{-1}$, and chicks fed a diet combining WGO with *B. subtilis* (CWB) at the same doses. The room temperature was set at 32°C for 48 h, then reduced to 31°C for another 48 h, after which the birds were kept at ambient temperature, averaging 31.4°C with 59.3 % relative humidity during the experiment. The temperature-humidity index (THI) was calculated following Tao and Xin (2003) equation: $THI = 0.85t_{db} + 0.15t_{wb}$, where t_{wb} and t_{db} are wet and dry bulb temperatures, respectively, as shown in Fig. 1. Lighting was maintained at 24 h (20+ lux) during the first week, then reduced to 20 h (10 lux) from day 8 until the end of the experiment. Fresh water and feed were provided *ad libitum* throughout the trial. A corn-soybean meal--based diet was formulated according to the nutrient requirements of the birds (Aviagen, 2018) in a two-phase feeding program: starter (0-21 d) and grower (22-35 d), as given in Table 1. *B. subtilis* strain was obtained from Cairo Agricultural Research Center, Microbiology Culture Collection, Faculty of Agriculture, Ain Shams University, Egypt, and the cold-pressed WGO was purchased from ALHAWAG Co., Egypt.

Growth performance and carcass traits

Live body weight (LBW) and feed intake (FI) were recorded on days 21 and 35. Mortality was recorded daily throughout the experiment, and body weight gain (BWG), and feed conversion ratio (FCR) were calculated. On day 35, 15 birds per group were randomly selected for sampling. These birds were weighed (representative weights for each group), slaughtered, and dissected for carcass characteristics, including carcass weight, liver, immune organs, and abdominal fat.

Nutrient digestibility

Five broilers from each group were separated and placed individually in digestion cages at 35 days of age. The digestion experiment lasted for 4 days. Fresh excreta samples were collected from beneath each bird every 8 h daily during the 4-day digestion experiment and then dried. Additionally, the amount of feed consumed during the digestion period was recorded to measure nutrient digestibility coefficients. The feed and excreta samples collected were analyzed at the Desert Research Center Laboratory in Egypt for dry matter (DM), nitrogen-free extract (NFE), ether extract (EE), and crude protein (CP) using the methods of AOAC (2000).

Blood biochemical analysis

Before slaughter, blood samples were collected from the jugular vein into heparinized tubes, then centrifuged at 3000 × g for 15 min to separate the plasma, which was stored at −20°C until further analysis. Plasma concentrations of triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and glucose were analyzed using an automatic biochemical analyzer (Amiri et al., 2020). In addition, levels of immunoglobulins M, G, and A (IgM, IgG, and IgA), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity were measured following Abdel-Moneim et al. (2020). Plasma samples were also used to detect concentrations of interleukin-10 and 6 (IL-10 and IL-6), and tumor necrosis factor-α (TNF-α) using commercial ELISA kits (MyBioSource, San Diego, CA), following the manufacturer's instructions. Plasma levels of triiodothyronine (T_3) and corticosterone were measured using specialized commercial kits as described by Abdel-Moneim et al. (2022b) and Emam et al. (2024), respectively.

Cecum microbial enumeration

During slaughter, approximately 2 g of cecal contents were collected to assess gut microbial diversity. One gram of each cecal sample was placed in 9 ml of phosphate-buffered saline, serially diluted, and then

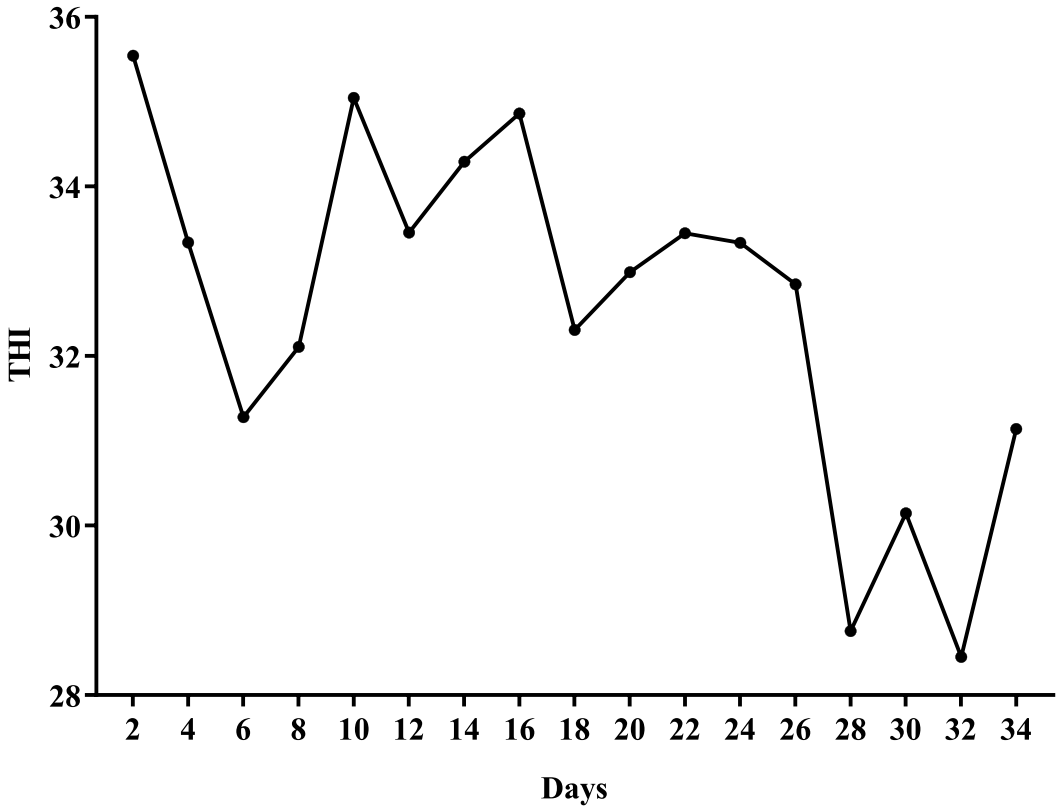

Fig. 1. Average temperature-humidity index (THI) during the experimental period.

Table 1
Feed ingredients and chemical composition of basal diets.

Ingredient, %	Starter (0-21d)	Grower (22-35d)
Yellow corn	55.40	59.20
Soybean meal	38.06	33.10
Corn Oil	2.380	4.050
Di-Calcium Phosphate	2.040	1.820
Calcium carbonate	1.270	1.060
Premix*	0.300	0.300
Salt	0.250	0.250
DL-Methionine	0.160	0.120
Hcl-Lysine	0.040	-
Sodium bicarbonate	0.100	0.100
Chemical composition		
Crude protein, %	23	21
Metabolizable energy, kcal.kg^{-1}	3000	3200
Calcium, %	1.045	0.941
Available phosphorus, %	0.497	0.451

* Premix: (1 %) provided the following (per Kilogram of complete diets). 1400 IU Vitamin A, 3000 IU Vitamin D3, 50 mg Vitamin E, 4 mg Vitamin K, 3 mg Vitamin B6, 6 mg Vitamin B12, 60 mg Niacin, 20 mg Pantothenic acid, 0.20 mg folic acid, 150 mg Choline, 48 mg Ca, 3.18 mg P, 100 mg Mn, 50 mg Fe, 80 mg Zn, 10 mg Cu, 0.25 mg Co, 1.5 mg Iodine.

cultured on agar specific to each microbe. The cultures were incubated at the required temperature and duration as described by Abdel-Moneim et al. (2022c). The microbial counts included *Clostridium perfringens* (on tryptose sulfite cycloserine agar), *Escherichia coli* (on MacConkey agar), and *Lactobacillus* (on MRS agar).

Gene expression

After slaughter, liver and ileum samples were collected from five chickens in each group at the end of the experiment to directly assess target gene expression. Samples were homogenized thoroughly for RT-PCR analyses using a Qiagen one-step RT-PCR kit (Qiagen, USA), following the manufacturer's guidelines. NanoDrop spectrophotometer was used to assess the purity and integrity of RNA. RNA with an A260/A280 ratio of 1.8 or greater was selected for cDNA synthesis. The RT-PCR reaction was conducted in a total volume of 25 µl for each gene of interest using a Bio-Rad thermal cycler (MyCycler, Germany) and included 40 cycles of denaturation (95°C, 30 s), annealing (60°C, 30 s), and extension (72°C, 30 s) using a RT-PCR System (Applied Biosystems, USA). The primer pairs for insulin-like growth factor 1 (IGF-1) and mucin 2 (MUC2) genes were designed based on the *Gallus gallus* sequence as follows: MUC2) 5-"F-TTCATGATGCCTGCTCTTGTG"−3; 5-"R-CCTGAGCCTTGGTACATTCTTGT"−3, while IGF-1) 5-"F-TGTACTG TGCTCCAATAAAGC"−3; 5-"RCTGTTTCCTGTGTTCCCTCTACTT"−3. Relative gene expression levels were calculated using the 2ˆ-ΔΔCt method, with normalization to the β-actin housekeeping gene, as described by Livak and Schmittgen (2001).

Statistical analysis

All experimental data were analyzed using a one-way ANOVA with the GLM procedure in SAS (9.4 software, SAS Institute Inc., Cary, NC, USA). The Tukey multiple comparison range test was performed to determine statistical differences between groups' means at $P < 0.05$. Data were presented as mean values with pooled SEM.

Results

Performance parameter

The LBW and BWG of the CON group at 3 and 5 weeks were reduced ($P < 0.05$) compared to the treated groups (Table 2). Chickens fed the experimental additives showed an increase in LBW and BWG compared to CON group, with the highest LBW and BWG ($P < 0.05$) observed in the CWB group that received WGO and *B. subtilis* mixture. Furthermore, FCR

Table 2
Effect of dietary supplementation of wheat germ cold-pressed oil, *B. subtilis* and their mixture on growth performance and carcass traits in broiler chickens under heat stress.

Parameter	CON	WGO	BS	CWB	SEM	P-value
Week 0-3						
LBW, g	692.2^{c}	713.1^{b}	721.9^{b}	747.0^{a}	3.814	>0.001
BWG, g.period^{-1}	651.6^{c}	672.5^{b}	681.3^{b}	706.1^{a}	3.797	>0.001
FI, g.period^{-1}	768.1	770.4	765.3	764.7	1.449	0.453
FCR, g feed.g gain^{-1}	1.179^{a}	1.146^{b}	1.123^{b}	1.082^{c}	0.010	>0.001
Week 3-5						
LBW, g	1797.2^{c}	2004.2^{b}	1995.8^{b}	2074.5^{a}	18.41	>0.001
BWG, g.period^{-1}	1105.0^{b}	1291.1^{a}	1273.9^{a}	1327.5^{a}	16.17	>0.001
FI, g.period^{-1}	2422	2476	2429	2473	12.01	0.243
FCR, g feed.g gain^{-1}	2.108^{a}	1.921^{b}	1.908^{b}	1.866^{b}	0.021	>0.001
Week 0-5						
BWG, g.period^{-1}	1756.6^{c}	1963.6^{b}	1955.2^{b}	2033.7^{a}	18.40	>0.001
FI, g.period^{-1}	3190	3246	3194	3237	11.61	0.202
FCR, g feed.g gain^{-1}	1.818^{a}	1.654^{b}	1.634^{bc}	1.593^{c}	0.016	<0.001
Mortality, %	11	9	8	5	-	-
Carcass traits, %						
Dressing	69.61	70.26	69.80	71.33	1.614	0.063
Abdominal fat	4.21^{a}	3.06^{c}	3.61^{b}	2.94^{c}	0.022	0.002
Liver	2.16	2.24	2.08	2.21	0.091	0.615

CON, Experimental basal diet without feed additive; WGO, Experimental basal diet with wheat germ oil at 200 mg.kg^{-1}; BS, Experimental basal diet with *Bacillus subtilis* at 500 mg.kg^{-1} containing 5 × 10^{8} CFU.g^{-1}; CWB, Experimental basal diet with wheat germ oil and *B. subtilis*; LBW, live body weight; FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio; SEM, standard error of the mean. Means in the same row with different superscripts are significantly different.

was lower in the treated groups compared to the CON group during Weeks 4-5 and Weeks 0-5. The CWB group achieved the best FCR compared to the other groups ($P < 0.05$), even though FI remained unaffected across all experimental groups during the study.

Relative abdominal fat weight decreased ($P < 0.05$) in the WGO, BS, and CWB groups, with the lowest level observed in the WGO and CWB groups. However, the relative weights of the liver and dressing were not statistically altered by the experimental feed additives (Table 2). Nevertheless, there was also a numerical elevation ($P < 0.063$) in the dressing in the CWB group compared to the remaining groups.

Nutrient digestibility

Table 3 demonstrates the impact of the dietary supplements on nutrient digestibility. Broilers fed the CWB, BS, and WGO diets showed increased digestibility of DM and CP ($P < 0.05$) compared to the control. Additionally, EE digestibility was higher ($P < 0.05$) in chicks fed the CWB and WGO diets compared to those fed the BS and CON diets. Nitrogen-free extract digestibility was higher ($P < 0.05$) in chicks fed the CWB diet compared to the other groups. Chicks fed the CWB diet exhibited the best digestibility coefficient of NFE, EE, DM, and CP.

Table 3
Effect of dietary supplementation of wheat germ cold-pressed oil, *B. subtilis* and their mixture on digestion coefficients of nutrient in broiler chickens under heat stress.

Parameter	CON	WGO	B.S	CWB	SEM	P-value
Dry matter	61.7^{c}	65.3^{b}	64.5^{b}	68.2^{a}	0.02	<0.001
Crude protein	66.9^{c}	67.5^{bc}	68.3^{b}	70.6^{a}	0.17	0.016
Ether Extract	54.1^{c}	63.7^{b}	56.2^{c}	67.4^{a}	0.09	<0.001
Nitrogen-free extract	63.0^{b}	65.2^{ab}	64.1^{ab}	66.8^{a}	0.51	0.035

CON, Experimental basal diet without feed additive; WGO, Experimental basal diet with wheat germ oil at 200 mg.kg^{-1}; BS, Experimental basal diet with *Bacillus subtilis* at 500 mg.kg^{-1} containing 5 × 10^{8} CFU.g^{-1}; CWB, Experimental basal diet with wheat germ oil and *Bacillus subtilis*; SEM, standard error of the mean. Means in the same row with different superscripts are significantly different.

Stress biomarkers and lipid profile

Glucose and corticosterone levels were lower ($P < 0.05$) in the WGO, BS, and CWB groups compared to the CON group, as presented in Table 4. Nevertheless, T_3 levels were higher ($P < 0.05$) in these groups than in the CON group, with the CWB group exhibiting the lowest glucose and corticosterone levels and the highest T_3 levels. Additionally, the dietary supplements significantly impacted the blood lipid profile, with chickens fed BS and CWB diets showing reduced ($P < 0.05$) plasma cholesterol levels. Plasma HDL levels were increased ($P < 0.05$), and LDL levels were reduced ($P < 0.05$) in the BS, WGO, and CWB groups compared to the CON group. However, triglyceride levels were not influenced by the experimental additives Table 5.

Immune response

Table 3 illustrates the impact of dietary supplementation of WGO, *B. subtilis,* or their mixture on the broiler's immune response. While IgM levels were not significantly affected, both IgA and IgG levels were higher ($P < 0.05$) in the BS and CWB groups compared to the other groups. The dietary supplements also significantly influenced blood immune cytokines: IL-10 levels increased in the WGO, BS, and CWB groups ($P < 0.05$) compared to the CON group, whereas IL-6 levels decreased ($P < 0.05$) in the BS and CWB groups compared to the WGO and CON groups. TNF-α levels remained unaffected by the supplements.

Antioxidant status

As depicted in Fig. 2, antioxidant status improved with experimental feed additives, as SOD levels increased ($P < 0.05$) and MDA levels decreased ($P < 0.05$) in the BS, WGO, and CWB groups compared to the CON group, while GPx levels were not affected by the dietary supplements.

Cecum microbial enumeration

The experimental treatments significantly altered the microbial

Table 4
Effect of dietary supplementation of wheat germ cold-pressed oil, *B. subtilis* and their mixture on stress biomarkers and lipid profile in broiler chickens under heat stress.

Parameter	CON	WGO	BS	CWB	SEM	P-value
Stress biomarkers						
Glucose, mg.dl^{-1}	94.61^{a}	81.40^{b}	86.13^{b}	75.24^{c}	2.630	0.029
T3, ng.ml^{-1}	0.567^{c}	0.813^{b}	0.757^{b}	1.061^{a}	0.061	0.006
COR, pg.ml^{-1}	413.4^{a}	325.3^{b}	283.7^{c}	279.0^{c}	18.25	0.004
Lipid profile, mg. dl^{-1}						
Triglyceride	24.44	23.78	24.10	23.21	0.449	0.842
Cholesterol	176.3^{a}	166.1^{ab}	154.2^{b}	148.8^{b}	4.738	0.010
LDL	60.20^{a}	53.42^{b}	49.11^{b}	44.67^{c}	2.059	0.016
HDL	82.30^{c}	106.1^{b}	97.60^{b}	111.5^{a}	4.482	<0.001

CON, Experimental basal diet without feed additive; WGO, Experimental basal diet with wheat germ oil at 200 mg.kg^{-1}; BS, Experimental basal diet with *Bacillus subtilis* at 500 mg.kg^{-1} containing 5 × 10^{8} CFU.g^{-1}; CWB, Experimental basal diet with wheat germ oil and *Bacillus subtilis*; T3, triiodothyronine; COR, corticosterone; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; SEM, standard error of the mean. Means in the same row with different superscripts are significantly different.

Table 5
Effect of dietary supplementation of wheat germ cold-pressed oil, *B. subtilis* and their mixture on the immune response of broiler chickens under heat stress.

Parameter[1]	CON	WGO	BS	CWB	SEM	P-value
Immunoglobulin						
IgA, ng.ml^{-1}	247.4^{b}	256.3^{b}	271.5^{a}	268.1^{a}	5.339	0.001
IgM, ng.ml^{-1}	12.53	11.89	12.92	13.11	0.429	0.192
IgG, μg.ml^{-1}	2.346^{c}	2.913^{b}	3.054^{a}	3.220^{a}	0.108	<0.001
Immune cytokines						
IL-10, pg.ml^{-1}	37.92^{c}	40.61^{b}	38.84^{bc}	42.63^{a}	0.679	<0.001
IL-6, pg.ml^{-1}	88.65^{a}	81.33^{ab}	72.91^{b}	71.17^{b}	2.150	0.001
TNF-α, pg.ml^{-1}	247.1	251.3	238.7	242.2	3.685	0.361

CON, Experimental basal diet without feed additive; WGO, Experimental basal diet with wheat germ oil at 200 mg.kg^{-1}; BS, Experimental basal diet with *Bacillus subtilis* at 500 mg.kg^{-1} containing 5 × 10^{8} CFU.g^{-1}; CWB, Experimental basal diet with wheat germ oil and *Bacillus subtilis*; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; SEM, standard error of the mean. Means in the same row with different superscripts are significantly different.

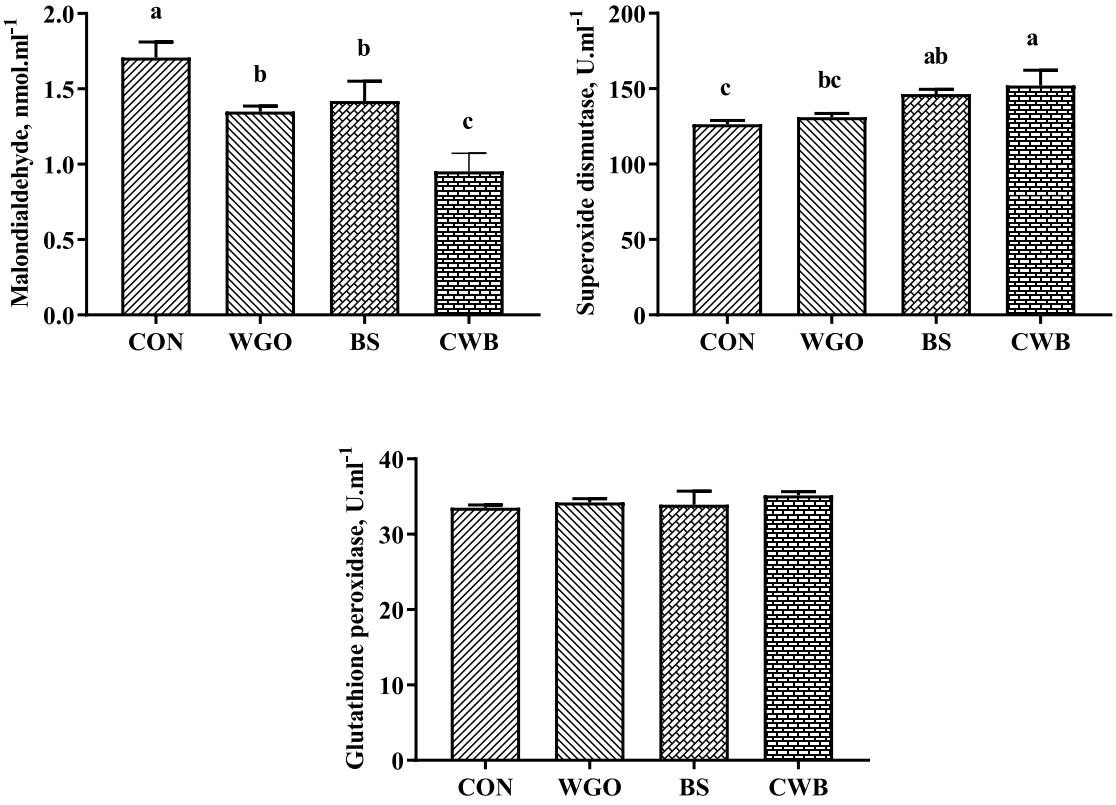

Fig. 2. Effect of dietary supplementation of wheat germ cold-pressed oil, *B. subtilis,* and their mixture on oxidative status of broiler chickens under heat stress. CON, Experimental basal diet without feed additive; WGO, Experimental basal diet with wheat germ oil at 200 mg.kg^{-1}; BS, Experimental basal diet with *Bacillus subtilis* at 500 mg.kg^{-1} containing 5 × 10^{8} CFU.g^{-1}; CWB, Experimental basal diet with wheat germ oil and *Bacillus subtilis* Data presented as mean values with their standard errors. Values with different superscript letters are statistically different ($P < 0.05$).

composition of the cecum, specifically affecting *C. perfringens, E. coli*, and *Lactobacillus*, as illustrated in Fig. 3. Chickens fed BS and CWB had a higher ($P < 0.05$) *Lactobacillus* count and lower ($P < 0.05$) *E. coli* and *C. perfringens* counts compared to the WGO and CON groups.

Gene expression

As shown in Fig. 4, the dietary supplementation of WGO, *B. subtilis,* or their combination significantly influenced gene expression of IGF-1 and MUC2. Heat stress reduced the expression of IGF-1 and MUC2. However, adding a WGO and *B. subtilis* mixture to the diet of heat-stressed broilers resulted in the highest ($P < 0.05$) upregulation of IGF-1 and MUC2 gene expression compared to the other groups. Specifically, MUC2 expression increased ($P < 0.05$) in broilers fed *B. subtilis*-supplemented diets (BS and CWB groups) compared to the CON and WGO groups. Additionally, IGF-1 expression was higher ($P < 0.05$) in the CWB-supplemented group than in the other experimental groups.

Discussion

Despite the great development in the poultry industry, environmental stressors, particularly high summer temperatures, continue to pose challenges. This highlights the need to explore natural alternatives with antioxidant, antimicrobial, and anti-inflammatory properties as an effective strategy to combat the harmful impacts of heat stress. The current study found that birds exposed to high ambient temperatures exhibited signs of morphological heat stress alongside elevated corticosterone and glucose levels and reduced T_3 levels in birds on a diet without experimental additives. As anticipated, the dietary combination of WGO and *B. subtilis* in this study demonstrated a positive impact in alleviating the negative impacts of heat stress on broilers.

Recent studies proposed that using natural antioxidants or probiotics

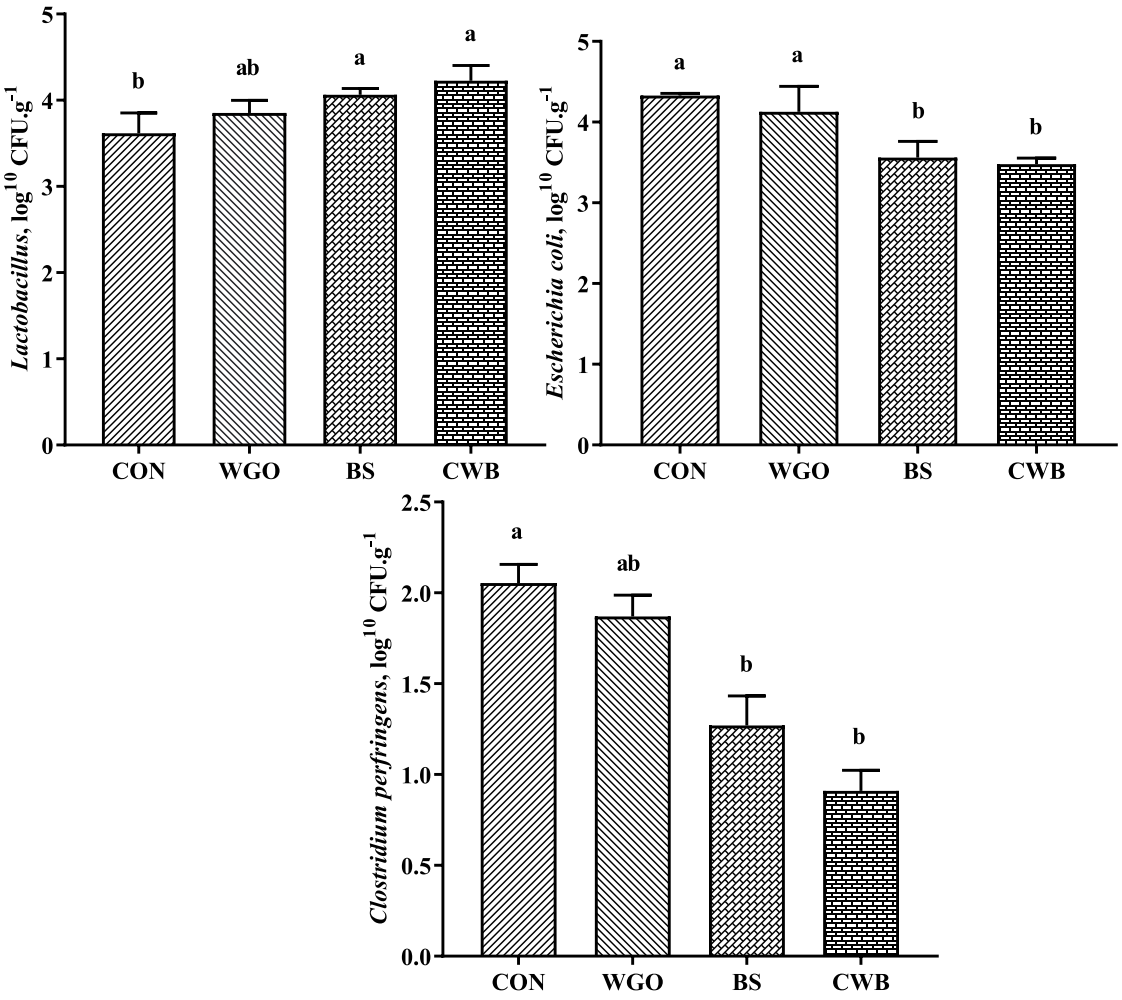

Fig. 3. Effect of dietary supplementation of wheat germ cold-pressed oil, *B. subtilis,* and their mixture on cecal microbial enumeration (Log^{10} CFU.g^{-1} wet weight) of broiler chickens under heat stress. CON, Experimental basal diet without feed additive; WGO, Experimental basal diet with wheat germ oil at 200 mg.kg^{-1}; BS, Experimental basal diet with *Bacillus subtilis* at 500 mg.kg^{-1} containing 5×10^8 CFU.g^{-1}; CWB, Experimental basal diet with wheat germ oil and *Bacillus subtilis* Data presented as mean values with their standard errors. Values with different superscript letters are statistically different ($P < 0.05$).

is an effective strategy for enhancing the performance and health of broiler chickens, particularly during heat stress (Abdel-Moneim et al., 2022a; Elbaz et al., 2023a). In our study, heat-stressed chickens fed a diet without additives exhibited poor growth, characterized by lower BW and higher FCR. However, dietary incorporation of WGO with *B. subtilis* improved BWG and reduced FCR, consistent with previous studies (Arshad et al., 2013; Bai et al., 2016). *B. subtilis* has been shown to enhance immune response during stress, supporting broiler growth (Abdel-Moneim et al., 2021), and WGO has been found to improve growth performance in rabbits and broilers (Arshad et al., 2013; Morshedy et al., 2021; Zou et al., 2023). The benefits of WGO may be due to its active compounds, such as α-lipoic acid, α-tocopherols, and vitamin C (Barakat et al., 2011), which promote health and performance. Additionally, WGO's antibacterial and antioxidant properties likely contribute to these improvements (Morshedy et al., 2021). The combination of WGO and *B. subtilis* under heat stress likely enhances growth by boosting antioxidative enzymes, immune response, nutrient utilization, and intestinal morphology, and modifying gut microbiota (Morshedy et al., 2021; Elbaz et al., 2022b).

Despite the growth performance improvement from feeding chickens WGO and *B. subtilis* supplements, carcass characteristics were largely unaffected except for abdominal fat. Chickens fed WGO alone or combined with *B. subtilis* (WGO and CWB groups) had less abdominal fat than other groups. In agreement with our results, Arshad et al. (2013) and Elbaz et al. (2021) reported that WGO and probiotics supplementation to broiler diets led to decreased abdominal fat. This reduction in abdominal fat may be due to the active chemicals in the oils that influence fat metabolism by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA), the rate-limiting enzyme involved in cholesterol synthesis, thereby reducing cholesterol production and fat deposition (Zhang et al., 2009). Gut microbiota also affects fat metabolism by reducing the production of acetyl-CoA carboxylase, which decreases lipogenesis. The reduction in abdominal fat indicates that the experimental supplements improved carcass quality by redistributing fatty acids within the carcass (Wood et al., 2008).

In this study, the digestive system performance was investigated to clarify the effectiveness of experimental additives on nutrient utilization. The results showed a notable improvement in the digestibility of DM, CP, EE, and NFE in chickens of the CWB group under heat stress. Previous studies have reported that feeding diets containing probiotics enhanced nutrient digestion in broilers by modifying the microbial environment, inhibiting pathogens, and supporting host metabolism (Elbaz et al., 2023b; Gelinas et al., 2024). Similar to our results, plant oils have been shown to significantly improve CP and EE digestibility (Ezzat and Hamed, 2012). For instance, adding vegetable oil to rabbit diets has been found to improve the digestibility of DM, OM, and CF (Omer et al., 2013). Morshedy et al. (2021) also confirmed that the CWB supplement improved digestible crude protein, total digestible

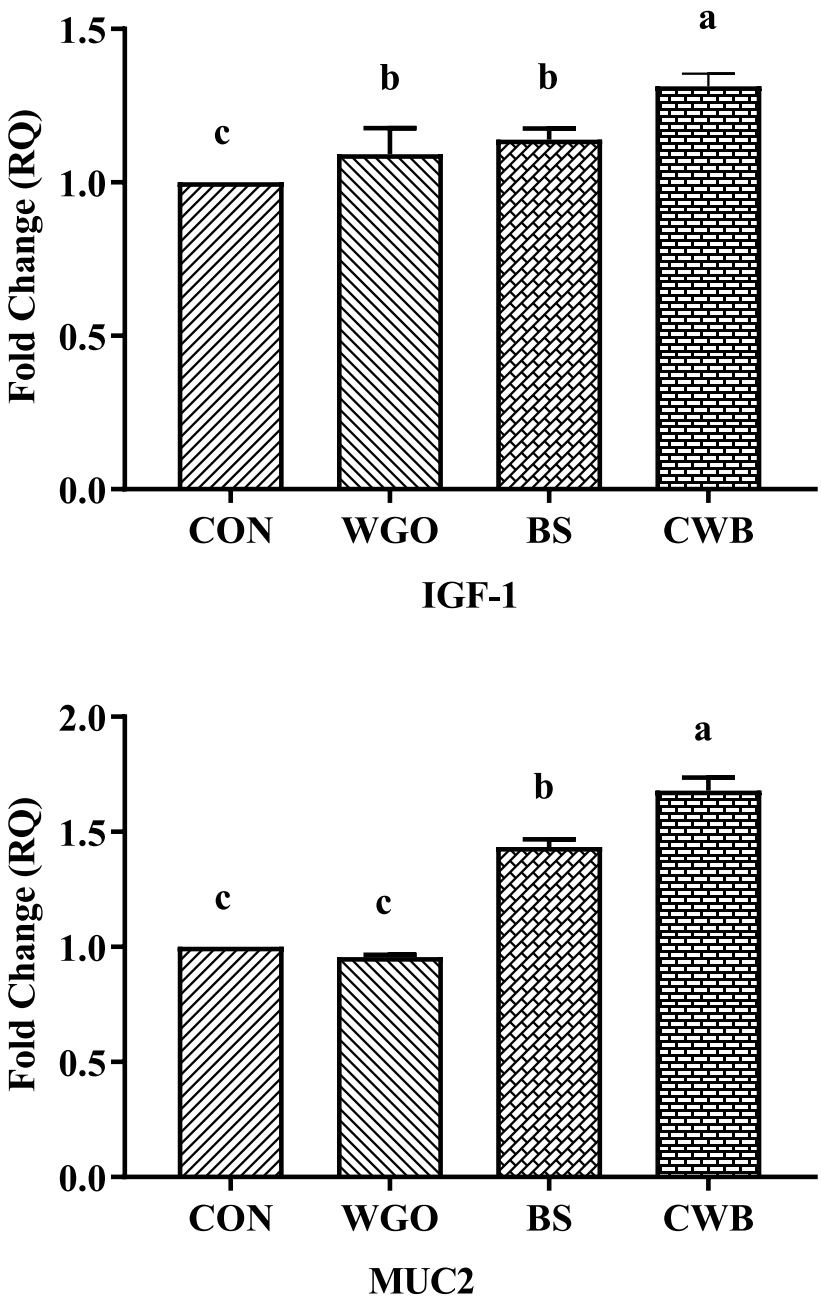

Fig. 4. Effect of dietary supplementation of wheat germ cold-pressed oil, *B. subtilis,* and their mixture on mRNA expression of hepatic IGF-1 and ileal MUC2 of broiler chickens under heat stress. CON, Experimental basal diet without feed additive; WGO, Experimental basal diet with wheat germ oil at 200 mg.kg^{-1}; BS, Experimental basal diet with *Bacillus subtilis* at 500 mg.kg^{-1} containing 5 × 10^{8} CFU.g^{-1}; CWB, Experimental basal diet with wheat germ oil and *Bacillus subtilis* Data presented as mean values with their standard errors. Values with different superscript letters are statistically different ($P < 0.05$).

nutrients, and digestible energy. These findings are in line with those of Lee et al. (2003), which demonstrated that active compounds in oil increase digestive enzyme activity in broilers, thereby enhancing nutrient digestibility.

Heat stress in broilers leads to major physiological changes, including increased corticosterone levels and decreased T_3 levels (Apalowo et al., 2024). This stress also damages the intestinal mucosa, closely linked to the elevated secretion of corticosterone in broilers (Law et al., 2019). In our experiment, broilers under heat stress in the control group showed significantly higher corticosterone and glucose levels and lower T_3 concentrations. However, the experimental feed additives significantly altered these stress markers by decreasing corticosterone and glucose levels and increasing T_3 concentrations in heat-stressed broilers. These findings align with Abdelnour et al. (2023), who reported that adding pumpkin essential oil to rabbits' diets reduced stress-related threats by modifying many physiological processes. The physiological changes induced by the WGO and *B. subtilis* supplement in heat-stressed chickens likely play a role in alleviating heat stress by increasing T_3 concentrations, which are crucial for regulating body metabolism and temperature (Quinteiro-Filho et al., 2012). Additionally, heat stress reduces the aerobic metabolism of glucose and fats while increasing glycolysis due to ROS production, which damages mitochondrial activity and explains the rise in glucose levels during heat stress (Estévez, 2015).

Heat stress has been shown to negatively affect lipid metabolism due to the activation of lipid peroxidation and electrolyte imbalances (Sokołowicz et al., 2016). Oxidative stress during heat stress triggers the production of ROS, altering lipid profiles in blood and meat (Apalowo

et al., 2024). In the present study, heat stress and experimental additives remarkably affected the blood lipid profile. Heat stress increases cholesterol and LDL levels and decreases HDL levels, consistent with findings by Abdel-Moneim et al. (2024), who reported similar changes under heat stress. However, supplementing broiler diets with a mixture of WGO and *B. subtilis* significantly reduced plasma cholesterol and LDL levels while increasing HDL levels compared to the remaining groups. These findings are in line with Kim et al. (2009), who observed a reduction in cholesterol levels in broilers fed probiotics, which might be attributed to the inhibition of cholesterol synthesis by probiotics. Similarly, Abd El-Moneim and Sabic (2019) found that feeding on *A. awamori* as a probiotic exhibited a positive effect on lipid metabolism by lowering cholesterol through the inhibition of HMG-CoA. Morshedy et al. (2021) also reported that WGO supplementation in rabbit diets increased HDL levels and decreased LDL levels. The hypocholesterolemic impact of WGO may be attributed to its high contents of phytosterols and vitamin E that reduce cholesterol synthesis and absorption by limiting the incorporation of bile and dietary cholesterol into micelles. The presence of linoleic acid in WGO, which abstracts cholesterol, further contributes to this effect (Zacchi et al., 2006).

Blood levels of immunoglobulins and cytokines are key indicators of broiler immunity. In the current study, chickens of the CWB group showed increased levels of IgA, IgG, and IL-10, while IL-6 levels decreased compared to other groups. Conversely, IL-6 increased and IL-10 decreased in the control group. In the same context, recent studies indicate that heat stress causes the release of pro-inflammatory cytokines (IL-6 and IL-10) through an imbalance in the gut microbiota and thus a disruption in physiological functions (Chen et al., 2024). Consistent with our findings, *B. subtilis* supplementation has been shown to boost IgA, IgG, and IgM levels and enhance innate immunity by reducing IL-6 and increasing IL-10 during heat stress (Wu et al., 2018). Moreover, many reports have documented the anti-inflammatory and antioxidant properties of WGO (Alamery et al., 2022; Zargar et al., 2023).

Oxidative stress is a major harmful effect of from heat stress, producing ROS that damage biological molecules, leading to impaired physiological performance (Abdel-Moneim et al., 2021). During oxidative stress, birds produce enzymes like SOD and GPx to neutralize these reactive species. Malondialdehyde, a key product of lipid peroxidation, serves as an indicator of oxidative damage, signaling that hydroxyl radicals have attacked fatty acids in membrane phospholipids, causing cell damage. In the current study, the heat-stressed chickens fed on diets containing a mixture of WGO and *B. subtilis* showed increased SOD activity and decreased MDA levels, although GPx activity remained unaffected. These findings align with previous studies, which demonstrated that *B. subtilis* supplementation boosted CAT, GSH, and SOD activities while reducing MDA in poultry (Bai et al., 2016; Abdel-Moneim et al., 2020a). Additionally, Arshad et al. (2013) noticed that incorporating WGO to broiler diets improved their antioxidant status by decreasing MDA levels. Consistent with these results, Zargar et al. (2023) reported that antioxidant capabilities and performance were significantly improved by the treatment of WGO.

Gut microorganisms play vital roles in many aspects of chickens' health including metabolism, immunity, and growth. The use of probiotics to mitigate stress has gained popularity, with studies highlighting their positive effects on modulating gut microbiota (Elbaz et al., 2021; Shehata et al., 2021). In the current study, the experimental supplements improved the microbial balance in the ceca of heat-stressed broilers. Chickens fed the diet with WGO and *B. subtilis* supplements mixture showed a noteworthy increase in *Lactobacillus* counts and a decrease in *C. perfringens* and *E. coli* counts. These findings align with previous studies that reported increased *Lactobacillus* and reduced *E. coli* enumeration when *B. subtilis* was added to broiler diets (Jeong and Kim, 2014). Additionally, *B. subtilis* has been shown to decrease *C. perfringens* and increase *Lactobacillus* and *Bifidobacteria* in the ileum and cecum (Whelan et al., 2019). Similarly, Zou et al. (2023) results documented that WGO incorporation decreased counts of *E. coli* and *Salmonella* and increased the enumeration of *Lactobacillus* in broiler feces. The antimicrobial effect of WGO may be attributed to its bioactive compounds. Cao et al. (2020) reported that compounds such as ß-acids, polyphenols flavonoids, and xanthohumol in plant extracts promote the growth of *Lactobacillus* bacteria and maintain gut health. Wheat germ extract also has various therapeutic and biomedical applications, including antimicrobial effects (Ryva et al., 2019). Based on these results, we conclude that the improved growth performance of heat-stressed broilers is due to the antioxidant and antibacterial properties of the WGO and *B. subtilis* mixture added to their diet.

Heat stress negatively impacts poultry productivity by reducing nutrient utilization and disrupting the intestinal mucosal barrier, leading to altered gene expression and changes in gut microbial content (Abdel-Moneim et al., 2021). Our findings revealed that dietary incorporation of WGO and *B. subtilis* mixture increased the mRNA expression of MUC2 and IGF-1 in heat-stressed broilers. Similar findings were reported by Aliakbarpour et al. (2012), who observed that probiotics enhanced the regulation of the intestinal epithelial barrier by upregulating MUC2 expression. Furthermore, Humam et al. (2021) noticed that adding probiotics under heat stress conditions exhibited antioxidative activities and modified IGF-1 mRNA expression. Salehizadeh et al. (2019) also reported that probiotic supplements elevated IGF-1 mRNA expression, and Kareem et al. (2016) found a positive correlation between increased hepatic IGF-1 expression and the addition of L. *plantarum* strains in broiler diets. Beneficial gut microbes are known to modulate IGF-1 gene expression by producing short-chain fatty acids, which stimulate liver and adipose tissue cells to increase IGF-1 levels, thereby promoting growth (Salehizadeh et al., 2019). Ginkgo biloba oil supplementation has also been shown to increase IGF-I expression in broilers (El-Kasrawy et al., 2023), likely due to the bioactive compounds in the oil that enhance growth performance. Our results indicate that supplementing WGO and *B. subtilis* mixture to the CWB group boosts IGF-1 expression, which binds to its growth hormone receptor, stimulating cell proliferation and leading to increased BW. The increased MUC2 expression in chickens of the CWB group could be attributed to improved modulation of beneficial gut bacteria, reducing inflammation, enhancing gut integrity, and optimizing nutrient utilization, thereby improving the growth performance of heat-stressed broilers.

This study demonstrates that dietary incorporation of a combination of WGO and *B. subtilis* can effectively mitigate the deleterious impacts of heat stress on broiler chickens. The dietary inclusion of this mixture improved immune response, nutrient digestibility, growth performance, and enhancing antioxidant capacity. The combination also modulated the gut microbial environment, reduced inflammation, and up-regulated mRNA expression of IGF-1 and MUC2, indicating enhanced physiological resilience and overall health in heat-stressed broilers.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abd El-Hack, M.E., El-Saadony, M.T., Shafi, M.E., Alshahrani, O.A., Saghir, S.A., Al-Wajeeh, A.S., Al-Shargi, O.Y., Taha, A.E., Mesalam, N.M., Abdel-Moneim, A.M.E., 2021. Prebiotics can restrict Salmonella populations in poultry: a review. Anim. Biotechnol. 33, 1668–1677. https://doi.org/10.1080/10495398.2021.1883637.

Abd El-Moneim, A.E., Sabic, E.M., 2019. Beneficial effect of feeding olive pulp and *Aspergillus awamori* on productive performance, egg quality, serum/yolk cholesterol and oxidative status in laying Japanese quails. J. Anim. Feed Sci. 28, 52–61. https://doi.org/10.22358/jafs/105537/2019.

Abd El-Moneim, E.A., El-Wardany, I., Abu-Taleb, A.M., Wakwak, M.M., Ebeid, T.A., Saleh, A.A., 2020. Assessment of in ovo administration of *bifidobacterium bifidum* and *bifidobacterium longum* on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. Probiotics Antimicrob. Proteins 12, 439–450. https://doi.org/10.1007/s12602-019-09549-2.

Abd El-Hack, M.E., El-Saadony, M.T., Shafi, M.E., Qattan, S.Y., Batiha, G.E., Khafaga, A. F., Abdel-Moneim, A.M.E., Alagawany, M., 2020. Probiotics in poultry feed: a comprehensive review. J. Anim. Physiol. Anim. Nutr. 104, 1835–1850. https://doi.org/10.1111/jpn.13454.

Abdel-Moneim, A.M.E., El-Saadony, M.T., Shehata, A.M., Saad, A.M., Aldhumri, S.A., Ouda, S.M., Mesalam, N.M., 2022a. Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi J. Biol. Sci. 29, 1197–1209. https://doi.org/10.1016/j.sjbs.2021.09.046.

Abdel-Moneim, A.M.E., Khidr, R.S., Badran, A.M., Amin, S.A., Badri, F.B., Gad, G.G., Thabet, H.A., Elbaz, A.M., 2024. Efficacy of supplementing Aspergillus awamori in enhancing growth performance, gut microbiota, digestibility, immunity, and antioxidant activity of heat-stressed broiler chickens fed diets containing olive pulp. BMC Vet. Res. 20, 205. https://doi.org/10.1186/s12917-024-04050-7.

Abdel-Moneim, A.M.E., Selim, D.A., Basuony, H.A., Sabic, E.M., Saleh, A.A., Ebeid, T.A., 2020a. Effect of dietary supplementation of *Bacillus subtilis* spores on growth performance, oxidative status and digestive enzyme activities in Japanese quail birds. Trop. Anim. Health Prod. 52, 671–680. https://doi.org/10.1007/s11250-019-02055-1.

Abdel-Moneim, A.M.E., Shehata, A.M., Khidr, R.E., Paswan, V.K., Ibrahim, N.S., El-Ghoul, A.A., Aldhumri, S.A., Gabr, S.A., Mesalam, N.M., Elbaz, A.M., 2021. Nutritional manipulation to combat heat stress in poultry–A comprehensive review. J. Therm. Biol. 98, 102915. https://doi.org/10.1016/j.jtherbio.2021.102915.

Abdel-Moneim, A.M.E., Shehata, A.M., Mohamed, N.G., Elbaz, A.M., Ibrahim, N., 2022b. Synergistic effect of Spirulina platensis and selenium nanoparticles on growth performance, serum metabolites, immune responses, and antioxidant capacity of heat-stressed broiler chickens. Biol. Trace Elem. Res. 200, 768–779. https://doi.org/10.1007/s12011-021-02662-w.

Abdel-Moneim, A.M.E., Shehata, A.M., Selim, D.A., El-Saadony, M.T., Mesalam, N.M., Saleh, A.A., 2022c. Spirulina platensis and biosynthesized selenium nanoparticles improve performance, antioxidant status, humoral immunity and dietary and ileal microbial populations of heat-stressed broilers. J. Therm. Biol. 104, 103195. https://doi.org/10.1016/j.jtherbio.2022.103195.

Abdel-Moneim, A.M.E., Siddiqui, S.A., Shehata, A.M., Biswas, A., Abougabal, M.S., Kamal, A.M., Mesalam, N.M., Elsayed, M.A., Yang, B., Ebeid, T.A., Teng, X., 2023. Impact of light wavelength on growth and welfare of broiler chickens: aan overview and future perspective. Ann. Anim. Sci. https://doi.org/10.2478/aoas-2023-0090.

Abdel-Moneim, A.E., Elbaz, A.M., Khidr, R.E., Badri, F.B., 2020b. Effect of in ovo inoculation of *bifidobacterium* spp. On growth performance, thyroid activity, ileum histomorphometry and microbial enumeration of broilers. Probiotics. Antimicrob. Proteins 12, 873–882. https://doi.org/10.1007/s12602-019-09613-x.

Abdelnour, S.A., Metwally, M.G., Bahgat, L.B., Naiel, M.A., 2023. Pumpkin seed oil–supplemented diets promoted the growth productivity, antioxidative capacity, and immune response in heat-stressed growing rabbits. Trop. Anim. Health Prod. 55, 55. https://doi.org/10.1007/s11250-023-03460-3.

Alamery, S., Zargar, S., Yaseen, F., Wani, T.A., Siyal, A., 2022. Evaluation of the effect of wheat germ oil and olmutinib on the thioacetamide-induced liver and kidney toxicity in mice. Life 12, 900. https://doi.org/10.3390/life12060900.

Aliakbarpour, H., Chamani, M., Rahimi, G., Sadeghi, A., Qujeq, D., 2012. The Bacillus subtilis and lactic acid bacteria probiotics influences intestinal mucin gene expression, histomorphology and growth performance in broilers. Asian-Australas. J. Anim. Sci. 25, 1285. https://doi.org/10.5713/ajas.2012.12110.

Anwar, M., Mohamed, N., 2015. Amelioration of liver and kidney functions disorders induced by sodium nitrate in rats using wheat germ oil. J. Radiat. Res. Appl. Sci. 8, 77–83. https://doi.org/10.1016/j.jrras.2014.11.004.

AOAC, 2000. Official Methods of Analysis of AOAC, 16th ed. AOAC, Gaithersburg, MD, USA.

Apalowo, O.O., Ekunseitan, D.A., Fasina, Y.O., 2024. Impact of heat stress on broiler chicken production. Poultry 3, 107–128. https://doi.org/10.3390/poultry3020010.

Arshad, M.S., Anjum, F.M., Khan, M.I., Shahid, M., Akhtar, S., Sohaib, M., 2013. Wheat germ oil enrichment in broiler feed with α-lipoic acid to enhance the antioxidant potential and lipid stability of meat. Lipids Health Dis. 12, 1–14. https://doi.org/10.1186/1476-511X-12-164.

Aviagen, R. 2018. Ross broiler management handbookAviagen Limited Newbridge Midlothian EH28 8SZ, Scotland, UK.

Bai, W.K., Zhang, F.J., He, T.J., Su, P.W., Ying, X.Z., Zhang, L.L., Wang, T., 2016. Dietary probiotic Bacillus subtilis strain fmbj increases antioxidant capacity and oxidative stability of chicken breast meat during storage. PLoS One 11, e0167339. https://doi.org/10.1371/journal.pone.0167339.

Barakat, I.A., Abbas, O.A., Ayad, S., Hassan, A.M., 2011. Evaluation of radio protective effects of wheat germ oil in male rats. J. Am. Sci. 7, 664–673.

Cao, G., Zeng, X., Liu, J., Yan, F., Xiang, Z., Wang, Y., Tao, F., Yang, C., 2020. Change of serum metabolome and cecal microflora in broiler chickens supplemented with grape seed extracts. Front. Immunol. 11, 610934. https://doi.org/10.3389/fimmu.2020.610934.

Chen, H., Wang, F., Wu, X., Yuan, S., Dong, H., Zhou, C., Feng, S., Zhao, Z., Si, L., 2024. Chronic heat stress induces oxidative stress and induces inflammatory injury in broiler spleen via TLRs/MyD88/NF-κb signaling pathway in broilers. Vet. Sci. 11, 293. https://doi.org/10.3390/vetsci11070293.

Dosoky, W.M., Zeweil, H.S., Ahmed, M.H., Zahran, S.M., Shaalan, M.M., Abdelsalam, N. R., Abdel-Moneim, A.M.E., Taha, A.E., El-Tarabily, K.A., Abd El-Hack, M.E., 2021. Impacts of onion and cinnamon supplementation as natural additives on the performance, egg quality, and immunity in laying Japanese quail. Poult. Sci. 100, 101482. https://doi.org/10.1016/j.psj.2021.101482.

Ebeid, T.A., Ketta, M., Al-Homidan, I.H., Barakat, H., Abdel-Moneim, A.M.E., 2023. In ovo feeding of nutraceuticals and its role in adjusting the gastrointestinal tract, antioxidative properties, immunological response, and performance in poultry: aan updated review. Czech J. Anim. Sci. 68. https://doi.org/10.17221/201/2022-CJAS.

El-Kasrawy, N.I., Majrashi, K.A., El-Naggar, K., Abd Elreheim, A.M., Essa, B.H., Mahmoud, S.F., Ibrahim, S.A., Raafat, M., Abd El-Hack, M.E., Aboghanima, M.M., 2023. Impacts of supplemental Ginkgo biloba oil on broilers' growth, blood indices, intestinal and hepatic morphology and expression of growth-related genes. Poult. Sci. 102, 102520. https://doi.org/10.1016/j.psj.2023.102520.

Elbaz, A.M., Ahmed, A.M., Abdel-Maqsoud, A., Badran, A.M., Abdel-Moneim, A.M.E., 2022a. Potential ameliorative role of Spirulina platensis in powdered or extract forms against cyclic heat stress in broiler chickens. Environ. Sci. Pollut. Res. 29, 45578–45588. https://doi.org/10.1007/s11356-022-19115-z.

Elbaz, A.M., Ashmawy, E.S., Salama, A.A., Abdel-Moneim, A.M.E., Badri, F.B., Thabet, H. A., 2022b. Effects of garlic and lemon essential oils on performance, digestibility, plasma metabolite, and intestinal health in broilers under environmental heat stress. BMC Vet. Res. 18, 1–12. https://doi.org/10.1186/s12917-022-03530-y.

Elbaz, A.M., El-Sheikh, S.E., Abdel-Maksoud, A., 2023a. Growth performance, nutrient digestibility, antioxidant state, ileal histomorphometry, and cecal ecology of broilers fed on fermented canola meal with and without exogenous enzymes. Trop. Anim. Health Prod. 55, 46. https://doi.org/10.1007/s11250-023-03476-9.

Elbaz, A.M., Farrag, B., Mesalam, N.M., Basuony, H.A., Badran, A.M., Abdel-Moneim, A. M.E., 2023b. Growth performance, digestive function, thyroid activity, and immunity of growing rabbits fed olive cake with or without saccharomyces cerevisiae or citric acid. Trop. Anim. Health Prod. 55, 376. https://doi.org/10.1007/s11250-023-03794-y.

Elbaz, A.M., Ibrahim, N.S., Shehata, A.M., Mohamed, N.G., Abdel-Moneim, A.M.E., 2021. Impact of multi-strain probiotic, citric acid, garlic powder or their combinations on performance, ileal histomorphometry, microbial enumeration and humoral immunity of broiler chickens. Trop. Anim. Health Prod. 53, 1–10. https://doi.org/10.1007/s11250-021-02554-0.

Emam, K., Ali, S.A., Morsy, A., Fouda, W.A., Elbaz, A.M., 2024. Role of Nannochloropsis Oculata supplement in improving performance, antioxidant status, blood metabolites, and egg quality of laying hens under hot environmental conditions. Sci. Rep. 14, 16884. https://doi.org/10.1038/s41598-024-66595-9.

Estévez, M., 2015. Oxidative damage to poultry: from farm to fork. Poult. Sci. 94, 1368–1378. https://doi.org/10.3382/ps/pev094.

Ezzat, W., Hamed, S., 2012. Influence of using rocket seed (Eruca sative) oil and onion seed (Allium cepa) oil on productive and physiological performance of growing rabbits under hot climate condition. J. Product. Dev. 17, 127–148.

Gelinas, A., Sudan, S., Patterson, R., Li, J., Huyben, D., Barta, J.R., Kiarie, E.G., 2024. Growth performance, organs weight, intestinal histomorphology, and oocyst shedding in broiler chickens offered novel single strain Bacillus subtilis isolated from camel dung and challenged with Eimeria. Poult. Sci. 103, 103519. https://doi.org/10.1016/j.psj.2024.103519.

Gumus, Z.P., Guler, E., Demir, B., Barlas, F.B., Yavuz, M., Colpankan, D., Senisik, A.M., Teksoz, S., Unak, P., Coskunol, H., 2015. Herbal infusions of black seed and wheat germ oil: ttheir chemical profiles, in vitro bio-investigations and effective formulations as phyto-nanoemulsions. Colloids Surf. B Biointerfaces 133, 73–80. https://doi.org/10.1016/j.colsurfb.2015.05.044.

Humam, A.M., Loh, T.C., Foo, H.L., Izuddin, W.I., Zulkifli, I., Samsudin, A.A., Mustapha, N.M., 2021. Supplementation of postbiotic RI11 improves antioxidant enzyme activity, upregulated gut barrier genes, and reduced cytokine, acute phase protein, and heat shock protein 70 gene expression levels in heat-stressed broilers. Poult. Sci. 100, 100908. https://doi.org/10.1016/j.psj.2020.12.011.

Jeong, J., Kim, I., 2014. Effect of Bacillus subtilis C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. Poult. Sci. 93, 3097–3103. https://doi.org/10.3382/ps.2014-04086.

Kareem, K.Y., Loh, T.C., Foo, H.L., Akit, H., Samsudin, A.A., 2016. Effects of dietary postbiotic and inulin on growth performance, IGF1 and GHR mRNA expression, faecal microbiota and volatile fatty acids in broilers. BMC Vet. Res. 12, 1–10. https://doi.org/10.1186/s12917-016-0790-9.

Kim, S.S., Galaz, G.B., Pham, M.A., Jang, J.W., Oh, D.H., Yeo, I.K., Lee, K.J., 2009. Effects of dietary supplementation of a meju, fermented soybean meal, and Aspergillus oryzae for juvenile parrot fish (Oplegnathus fasciatus). Asian-Australas. J. Anim. Sci. 22, 849–856. https://doi.org/10.5713/ajas.2009.80648.

Law, F., Zulkifli, I., Soleimani, A., Liang, J., Awad, E., 2019. Effects of reduced-protein diets supplemented with protease in broiler chickens under high stocking density. Anim. Product. Sci. 59, 2212–2221. https://doi.org/10.1071/AN18477.

Lee, K.W., Everts, H., Kappert, H., Frehner, M., Losa, R., Beynen, A., 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br. Poult. Sci. 44, 450–457. https://doi.org/10.1080/0007166031000085508.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Mesalam, N.M., Aldhumri, S.A., Gabr, S.A., Ibrahim, M.A., Al-Mokaddem, A.K., Abdel-Moneim, A.M.E., 2021. Putative abrogation impacts of Ajwa seeds on oxidative damage, liver dysfunction and associated complications in rats exposed to carbon

tetrachloride. Mol. Biol. Rep. 48, 5305–5318. https://doi.org/10.1007/s11033-021-06544-1.

Morshedy, S.A., Abdelmodather, A.M., Basyony, M.M., Zahran, S.A., Hassan, M.A., 2021. Effects of rocket seed oil, wheat germ oil, and their mixture on growth performance, feed utilization, digestibility, redox status, and meat fatty acid profile of growing rabbits. Agriculture 11, 662. https://doi.org/10.3390/agriculture11070662.

Mountzouris, K., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., Fegeros, K., 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult. Sci. 89, 58–67. https://doi.org/10.3382/ps.2009-00308.

Omer, H., El-Nomeary, Y., El-Kady, R., Badr, A.M., Ali, F., Ahmed, S.M., El-Allawy, H., Ibrahim, S.A., 2013. Improving the utilization of rabbit diets containing vegetable oil by using fennel (Foeniculum vulgare) and oregano (Origanum vulgare L.) as feed additives. Life Sci. J. 10, 2625–2636.

Quinteiro-Filho, W., Calefi, A., Cruz, D., Aloia, T., Zager, A., Astolfi-Ferreira, C., Ferreira, J.P., Sharif, S., Palermo-Neto, J., 2017. Heat stress decreases expression of the cytokines, avian β-defensins 4 and 6 and toll-like receptor 2 in broiler chickens infected with Salmonella Enteritidis. Vet. Immunol. Immunopathol. 186, 19–28. https://doi.org/10.1016/j.vetimm.2017.02.006.

Quinteiro-Filho, W.M., Gomes, A., Pinheiro, M.L., Ribeiro, A., Ferraz-de-Paula, V., Astolfi-Ferreira, C.S., Ferreira, A.J.P., Palermo-Neto, J., 2012. Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with Salmonella Enteritidis. Avian Pathol. 41, 421–427. https://doi.org/10.1080/03079457.2012.709315.

Ryva, B., Zhang, K., Asthana, A., Wong, D., Vicioso, Y., Parameswaran, R., 2019. Wheat germ agglutinin as a potential therapeutic agent for leukemia. Front. Oncol. 9, 100. https://doi.org/10.3389/fonc.2019.00100.

Salehizadeh, M., Modarressi, M.H., Mousavi, S.N., Ebrahimi, M.T., 2019. Effects of probiotic lactic acid bacteria on growth performance, carcass characteristics, hematological indices, humoral immunity, and IGF-I gene expression in broiler chicken. Trop. Anim. Health Prod. 51, 2279–2286. https://doi.org/10.1007/s11250-019-01935-w.

Shehata, A.M., Paswan, V.K., Attia, Y.A., Abdel-Moneim, A.M.E., Abougabal, M.S., Sharaf, M., Elmazoudy, R., Alghafari, W.T., Osman, M.A., Farag, M.R., 2021. Managing gut microbiota through in ovo nutrition influences early-life programming in broiler chickens. Animals 11, 3491. https://doi.org/10.3390/ani11123491.

Shehata, A.M., Paswan, V.K., Attia, Y.A., Abougabal, M.S., Khamis, T., Alqosaibi, A.I., Alnamshan, M.M., Elmazoudy, R., Abaza, M.A., Salama, E.A., Abdel-Moneim, A.M. E., 2022. In ovo inoculation of Bacillus subtilis and raffinose affects growth performance, cecal microbiota, volatile fatty acid, ileal morphology and gene expression, and sustainability of broiler chickens (Gallus gallus). Front. Nutr. 9, 903847. https://doi.org/10.3389/fnut.2022.903847.

Siddiqui, S.A., Bahmid, N.A., Taha, A., Abdel-Moneim, A.M.E., Shehata, A.M., Tan, C., Kharazmi, M.S., Li, Y., Assadpour, E., Castro-Muñoz, R., 2022. Bioactive-loaded nanodelivery systems for the feed and drugs of livestock; purposes, techniques and applications. Adv. Colloid Interface Sci., 102772 https://doi.org/10.1016/j.cis.2022.102772.

Sokołowicz, Z., Krawczyk, J., Świątkiewicz, S., 2016. Quality of poultry meat from native chicken breeds–a review. Ann. Anim. Sci. 16, 347–368. https://doi.org/10.1515/aoas-2016-0004.

Tao, X., Xin, H., 2003. Acute synergistic effects of air temperature, humidity, and velocity on homeostasis of market–size broilers. Trans. ASAe 46, 491. https://doi.org/10.13031/2013.12971.

Whelan, R.A., Doranalli, K., Rinttilä, T., Vienola, K., Jurgens, G., Apajalahti, J., 2019. The impact of Bacillus subtilis DSM 32315 on the pathology, performance, and intestinal microbiome of broiler chickens in a necrotic enteritis challenge. Poult. Sci. 98, 3450–3463. https://doi.org/10.3382/ps/pey500.

Wood, J., Enser, M., Fisher, A., Nute, G., Sheard, P., Richardson, R., Hughes, S., Whittington, F., 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat. Sci. 78, 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019.

Wu, Q., Liu, N., Wu, X., Wang, G., Lin, L., 2018. Glutamine alleviates heat stress-induced impairment of intestinal morphology, intestinal inflammatory response, and barrier integrity in broilers. Poult. Sci. 97, 2675–2683. https://doi.org/10.3382/ps/pey123.

Yang, B., Li, X., Baran, A.M., Abdel-Moneim, A.M.E., 2023. Effects of dietary incorporation of Radix rehmanniae praeparata polysaccharide on growth performance, digestive physiology, blood metabolites, meat quality, and tibia characteristics in broiler chickens. Poult. Sci. 102, 103150. https://doi.org/10.1016/j.psj.2023.103150.

Zacchi, P., Daghero, J., Jaeger, P., Eggers, R., 2006. Extraction/fractionation and deacidification of wheat germ oil using supercritical carbon dioxide. Braz. J. Chem. Eng. 23, 105–110. https://doi.org/10.1590/S0104-66322006000100011.

Zargar, S., Wani, T.A., Ahamad, S.R., 2023. An insight into wheat germ oil nutrition, identification of its bioactive constituents and computer-aided multidimensional data analysis of its potential anti-inflammatory effect via molecular connections. Life 13, 526. https://doi.org/10.3390/life13020526.

Zhang, G., Yang, Z., Wang, Y., Yang, W., Jiang, S., Gai, G., 2009. Effects of ginger root (Zingiber officinale) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. Poult. Sci. 88, 2159–2166. https://doi.org/10.3382/ps.2009-00165.

Zou, Q., Meng, W., Li, C., Wang, T., Li, D., 2023. Feeding broilers with wheat germ, hops and grape seed extract mixture improves growth performance. Front. Physiol. 14, 1144997. https://doi.org/10.3389/fphys.2023.1144997.