



Fermented Shallot (*Allium cepa* L.) Extract with *Lactiplantibacillus plantarum* Improves Growth Performance and Attenuates Physiological Stress in Broiler Chickens under Chronic Natural Summer Heat Exposure

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ABSTRACT

Heat stress has remained one of the gravest limitations on poultry production under tropical climatic conditions. It stimulates increased stress hormone levels, oxidative damage, and impaired metabolism, which ultimately reduce growth performance. This study aimed to evaluate whether *Lactiplantibacillus plantarum* 1582-fermented shallot bulb extract (LPFS), supplied as drinking water, could improve growth performance and physical resilience in yellow-feathered Rilai broilers exposed to chronic natural summer heat in Central Vietnam. In a completely randomized study design, 300 day-old male chicks were randomly assigned to six treatments (five replications of 10 birds each) and raised for 90 days in an open-sided house. The birds were fed plain drinking water (negative control), oxytetracycline in feed (100 mg/kg diet, positive control), or LPFS at 25, 50, 75, or 100 mg/L in drinking water. The growth performance was measured weekly, and blood samples were taken after 90 days and analyzed to determine lipid profiles, liver enzymes, acute-phase proteins, and stress/oxidative biomarkers. Body weight gain and feed conversion ratio were significantly higher with LPFS supplementation compared to the negative control ($p < 0.05$), with the most significant benefits observed in the later growth phase. Also, LPFS significantly decreased the levels of circulating corticosterone, malondialdehyde, and heat shock protein 70 in addition to increasing the activities of several major antioxidant enzymes. The serum cholesterol and triglyceride levels were also lower in the groups treated with LPFS, with positive changes in liver enzyme activities. In conclusion, LPFS acts as a postbiotic–phytochemical water additive that supports productivity and metabolic resilience during prolonged natural summer heat exposure and may partially substitute for antibiotic-associated benefits in tropical poultry production.

Keywords: broiler chicken; fermented shallot extract; heat stress; *Lactiplantibacillus plantarum*; oxidative stress

INTRODUCTION

The rising global temperatures are indeed a major challenge to the poultry industry, especially in tropical and subtropical latitudes, where heat stress (HS) is one of the major factors leading to reduced productivity. Physiological indicators of HS include inhibited feed consumption, impaired immune competence, and elevated mortality rates (Wasti *et al.*, 2020; Teyssier *et al.*, 2022). On the cellular level, thermal challenges result in excessive production of reactive oxygen species (ROS) that disrupts the endogenous antioxidant system, impairs the integrity of the intestinal barrier (often referred to as leaky gut), and alters lipid metabolism (Surai *et al.*, 2019; Mishra & Jha, 2019). Although in the past, antibiotic growth promoters (AGPs) were used to overcome these stressors, the global shift towards eliminating

AGPs due to concerns about antimicrobial resistance has necessitated the identification of sustainable, natural alternatives (Sugiharto & Ranjitkar, 2019).

Phytogenic additives, or those of the *Allium* genus, are a promising avenue since they have inherent antioxidant and antimicrobial properties. Shallots (*Allium cepa* L. var. *aggregatum*) are a major source of bioactive organosulfur compounds and flavonoids, including quercetin (Kothari *et al.*, 2019; Hai *et al.*, 2024). However, low palatability and low bioavailability of glycosylated flavonoids often inhibit the efficacy of raw extracts (Adli *et al.*, 2024). In order to avoid these limitations, it is possible to use the probiotic fermentation - i.e., the use of *Lactiplantibacillus plantarum* - to biotransform phytochemicals into more readily absorbable aglycones through bacterial 8-glucosidase activity (Huynh *et al.*, 2014; Hai *et al.*, 2025a). The result

of this process is functional postbiotics, which strengthen gut health and resilience to systemic stress.

Since very little information is available on the effectiveness of fermented shallot extracts in yellow-feathered chickens under natural tropical heat loads, this experiment aimed to determine the efficacy of fermented shallot extracts as water additives in this poultry model to enhance growth and stress biomarkers.

MATERIALS AND METHODS

Ethics Approval

The protocols of the research were formally approved by the Animal Ethics Advisory Committee of Hue University, Vietnam (Approval No. HUVNO39.C; 20 March 2025).

Birds, Housing, and Experimental Design

Three hundred male yellow-feathered Rilai one-day-old chicks were purchased, individually weighed, and randomly assigned to six treatments with five replicate pens of ten birds each in a completely randomized design. The experiment was performed in the natural summer season in Central Vietnam in an open-sided house. For the first three days, all the chicks were fed on an anti-stress additive in drinking water. During the 90-day trial, the birds were freely exposed to commercial corn-soybean meal diets, which were formulated to meet the feeding requirements of the Vietnamese. The treatments included: (1) plain drinking water (negative control), (2) oxytetracycline at 100 mg/kg diet (positive control), or (3-6) LPFS supplemented in drinking water at 25, 50, 75, or 100 mg/L, respectively. The fermented extract was made fresh every morning and given *ad libitum*.

Preparation of Fermented Shallot Extract

Fresh shallot (*Allium cepa* L.; GenBank ID: NC_057575.1) bulbs, which are harvested at 4-5 months of maturity and produced according to VietGAP biosecurity regulations (TCVN 11892-1:2017), were chosen as the starting material. The outer dry scales were removed, the

bulbs were washed, and the edible part was homogenized to allow fermentation. The procedure described by Hai *et al.* (2024), with slight modifications, was followed for lactic acid fermentation. *L. plantarum* strain 1582 at the initial concentration of 1×10^8 CFU/mL was inoculated in shallot mash. The fermentation substrate contained 5% (w/v) NaCl and 3% (w/v) glucose, and it was incubated at 37 °C over 72 h under anaerobic conditions with mild agitation at 60 rpm. The fermented solution was then filtered to get the aqueous extract, which was then diluted in the drinking water and used. The final fermented extract had an estimated viable *Lactiplantibacillus* spp. of $2.7\text{--}3.0 \times 10^8$ CFU/mL. Major nutritional and bioactive components of the extract are shown in Table 1.

Diet Composition and Environmental Conditions

From the first day to the last day (day 35): The initial day and last day (day 35) were assigned to the birds as a control group. During the 36th to 90th day, they were fed on a finisher diet. In T1, the baseline diet was provided to the birds without antibiotics, and in T2, the birds were fed the baseline diet with the addition of 100 mg/kg diet of oxytetracycline. In T3 and T4, T5, and T6, the basal diet (no antibiotics) was administered to birds, and their drinking water was fed daily with fermented shallot extract at 25, 50, 75, and 100 mg/L. New dilutions were made daily in the morning and served *ad libitum*. The composition of the diet complies with the feeding standards of the Vietnam Ministry of Agriculture and Rural Development (TCVN 2265:2020) and is summarized in Table 2.

During the experiment, feed and water were given freely. Ambient dry-bulb temperature and relative humidity were measured at 30-minute intervals with a digital thermo-hygrometer set at bird height. In Figure 1, the temporal thermal pattern is depicted. Heat load was estimated using the temperature-humidity index according to Habeeb (2018): $\text{THI} = \text{Tdb} - [(0.31 - 0.31 \times \text{RH}) \times (\text{Tdb} - 14.4)]$, where $\text{RH} = \text{RH}\%/100$. Interpretation of THI values was made as follows: no heat stress, $\text{THI} < 27.8$; moderate heat stress, $27.8 \leq \text{THI} < 28.9$; severe heat stress, $28.9 \leq \text{THI} < 30.0$; and extremely severe heat stress, $\text{THI} \geq 30.0$. The records of the environment revealed that there was extremely severe heat load in

Table 1. Nutritional and bioactive composition of *Lactiplantibacillus plantarum*-fermented shallot bulb extract

Component/compound	Value (mean $\pm$ SEM)	Determination method
Polyphenol (mg/g)	16.25 $\pm$ 0.22	Folin - Ciocalteu
Quercetin (mg/g)	4.67 $\pm$ 0.06	UV - Vis
Allicin (mg/kg)	1.25 $\pm$ 0.05	GC - MS
Thiosulfate (mg/g)	2.21 $\pm$ 0.08	GC
S-allyl cysteine (mg/g)	1.98 $\pm$ 0.05	GC - MS
Lactic acid (%)	1.32 $\pm$ 0.04	HPLC
Acetic acid (%)	0.46 $\pm$ 0.01	HPLC
Citric acid (%)	0.69 $\pm$ 0.02	HPLC
Crude protein (g/kg)	17.89 $\pm$ 1.01	AOAC
Crude fat (g/kg)	3.56 $\pm$ 0.09	AOAC
Crude fiber (g/kg)	21.4 $\pm$ 1.13	AOAC
Metabolizable energy (MJ/kg)	10.44 $\pm$ 0.23	AOAC

Note: SEM= Standard error of mean.

Table 2. Ingredients, composition, and nutritional composition of the diet for broiler chickens (as-fed basis)

Items	Starter phase (<30 days of age)	Finisher phase (≥30 days of age)
Ingredient composition (%)		
Yellow corn	47.1	56.4
Soybean meal (35.1% crude protein)	44.1	35.7
Fish meal	5.6	4.6
CaCO ₃ (39%)	1.3	1.4
CaHPO ₄	1.1	1.0
Sodium chloride	0.2	0.2
Choline chloride (51%)	0.02	0.02
DL-Methionine (98.5%)	0.2	0.2
Vitamin premix ¹	0.2	0.2
Mineral premix ²	0.2	0.2
Calculated nutritional values (%)		
Crude protein	221.7	20.0
Crude fat	4.18	5.37
Crude fiber	4.25	4.05
Calcium	1.20	1.01
Phosphorus	0.31	0.44
Lysine	1.22	1.14
Methionine	0.70	0.45
Methionine + Cystine	0.92	0.82
Metabolizable energy (kCal/kg)	3100.0	3150.0

Note: Each kilogram of vitamin premix contained 10 mg nicotinamide, 0.02 mg cholecalciferol, 0.3 mg folic acid, 2 mg pyridoxine HCl, 1.8 mg all-trans-retinyl acetate, 8 mg cyanocobalamin, 2.2 mg menadione, 8.3 mg alpha-tocopherol acetate, 160 mg choline chloride, and 20 mg D-biotin. Each kilogram of mineral premix contained 60 µg selenium (Se), 200 µg cobalt (Co) from CoSO₄, 800 µg iodine (I) from KI, 2 mg copper (Cu) from CuSO₄·5H₂O, 24 mg zinc (Zn) from ZnO, and 16 mg iron (Fe) from FeSO₄.

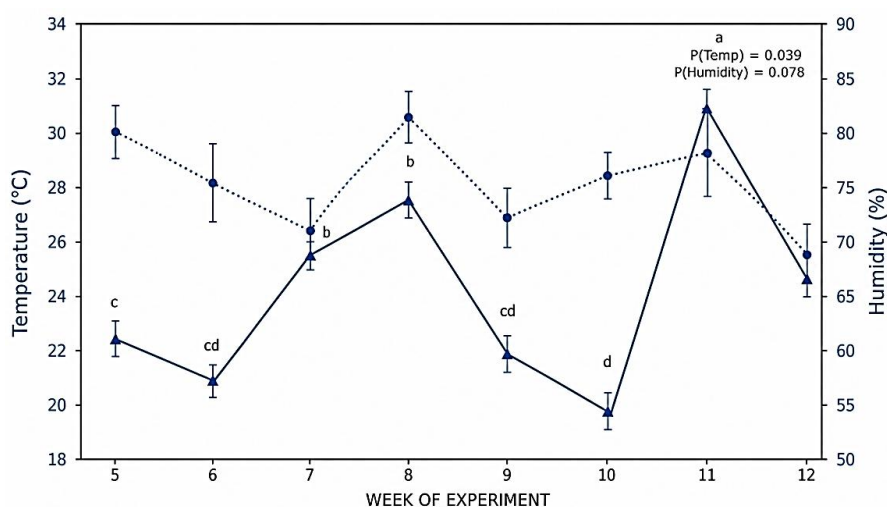


Figure 1. Temporal profile of ambient dry-bulb temperature, relative humidity, and derived temperature-humidity index (THI) during the experimental period based on repeated house measurements. —▲— Temperature (°C) ± SEM, ••• Humidity (%) ± SEM

June and July and severe heat load in August, which confirmed the chronic natural summer heat challenge during the study period.

Growth Performance and Sampling

To measure the body weight gain and feed conversion ratio (FCR), the total feed intake and body weight for each replicate were recorded weekly on a replicate basis throughout the experiment by using standard procedures as described by Hai *et al.* (2025b). One bird was randomly selected from each replicate pen at day 90, resulting in five birds per treatment (n = 5) and 30 birds in total. Blood samples were taken at

the time of slaughter and examined for the lipid profile, liver function, acute phase proteins, corticosterone, and heat shock protein concentrations.

Serum Biochemistry

Total serum cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were measured using an automatic analyzer (Automatic analyzer 902, Hitachi, Germany), and very low-density lipoprotein cholesterol (VLDL) and low-density lipoprotein cholesterol (LDL) were estimated using the Friedewald equations: LDL = Total cholesterol - HDL - VLDL, where VLDL = Triglycerides/5 (Friedewald *et al.*,

1972). The following parameters for liver function were determined using a BA400 biochemical and turbidimetry analyzer from Spain (Manual code TEUS00048-07-EN): alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST).

Stress and Oxidative Biomarkers

Using commercial enzyme-linked immunosorbent assay (ELISA) kits (QAYEE-BIO, China), the concentrations of serum amyloid A (SAA), alpha-1-acid glycoprotein (AGP), ceruloplasmin (CP), and heat shock protein 70 (HSP 70) in each blood serum sample were measured. Although the working phases of all the ELISA kits are similar, each kit uses a unique set of standards with known concentrations. A microplate reader (Bio-Rad Microplate Reader, USA) was used to determine optical density (OD) at 450 nm. The standard curve linear regression equation was established using the concentration of the standards and the corresponding OD values, and the sample concentration was computed using this same equation.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS version 21. For growth performance traits, the replicate pen was considered the experimental unit (five replicates per treatment). Weekly measurements were collected throughout the trial for management and monitoring purposes; however, the inferential analysis reported in Table 3 focused on biologically meaningful phase endpoints, namely day 35 (starter phase) and day 90 (overall final performance). For serum biochemical and stress-related measurements, one bird sampled from each replicate pen was used as the experimental observation ($n = 5$ per treatment). Because the primary objective was to compare treatment effects at these prespecified phase endpoints rather than model repeated trajectories over time, Time/Week was not included as a fixed factor in the primary model. When treatment effects were significant, means were separated

using Tukey's post hoc test. Results are presented as mean $\pm$ SE, and differences were considered significant at $p < 0.05$.

RESULTS

Growth Performance

The effects of LPFS supplementation on the growth performance of Rilai chickens reared under chronic natural summer heat exposure are presented in Table 3. The sustained thermal load documented by the environmental profile (Figure 1) adversely affected the control group (T1), which exhibited the lowest body weight gain and the poorest overall feed efficiency. LPFS supplementation significantly mitigated these negative effects ($p < 0.05$). During the starter phase, treatment differences were already evident for final body weight and body weight gain, whereas the clearest treatment-related improvements in cumulative feed conversion became apparent by the final 90-day evaluation. Overall, birds receiving the fermented extract used nutrients more efficiently and grew better than untreated birds despite a sustained summer heat challenge.

Blood Biochemical Parameters

As shown in Figure 2, heat load was associated with unfavorable changes in serum lipid profile and liver-related enzymes. The control group exhibited the highest levels of total cholesterol, triglycerides, and LDL-cholesterol. In contrast, LPFS supplementation dose-dependently ameliorated these parameters. Groups T5 and T6 showed a significant reduction in serum cholesterol and triglycerides compared to the control ($p < 0.05$), while HDL-cholesterol levels tended to increase. Additionally, serum activities of AST and ALT, which are markers of hepatocellular damage, were significantly elevated in the control group but were effectively maintained at lower levels in the T5 and T6 groups ($p < 0.05$), suggesting a hepatoprotective effect of LPFS against heat-induced oxidative damage.

Table 3. Growth performance of chickens receiving *Lactiplantibacillus plantarum* 1582-fermented shallot extract supplementation in drinking water

Variables	<i>L. plantarum</i> 1582 supplementation in drinking water						SEM	P-value
	T1	T2	T3	T4	T5	T6		
35 days old (Starter phase)								
Initial body weight (g)	44.5	45.1	44.4	44.5	45.2	44.7	0.02	0.878
Final body weight (g)	898.5 ^d	906.3 ^{cd}	946.3 ^a	934.5 ^{ab}	919.2 ^{bc}	907.9 ^{cd}	9.32	<0.001
Body weight gain (g)	853.3 ^d	861.3 ^{cd}	901.4 ^a	889.4 ^{ab}	874.3 ^{bc}	862.7 ^{cd}	8.19	<0.001
Feed intake (kg)	1.200	1.202	1.237	1.232	1.244	1.243	0.05	0.813
Cumulative FCR	1.40 ^{ab}	1.39 ^{ab}	1.37 ^{ab}	1.38 ^{ab}	1.42 ^{ab}	1.44 ^a	0.03	0.091
90 days old (Finisher phase)								
Final body weight (g)	2230.3 ^b	2230.5 ^b	2298.3 ^a	2219.2 ^b	2219.6 ^b	2230.5 ^b	0.03	<0.001
Body weight gain (g)	2185.3 ^b	2185.5 ^b	2253.1 ^a	2174.2 ^b	2173.6 ^b	2185.5 ^b	0.03	<0.001
Feed intake (kg)	4.235 ^a	4.119 ^c	4.175 ^b	4.153 ^{bc}	4.145 ^{bc}	4.133 ^c	0.02	<0.001
Cumulative FCR	2.13 ^{ab}	2.09 ^{bc}	2.05 ^c	2.11 ^b	2.10 ^b	2.08 ^{bc}	0.01	0.004

Note: Within a row, values with different superscripts differ at $p < 0.05$. FCR: Feed conversion ratio; T1: Negative control (basal diet plus plain drinking water without antibiotic or *Lactiplantibacillus plantarum* 1582-fermented shallot bulb extract (LPFS)); T2: Positive control (basal diet plus oxytetracycline at 100 mg/kg diet); T3, T4, T5, and T6, basal diet plus LPFS in drinking water at 25, 50, 75, and 100 mg/L, respectively.

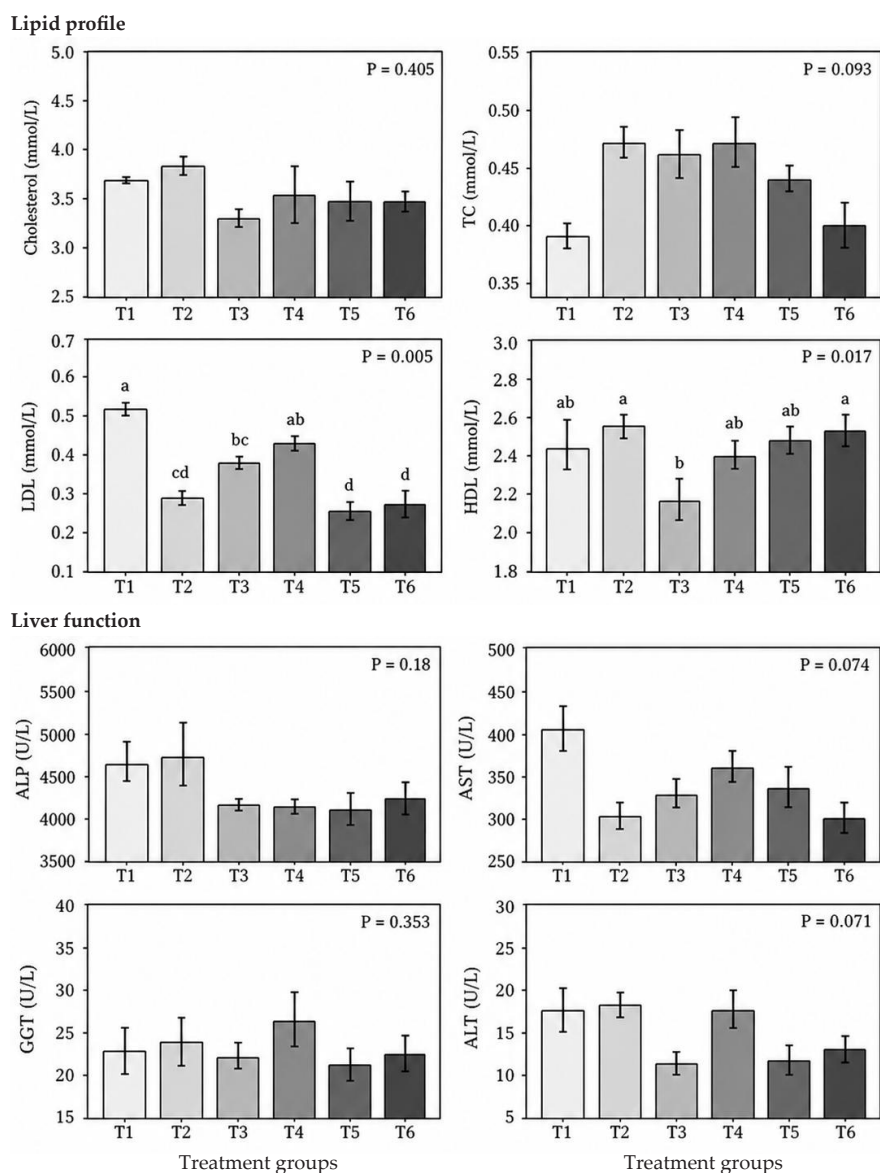


Figure 2. Serum lipid indices and liver enzymes of chickens at day 90 receiving *Lactiplantibacillus plantarum* 1582-fermented shallot extract supplementation in drinking water. T1, negative control (basal diet plus plain drinking water without antibiotic or *L. plantarum* 1582-fermented shallot bulb extract (LPFS)); T2, oxytetracycline (100 mg/kg diet); T3-T6, LPFS at 25, 50, 75, and 100 mg/L, respectively.

Stress Biomarkers and Antioxidant Status

DISCUSSION

The impact of LPFS on physiological stress and oxidative status is summarized in Figure 3. Chickens in the control group exhibited significantly elevated serum Corticosterone and Heat Shock Protein 70 (HSP70) levels ($p < 0.05$), confirming a state of acute heat stress. However, supplementation with LPFS significantly downregulated these stress biomarkers, with the lowest levels observed in the T6 group. Regarding oxidative stability, the malondialdehyde (MDA) content (or TBARS value), a marker of lipid peroxidation, was significantly lower in the LPFS-treated groups compared to the control ($p < 0.05$). At the same time, antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) were enhanced, especially in T5 and T6. Together, these responses indicate that the fermented extract strengthened endogenous antioxidant defense in chickens exposed to chronic summer heat.

Heat stress remains one of the most urgent environmental issues in tropical poultry production. It interferes with the overall physiological balance, which promotes oxidative damage, impairs intestinal integrity, and suppresses immune function (Nawab *et al.*, 2018). The current study involved the rearing of birds in open-sided house conditions in real-field conditions instead of in a controlled climatic chamber. However, the experimental recording of temperature-humidity index stayed within the severe to extremely severe range in most of the experimental periods, thus confirming the fact that the flock was subjected to sustained natural heat load. The significant decrease in growth performance and the increase in stress biomarkers in the untreated control group are in line with the well-documented metabolic shift that involves the redistribution of energy between growth and thermoregulation and inflammatory pro-

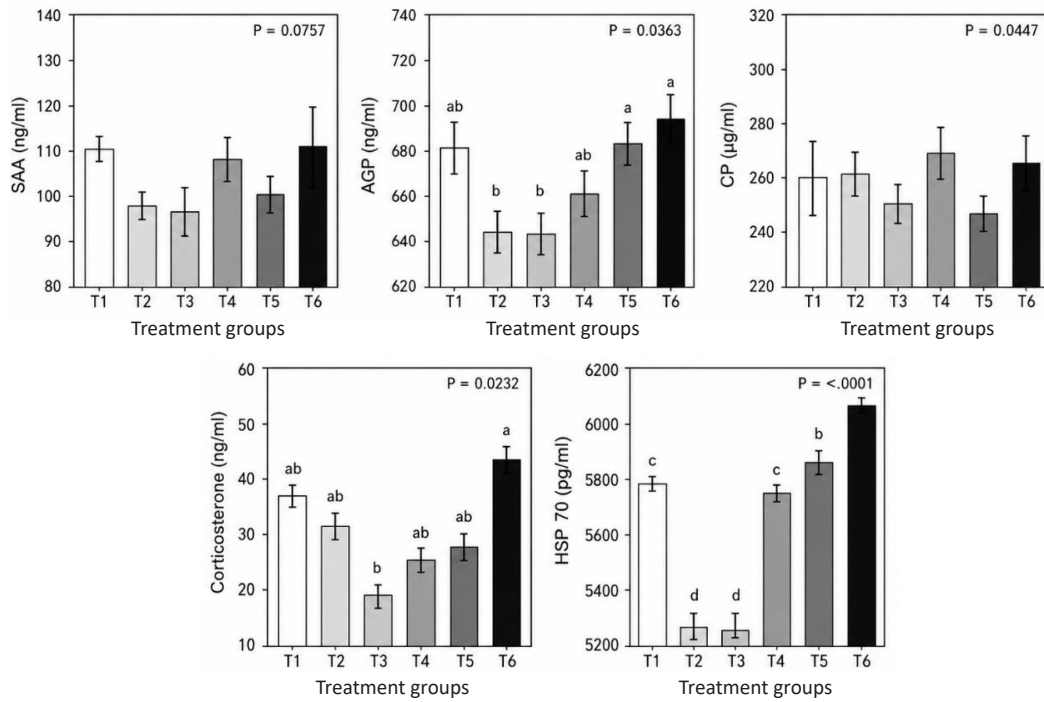


Figure 3. Serum stress- and immune-related biomarkers of chickens at day 90, including serum amyloid A (SAA), alpha-1-acid glycoprotein (AGP), ceruloplasmin (CP), and heat shock protein 70 (HSP 70), receiving *Lactiplantibacillus plantarum* 1582-fermented shallot extract supplementation in drinking water. T1, negative control (basal diet plus plain drinking water without antibiotic or *L. plantarum* 1582-fermented shallot bulb extract (LPFS)); T2, oxytetracycline (100 mg/kg diet); T3 - T6, LPFS at 25, 50, 75, and 100 mg/L, respectively.

cesses (Wasti *et al.*, 2020). These negative effects were evidently mitigated by supplementation with the fermented shallot extract (LPFS), implying the product has protective effects through a number of complementary pathways relating to gut health, antioxidant effects, and metabolic regulation.

The observed benefits of the increase in body weight gain and the ratio of feed to metabolite among the birds fed probiotics and prebiotics can be largely attributed to a synbiotic-like interaction between the prebiotic substrates (shallots) and the metabolites produced by *L. plantarum*. Heat stress has been found to compromise tight-junction proteins and cause a leaky-gut system, which reduces nutrient absorption and increases systemic exposure to endotoxins (Song *et al.*, 2014). The postbiotic compounds that are produced during the process of fermentation, including the short-chain fatty acids and the bacteriocin-like compounds, seem to have contributed to stabilizing the intestinal barrier and to increasing the efficiency of digestion (Sugiharto & Ranjitkar, 2019). Interestingly, the positive action on cumulative feed conversion was more pronounced in the later stages of the trial, which suggests that the full physiological usefulness of the fermented extract may require prolonged exposure to the fermented extract under a persistent natural heat stress.

Another interesting observation was the high drop in serum corticosterone levels in the birds subjected to LPFS. Their downregulation would be expected to conserve lean tissue accretion and facilitate overall growth even in stressful conditions due to glucocorticoids promoting muscle proteolysis and gluconeogenesis (Mishra & Jha, 2019).

It was also important that the antioxidant defense system (enhanced SOD and GPx activities) was improved along with the reduction of the malondialdehyde and heat shock protein 70 levels. We propose that the fermentation step was a key factor in this result. *L. plantarum* has an activity of β -glucosidase, which has the capacity to transform shallot flavonoid glycosides into more bioactive aglycones like quercetin (Huynh *et al.*, 2014; Hai *et al.*, 2024). Upon absorption, the compounds are most likely to activate the Nrf2 signaling pathway, which increases the expression of the key antioxidant enzymes (Sun *et al.*, 2020). The consequent increase in cellular redox balance would subsequently decrease the necessity of excessive HSP70 induction, as oxidative stress is a significant trigger of the heat-shock response (Zaboli *et al.*, 2019). Practically, these changes imply that the degree of acquired thermotolerance in the LPFS-supplemented birds was greater than in the unsupplemented control group.

Lastly, the observed dose-dependent decreases in serum cholesterol and triglycerides observed in the LPFS groups are in line with the known hypolipidemic properties of *Allium* species.

Two primary mechanisms are likely involved: (1) Steroidal saponins in shallot form insoluble complexes with cholesterol in the gut, inhibiting its absorption (Kothari *et al.*, 2019); and (2) Probiotic metabolites inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (Ooi & Liong, 2010; Alagawany *et al.*, 2018). Additionally, the normalization of liver enzymes (AST, ALT) confirms that LPFS protected hepatocytes from oxidative injury and lipid infiltration.

CONCLUSION

Lactiplantibacillus plantarum-fermented shallot bulb extract (LPFS) administered in drinking water improved growth performance and reduced circulating stress and oxidative damage biomarkers in Rilai chickens reared under prolonged natural summer heat exposure. The most consistent responses were observed at 75–100 mg/L. LPFS in drinking water may therefore represent a practical postbiotic–phytochemical strategy to support chicken productivity in hot climates and to reduce reliance on antibiotic growth promoters, but further dose-response and biomarker-validation studies are needed.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

We state that generative AI and AI-assisted technologies were used for language refinement. All the content has been critically reviewed and edited by the authors.

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