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# Dried plum supplementation enhanced the expression of liver antioxidant capacity, metabolism, and epigenetic-related gene markers in broiler chickens under heat stress conditions
# Dried plum increased liver metabolism in broiler

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ABSTRACT

Heat stress (**HS**) poses a substantial challenge in the poultry sector, resulting in considerable economic losses as it negatively impacts the well-being and productivity of chickens. Dried plum (**DP**) is a rich source of minerals, vitamins, antioxidants, and phenolic compounds. Studies have indicated that DP offers various health advantages, including preserving the body's redox system, immune function, and calcium balance. In our previous study, DP supplementation improved overall growth performance and intestinal health metrics in heat-stressed broilers. Considering the beneficial effects of DP on health, we hypothesized that adding DP to the diet would mitigate the harmful impacts of heat stress in the liver of broiler chickens. Day-old unsexed broiler chicks (n = 72) were raised under standard conditions and randomly assigned to three treatment groups (n = 24/group): 1) Control, 2) heat stress with basal diet (HS), and 3) heat stress with supplement (DP). During the finisher stage, the DP group received feed containing 2.5% DP during treatment, while the other groups were given a standard finisher diet. After 21 d, birds in the HS and DP groups were subjected to cyclic heat stress conditions for 3 wk. The heat stress conditions involved exposing the birds to a temperature of 33-35°C for 8 h during the daytime. In contrast, the birds in the Control group were raised under normal conditions with temperatures ranging from 22-24°C. DP supplementation significantly increased ($P < 0.05$) heat shock factor 1 (***HSF1***) expression in the liver compared to the Control group. DP supplementation significantly increased ($P < 0.05$) thioredoxin (***TXN***), peroxiredoxin (***PRDX***), insulin-like growth factor 1 (***IGF1***), and methyl-CpG binding domain (***MBD4***) expression in the DP group compared to the HS group. Fructose-1,6-bisphosphatase 1 (***FPB1***) expression was significantly decreased ($P < 0.05$) in the DP group compared to the HS group. Solute Carrier Family 3 Member 1 (***SLC3A1***), DNA methyltransferase 1 (***DNMT1***), DNA methyltransferase 3 alpha (***DNMT3A***), ten-eleven translocation methylcytosine dioxygenase 2 (***Tet2***), ten-eleven translocation methylcytosine dioxygenase (***Tet3***), and thymine DNA glycosylase (***TDG***) expression were significantly increased ($P < 0.05$) in the DP group compared to the other treatment groups. In conclusion, post-hatch DP supplementation lessened the negative effects of HS on broiler chickens by upregulating genes related to heat shock, antioxidants, growth, nutrient transporters, and epigenetics in the liver.

## Introduction

Prolonged droughts, unpredictable rainfall, rising temperatures, and an increased frequency of severe weather events pose significant challenges to food production and security (Sundström et al., 2014). The tropics, with rich biodiversity, are highly vulnerable to climate change impacts due to their elevated temperatures (Thomson et al., 2015). The dynamic climate change substantially impedes optimal broiler growth and production. Industry can surmount numerous challenges by embracing cutting-edge technology and environmentally conscious practices. Enhanced production efficiency can be attained by employing heat-resistant broiler breeds, optimized feed formulations, thermal

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manipulation during incubation, and modern management systems (Abdel-Moneim et al., 2021; Abd El-Hack et al., 2019; Al Amaz and Mishra, 2024; Al Amaz et al., 2025, Nawab et al., 2018; Oke et al., 2024; Wasti et al., 2020).

The liver is a critical organ responsible for diverse functions ranging from metabolism to immunity. Detoxification to other functions of metabolic activity exhibits a high susceptibility to atypical fluctuations in temperature and is a primary site for tissue injury during heat stress (**HS**) (Chen et al., 2023). During HS, the liver enhances the mobilization of energy and the breakdown of proteins and carbohydrates to meet the increased energy needs of the body (Gonzalez-Rivas et al., 2020). In addition, the increased metabolic rate in the liver results in an overproduction of reactive oxygen species (**ROS**) within the mitochondria, leading to oxidative stress and disrupting the liver's normal functioning (Akbarian et al., 2016). HS may cause oxidative stress and dysfunctional autophagy in the liver, leading to liver damage in broiler chickens (Tang et al., 2022). HS also decreases hepatic gluconeogenesis and lipid metabolism (Al Amaz et al., 2024b) and increases abdominal adipose deposition, which in turn leads to an increase in hepatic de novo lipogenesis and encourages the differentiation of preadipocytes within abdominal adipose (Lan et al., 2022). In Chinese indigenous broilers, HS modified hepatic lipid metabolism primarily via the linoleic acid, alpha-linolenic acid, glycerolipid, and glycerophospholipid metabolic pathways (Guo et al., 2021).

Among the most consumed fruits and vegetables, dried plum (**DP**) (*Prunus domestica L.)* has one of the highest ratings for oxygen radical absorbance capacity. They are exceptionally high in polyphenols (1.1–2.6 g/kg) (Kayano et al., 2004). The polyphenols found in DP inhibited the formation and function of osteoclasts in various conditions, including regular oxidative stress and inflammatory conditions (Bu et al., 2008). In addition to its antioxidant properties, DP improves immunological functions, gut health, and calcium metabolism (Arjmandi et al., 2017). DP is rich in sorbitol, quinic acid, chlorogenic acids, vitamin K1, boron, copper, and potassium. The combined effect of these and other compounds, also found in DP in smaller quantities, may have positive health benefits when DP is consumed regularly (Stacewicz-Sapuntzakis, 2013). Given its purported benefits and widespread availability at a reasonable price globally, DP is also used as a supplemental feed additive for animals and birds. A previous study from our lab reported that DP significantly increased the body weight, average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), histomorphology of the ileum, production of volatile fatty acids (VFA) in the cecum, and expression of genes related to heat shock, antioxidant, immune response, and tight junctions in the heat-stressed birds. In addition, DP also enhanced the prevalence of beneficial bacteria in the chicken gut (Wasti et al., 2021).

So, in this follow-up study, considering its antioxidant profile, health benefits, and previous evidence, we hypothesized that dietary DP supplementation alleviates oxidative stress, lowers gluconeogenesis, increases growth, nutrient transportation, and epigenetics markers-related gene expression in the liver under the HS condition in broiler chickens.

## Material and methods

### *Animals and husbandry practices*

The animal experiments were carried out under a protocol approved by the University of Hawaii Institutional Animal Care and Use Committee (IACUC) (Approval No. 17-2605). A dosage of 2.5% DP was selected for its antioxidant properties and based on dosages used in earlier studies involving both humans and rodents. A total of 72 day-old, unsexed Cobb-500 chicks were sourced from Asagi Hatchery Inc. (Honolulu, HI). Each chick was weighed individually, tagged on the wing, and randomly divided into 18 pens, with each pen housing four birds. This setup provided 6 replicates per treatment, with 24 birds allocated to each treatment group. The average initial body weight of the chicks was 40-41 grams. The experimental design consisted of 3 treatment groups: 1) Control group, 2) heat stress group fed a basal diet (HS), and 3) heat stress group supplemented with DP (DP). The chicks were reared on a floor pen system following standard guidelines for broiler management during the first 21 days. Afterward, the HS and DP groups were exposed to high daytime temperatures (33–35°C from 9:00 AM to 5:00 PM) followed by cooler nighttime temperatures (22–24°C) for 3 weeks, with a relative humidity (RH) of 50%. In contrast, the Control group was housed in different rooms but maintained under standard room temperatures (22–24°C) and the same humidity conditions. The health of the birds was monitored twice daily. This study employed a completely randomized design, with pens measuring 1 m by 0.61 m and providing a stocking density of 1,500 $cm^2$ per bird. The lighting schedule allowed for 23 hours of light followed by 1 hour of darkness each day.

### *Diet*

The birds were given ad libitum access to feed and water. The diets were formulated in two phases: the starter phase, which lasted from d 1 to d 21, and the finisher phase, which lasted from d 22 to d 42. These dietary formulations were designed to fulfill the specific nutrient needs of broiler chickens (National Research Council (U.S.), 1994) and dietary guidelines of the Cobb 500 breed (Cobb Broiler Management Guide, 2021). The birds were given the standard starter diet for the first 14 d. Subsequently, for 14 to 21 d, the DP group had 2.5% DP added to their starter diet, while the other two groups were given the standard starter diet. Between d 21 and d 42, the Control and HS birds were given regular finisher diets. The DP group had the regular finisher diet with an

**Table 1**
Nutrient composition of dried plum.

| Items | Dried plums |
|---|---|
| Serving, g | 100 |
| Energy, kcal | 240 |
| Total carbohydrate, g | 63.88 |
| Total sugars, g | 38.13 |
| Glucose, g | 25.46 |
| Fructose, g | 12.45 |
| Sucrose, g | 0.15 |
| Starch | 5.11 |
| Total dietary fiber, g | 7.1 |
| Sorbitol, g | 12 |
| Protein, g | 2.18 |
| Fat, g | 0.38 |
| Moisture, g | 30.92 |
| Ca, mg | 43 |
| K, mg | 732 |
| Fe, mg | 0.93 |
| Mg, mg | 41 |
| P, mg | 69 |
| Cu, mg | 0.281 |
| Mn, mg | 0.299 |
| Se, µg | 0.3 |
| Zn, mg | 0.44 |
| Vitamin A, µg RAE | 39 |
| Beta-carotene, µg | 394 |
| Alpha-carotene, µg | 57 |
| Beta-cryptoxanthin, µg | 93 |
| Lutein + zeaxanthin, µg | 148 |
| Vitamin C, mg | 0.6 |
| Vitamin E, mg (α-tocopherol) | 0.43 |
| Vitamin $K_1$, µg | 59.5 |
| Thiamin ($B_1$), mg | 0.051 |
| Riboflavin ($B_2$), mg | 0.186 |
| Niacin, mg | 1.882 |
| Panthotenic acid, mg | 0.422 |
| Vitamin $B_6$, mg | 0.205 |
| Folate, µg | 4 |
| Choline, mg | 10.1 |

Data from Stacewicz-Sapuntzakis (2013)

additional 2.5% DP included; feed formulation is added in Table 2. The nutrient composition of the DP is presented in Table 1.

*Sample collection*

At the end of the animal experimentation (42 d), 6 birds per treatment were euthanized by carbon dioxide asphyxiation. This involved selecting one bird from each pen. For the gene expression study, we collected small liver samples (n=6 per treatment; one from each pen), quickly froze them, and stored them at -80°C until we could extract the RNA.

*Quantitative real-time PCR*

Total RNA was isolated from liver tissue, converted into cDNA, and analyzed using qPCR following the protocol described by (Wasti et al., 2021). RNA concentration was measured with a NanoDrop™ spectrophotometer (ThermoFisher Scientific, Madison, WI). The primer sequences used for gene expression analysis are listed in Supplement Table 1. After amplification, the cycle threshold (Ct) values were recorded, and gene expression levels were calculated using beta-actin (β-actin) as the reference gene, following the $2^{-\Delta\Delta Ct}$ method.

**Table 2**
. Ingredients and nutrient composition of the experimental diets.

| | Starter | | Finisher | |
|---|---|---|---|---|
| Ingredients % | Control | with DP | Control | with DP |
| Corn | 54.86 | 53.36 | 63.14 | 61 |
| Soybean meal | 39.5 | 38.5 | 29.6 | 29 |
| Dry plum | 0 | 2.5 | 0 | 2.5 |
| Soybean oil | 2 | 2 | 4.5 | 4.74 |
| Limestone | 1.27 | 1.27 | 0.85 | 0.85 |
| Monocalcium phosphate | 0.75 | 0.75 | 0.5 | 0.5 |
| Lysine | 0.23 | 0.23 | 0.18 | 0.18 |
| Methionine | 0.14 | 0.14 | 0.12 | 0.12 |
| Threonine | 0.2 | 0.2 | 0.16 | 0.16 |
| NaCl | 0.43 | 0.43 | 0.35 | 0.35 |
| Sodium bicarbonate | 0.12 | 0.12 | 0.1 | 0.1 |
| Vitamin + Mineral mix[1] | 0.5 | 0.5 | 0.5 | 0.5 |
| Calculated nutrient contents, % | | | | |
| Metabolizable energy, kcal/kg | 2909 | 2903 | 3203 | 3207 |
| Crude Protein | 22.09 | 21.96 | 18.07 | 18.08 |
| Ca | 0.75 | 0.75 | 0.52 | 0.52 |
| Total P | 0.57 | 0.56 | 0.47 | 0.46 |
| dig P | 0.30 | 0.30 | 0.23 | 0.23 |
| Lysine | 1.39 | 1.36 | 1.10 | 1.08 |
| Methionine | 0.48 | 0.47 | 0.41 | 0.41 |
| Cystine | 0.43 | 0.41 | 0.38 | 0.37 |
| Threonine | 1.03 | 1.01 | 0.85 | 0.83 |
| Tryptophan | 0.33 | 0.32 | 0.26 | 0.25 |
| Methionine + Cysteine | 0.91 | 0.89 | 0.8 | 0.78 |
| Arginine | 1.61 | 1.57 | 1.31 | 1.28 |
| Valine | 1.22 | 1.19 | 1.03 | 1.00 |
| Isoleucine | 0.93 | 0.91 | 0.76 | 0.75 |
| Leucine | 1.89 | 1.84 | 1.63 | 1.59 |
| Choline (mg/kg) | 1419 | 1382 | 1200 | 1170 |
| dig Lysine | 1.25 | 1.22 | 0.99 | 0.97 |
| dig Methionine | 0.45 | 0.45 | 0.39 | 0.38 |
| dig Threonine | 0.85 | 0.83 | 0.69 | 0.68 |
| Neutral Detergent Fiber | 9.13 | 8.89 | 8.78 | 8.53 |
| Crude Fiber | 3.97 | 4.29 | 3.46 | 3.8 |
| Na | 0.22 | 0.22 | 0.18 | 0.18 |
| Cl | 0.30 | 0.30 | 0.25 | 0.25 |

[1] Provides following nutrients (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin $D_3$ (cholecalciferol), 3,000 IU; vitamin E (all-*rac*-tocopherol-acetate), 30 mg; vitamin $B_1$, 2 mg; vitamin $B_2$, 8 mg; vitamin $B_6$, 4 mg; vitamin $B_{12}$ (cyanocobalamin), 0.025 mg; vitamin $K_3$ (bisulfate menadione complex), 3mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; betaíne anhydrous, 80 mg; D-biotin, 0.15 mg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg iron ($FeCO_3$), 60 mg; copper ($CuSO_4 \cdot 5H_2O$), 8 mg; iodine (KI), 2 mg; selenium ($Na_2SeO_3$), 0.2 m

*Statistical analyses*

Gene expression was analyzed utilizing GraphPad and is presented as mean ± SEM. The Tukey-HSD test was employed to compare the means of various treatment groups following a one-way analysis of variance (ANOVA). The criterion for statistical significance was set at $P < 0.05$.

## Results

*Effects of dried plum on heat-shock-related gene expression*

The expression of the heat shock protein-related genes (*HSF1, HSF3, HSP70*, and *HSP90*) among the treatment is summarized in Fig. 1. The expression of *HSF1* mRNA was significantly increased ($P < 0.05$) in the heat-stressed birds supplemented with the DP than the Control group.

*Effects of dried plum on antioxidant-related gene expression*

The expression profile of the antioxidant-related genes (*SOD1, SOD2, GPX1, GPX3, TXN, PRDX1,* and *NRF2*) is presented in Fig. 2. The dietary supplementation of DP significantly increased ($P < 0.05$) the expression of *TXN* and *PRDX1* in the DP group compared to the HS birds that consumed basal diet.

*Effects of dried plum on gluconeogenesis-related gene expression*

The expression of the gluconeogenesis-related genes (*PC, PCKc, PCKm,* and *FBP1*) is presented in Fig. 3. *FPB1* expression was significantly higher ($P < 0.05$) in the HS group than in the DP group.

*Effects of dried plum on growth and nutrient transporter-related gene expression*

The expression of the growth and nutrient transporter-related genes (*IGF1, IGFR, SLC1A1, SLC3A1, SLC6A14, SLC7A1, SLC7A6,* and *SLCAL*) is presented in Fig. 4. *IGF1* expression was significantly increased ($P < 0.05$) in DP group compared to the HS group. *SLC3A1* expression was significantly increased ($P < 0.05$) in the DP group compared to the Control and HS groups.

*Effects of dried plum on epigenetics-related gene expression*

The expression of the epigenetics-related genes (*DNMT1, DNMT3A, DNMT3B, Tet1, Tet2, Tet3, GFAdd45B, TDG* and *MBD4*) is presented in Fig. 5. *DNMT1, DNMT3A, Tet2, Tet3* and *TDG* expression was significantly increased ($P < 0.05$) in the DP group compared to the Control and HS groups. *MBD4* was significantly increased ($P < 0.05$) in the DP group compared to the HS group.

## Discussion

The HS response initiates with the phosphorylation and trimerization of heat shock factors (HSF). Subsequently, the trimers translocate to the nucleus and bind to the heat shock elements in the promoter region of heat shock protein (HSP) genes, modulating HSP transcription (Inouye et al., 2003). Four heat shock factors (HSF1-4) control the expression of HSPs (Morimoto, 1998; Chaudhary and Mishra, 2024). The mechanisms of cellular adaptation to heat shock, mediated by HSF1 and HSF3, have been thoroughly examined in chicken cells (Fujimoto and Nakai, 2010). While HSF3 remains activated at higher temperatures and longer exposure times, HSF1 is activated at lower temperatures (Tanabe et al., 1997). HSF1 enhances the transcription of *HSP70* (Inouye et al., 2003), while HSF3 can stimulate the transcription of all heat shock proteins (HSPs) in chickens (Tanabe, 1998). **In chickens, HSP70 is the most**

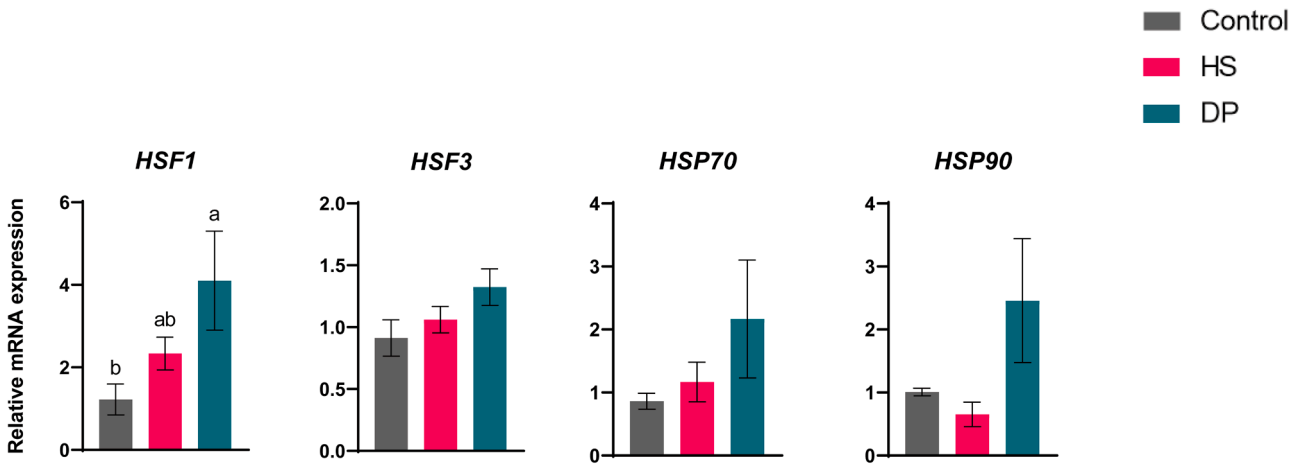


**Fig. 1.** Effects of the dried plum supplement on the mRNA expression of heat shock protein-related genes in the liver. Data showed as mean ± SEM. Different letters indicate a significant difference among the treatment groups.

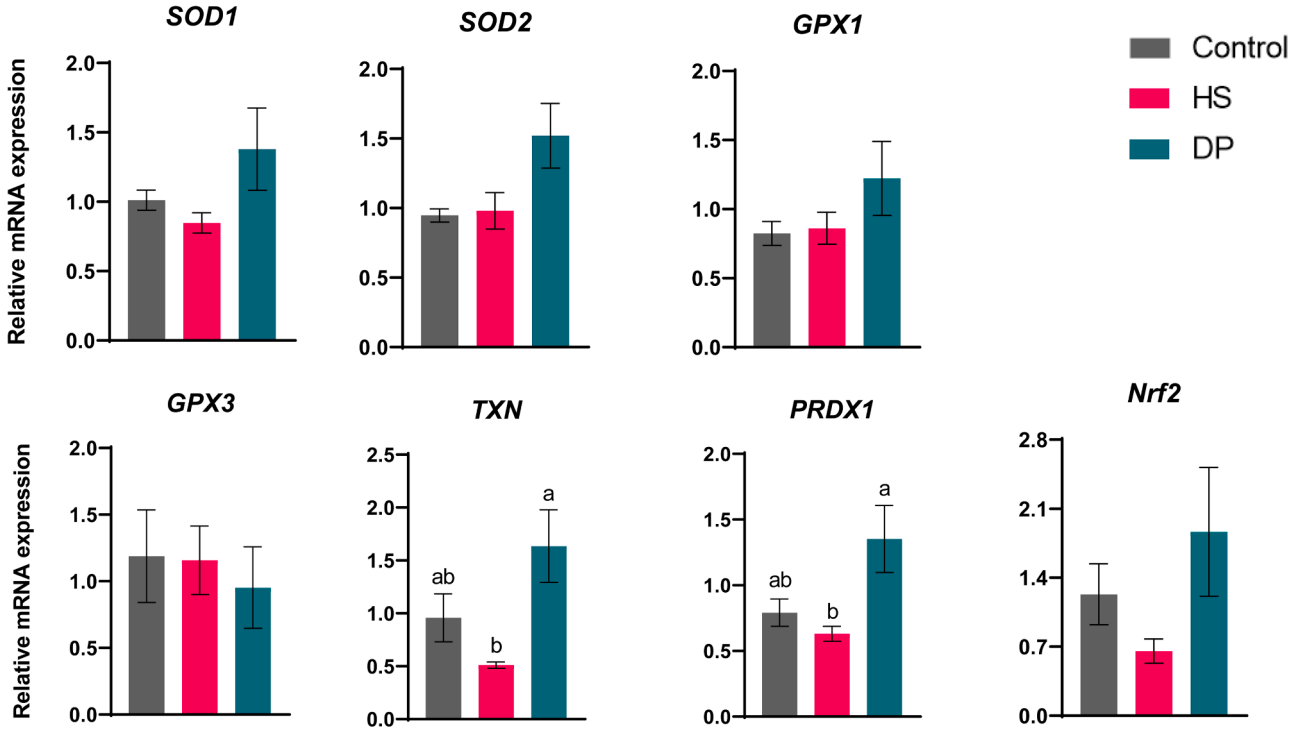


**Fig. 2.** Effects of the dried plum supplement on the mRNA expression of antioxidants-related genes in the liver. Data showed as mean ± SEM. Different letters indicate a significant difference among the treatment groups.

**prevalent and conservative member of the HSP family. *HSP70* significantly contributes to the adaptive response to thermal stress in broiler chickens by enhancing antioxidant capacity, inhibiting lipid peroxidation, and promoting the activity of digestive enzymes** (Hao et al., 2012; Al Amaz et al., 2024a). *HSP90* inhibits protein aggregation and degradation and promotes the activation of signal transduction molecules (Gonzalez-Rivas et al., 2020). In our study, significantly increased *HSF1* in DP than in the Control group might help improve the *HSP70* expression in the DP group under the HS condition. Also, numerically increased *HSF3* expression might have influenced the *HSP90* expression in the DP group. These findings indicate that DP supplementation did not affect thermotolerance capacity in the liver under the HS condition.

Oxidative stress is a significant factor contributing to the buildup of lipids and liver damage via intricate signal transduction pathways (Gu et al., 2021). Superoxide dismutase 1 (*SOD1)* and superoxide dismutase 2 (*SOD2*) are considered the first line of defense in the antioxidant defense system. It detoxifies superoxide radicals to hydrogen peroxide, which is subsequently transformed by CAT into water or GPX into nontoxic hydroxyl compounds by using glutathione as a redundant energy source (He et al., 2017). GPXs possess antioxidant properties in various cellular compartments. *GPX1* is found throughout the cytosol and mitochondria, while *GPX3* is in the plasma (Vašková et al., 2023). The TXN-PRDX antioxidant system eliminates hydrogen peroxides produced during mitochondrial cellular respiration and the superoxide dismutase activity of the enzyme (Mailloux et al., 2013). Factor nuclear erythroid 2-related factor 2 (*NRF2*) is a transcription factor that plays a crucial role in directly controlling the expression of antioxidant proteins. A minimal level of oxidative stress will initiate the activation of *NRF2*, leading to the induction of gene expression, which is crucial for

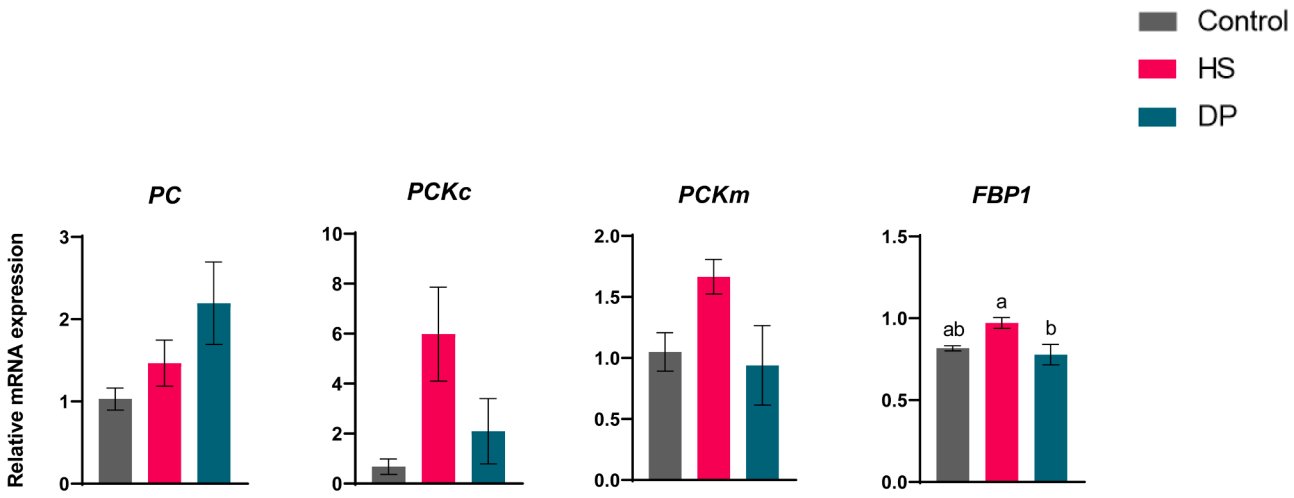


**Fig. 3.** Effects of the dried plum supplement on the mRNA expression of gluconeogenesis-related genes in the liver. Data showed as mean ± SEM. Different letters indicate a significant difference among the treatment groups.

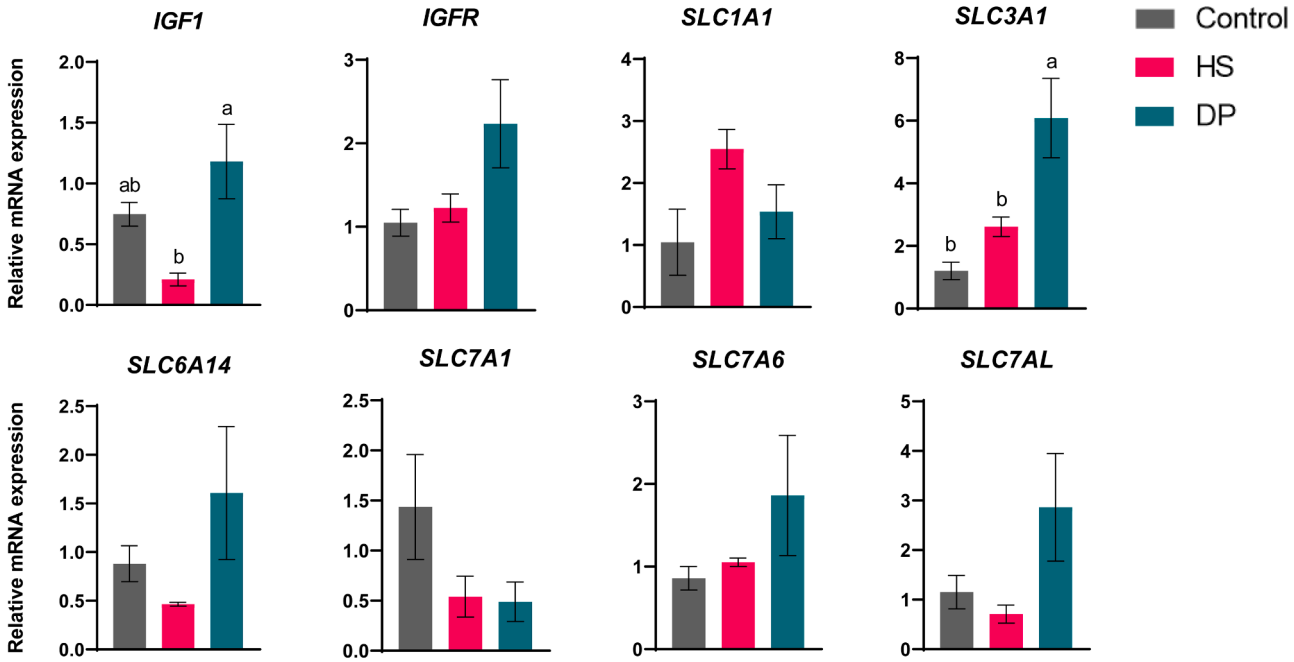


**Fig. 4.** Effects of the dried plum supplement on the mRNA expression of growth and nutrient transporter-related genes in the liver. Data showed as mean ± SEM. Different letters indicate a significant difference among the treatment groups.

protecting cells and their survival. This includes gene expression in cysteine uptake and heme oxygenase 1 (Niture et al., 2014). As the liver is one of the most vulnerable organs to oxidative stress, significant upregulation of *TXN* and *PRDX1* in the DP group compared to the HS group showed a potent antioxidant capacity of the DP supplements. Thus, DP played an essential role in helping the TXN-PRDX system scavenge the free radicals more rapidly under HS conditions.

The liver has a vital function in regulating energy and glucose balance. The enzymes pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PCK), and fructose-1,6-bisphosphatase 1 (FBP1) are the primary regulators of gluconeogenesis. Insulin signaling, gluconeogenesis, lipogenesis, and other metabolic processes are influenced by pyruvate carboxylase, a protein highly expressed in the liver. It accelerates the transformation of pyruvate into oxaloacetate (Valle, 2017). PCK exists in two genotypes: PCKm, located in the mitochondrial matrix, and PCKc, located in the cytosol (Parsanejad et al., 2003). The *PCK* gene promoter region contains binding sites for the glucocorticoid receptor. Corticosterone can potentially enhance hepatic gluconeogenesis by increasing the expression of the *PCK* gene (Cawley, 2012). In our study, significantly lower ($P < 0.05$) *FBP1* expression in the DP group compared to the HS group indicates a lower gluconeogenesis rate in HS birds. That means, in the HS birds' glucose was not produced from non-hexoses precursors, especially from glycerol, lactate, pyruvate, propionate, and glucogenic amino acids, even though they were in the HS condition. It articulates that DP-supplemented birds did not sacrifice the metabolic process in HS.

Insulin-like growth factors (IGFs) regulate embryonic and post-natal development in vertebrates (Al-Samerria and Radovick, 2021). IGFs enhance glycogen production in the liver, stimulate DNA synthesis, and facilitate chicken tissue growth (McMurtry et al., 1997; Amaz et al., 2024). The genotype significantly impacts *IGF1*, indicating that it plays a crucial role in regulating the growth rate of broiler chickens (Beccavin

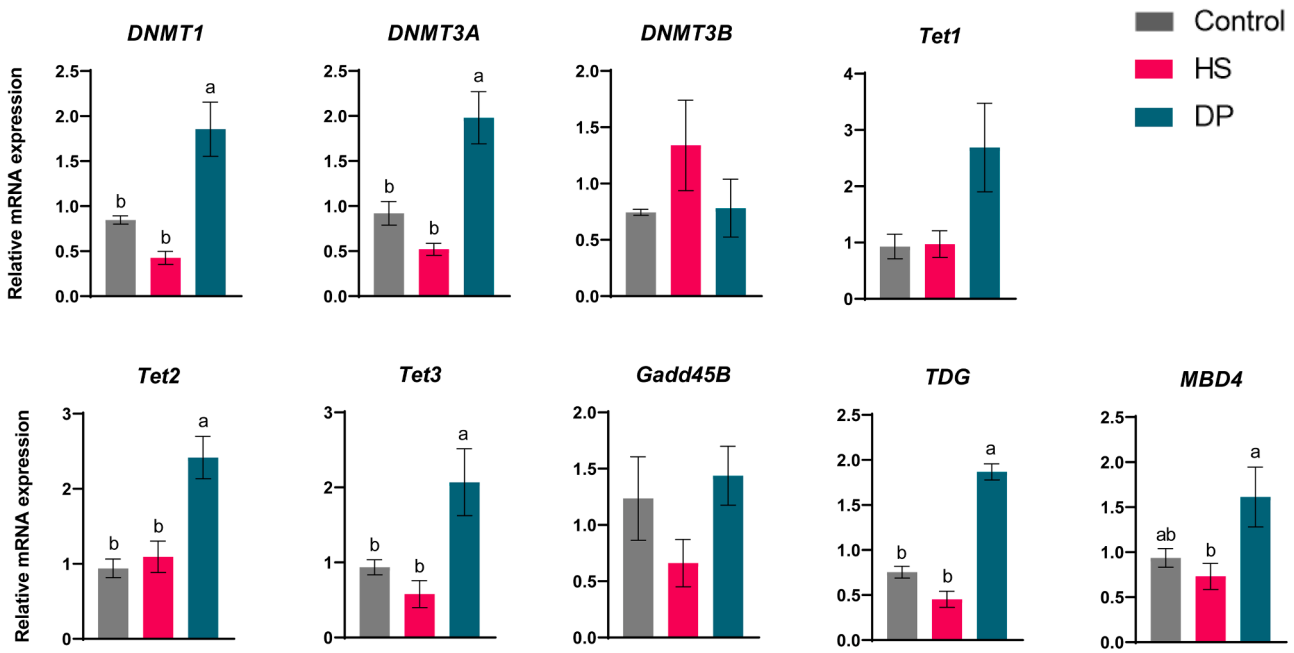


**Fig. 5.** Effects of the dried plum supplement on the mRNA expression of epigenetics-related genes in the liver. Data showed as mean ± SEM. Different letters indicate a significant difference among the treatment groups.

et al., 2001). The liver is a metabolically active organ that regulates amino acid metabolism, gluconeogenesis, and other metabolic processes to allocate energy resources efficiently (Taub, 2004). The liver is primarily responsible for transaminating amino acids, essential for gluconeogenesis and energy production (Brosnan, 2000). The liver absorbs amino acids through amino acid transporters. These transporters play a crucial role in regulating cysteine levels inside cells, which is the amino acid that limits the rate of glutathione synthesis (Wu et al., 2020). Therefore we studied the genes associated with the solute carrier (SLC) family. *SLC1A1*, also known as excitatory amino acid transporter 3 (EAAT3), moves excitatory amino acids by shifting the substrate binding region of the transport domain across the plasma membrane (Bjørn-Yoshimoto and Underhill, 2016). *SLC3A1* refers to a group of transporters responsible for cysteine uptake (Wu et al., 2020). *SLC6A14* facilitates the entry of neutral and cationic amino acids into cells by simultaneously transporting $Na^+$ and $Cl^-$ in the same direction (Bhutia et al., 2014). *SLC7A1* is a cationic amino acid transporter that does not require sodium and facilitates the absorption of arginine and lysine (Fernandez et al., 2001). *SLC7A6* functions as a supplementary subunit alongside SLC7A7 to form the y+L transport system responsible for transporting cationic and neutral amino acids, including glutamine (Milewski et al., 2017). The catalytic light chain of the heterodimeric amino acid transporter complex is identified as *SLC7AL*. It has been demonstrated to facilitate neutral amino acid uptake when co-expressed with 4F2hc (Sperandeo et al., 2008). In this study, significant upregulation ($P < 0.05$) of *IGF1, SLC3A1,* and numerically increased *IGFR, SLC6A14, SLC7A6,* and *SLC7AL* gene expression bolsters the findings of a significantly higher ($P < 0.05$) growth performance in HS condition in our previous study (with the same DP supplement and the same set of birds) (Wasti et al., 2021). More specifically, the DP supplement was able to accelerate the metabolic process by upregulating the solute carrier genes which aided in upregulating the *IGF1* expression, thus ensuring optimum growth performance under the HS condition.

DNA methylation is widely acknowledged as a significant epigenetic factor that profoundly impacts gene activities (Ju et al., 2023). DNA methylation has been identified as a potential mechanism for adapting to stress by suppressing the activity of specific genes that are activated during stressful conditions (Mifsud et al., 2011; Kim and Costello, 2017). In the liver, downregulation of *DNMT1, DNMT3A,* and *DNMT3B* expression indicates nutritional stress in the chicken (Kang et al., 2017). In this study, significant upregulation ($P < 0.05$) of *DNMT1* and *DNMT3A* in the DP group compared to the other treatment group suggested no nutritional stress during the HS in DP-supplemented birds. *Tet1, Tet2*, and *Tet3* are the evolutionary conserved TET proteins catalyzing active DNA demethylation. They mediate the iterative oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) and then to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). The excised DNA is subsequently removed by the protein thymine DNA glycosylase (TDG) and base excision repair equipment (Ito et al., 2011). *GADD45b* plays a role in cell death/survival pathways by interacting with other molecules through a posttranslational regulation mechanism. It also is a crucial mediator of hepatic oxidative stress (Kim et al., 2014). In mice, *TDG* acts as a tumor suppressor for hepatocellular carcinoma by controlling a transcriptional program that safeguards against the formation of glucose intolerance and the accumulation of bile acids in the liver (Hassan et al., 2020). *MBD4* is a DNA repair protein that functions as a thymine glycosylase and specifically recognizes and repairs sites where cytosine has undergone deamination (Hendrich and Bird, 1998). In chicken, *MBD4* acts as a uracil glycosylase and is considered a compelling candidate for its potential role in somatic hypermutation (Costello et al., 2019). *Tet2, Tet3, TDG,* and *MBD4* expression was significantly higher ($P < 0.05$) in this study. It indicates that chronic HS can influence DNA methylation in the postnatal period, and antioxidant supplements like DP can reverse this process in HS conditions. However, further investigation is necessary to conclude this mechanism in heat-stressed chickens.

## Conclusion

Dietary supplementation with dried plum has been shown to enhance the expression of genes associated with antioxidants, nutrient transporters, and epigenetic regulation in the livers of heat-stressed broiler chickens. Furthermore, dried plum supplementation reduced the expression of gluconeogenesis-related gene markers, indicating an increase in metabolic activity in the liver of heat-stressed broiler chickens. Therefore, incorporating dried plums into poultry diets represents a promising strategy for alleviating the adverse effects of heat stress in broiler chickens.

## Declaration of competing interest

The authors declare no conflict of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.104911.

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