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Evaluation of probiotic effects on physiological parameters in healthy and stressed broilers

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ABSTRACT

Clarifying the impact of probiotics on healthy and transported stressed broilers was the goal of the current study. Two hundred and ten male broiler chicks, 1-day-old were split up into 3 groups in 7 pens, each 10 broilers: a standard feed combined with the probiotic, Bacillus subtilis PB6, control (C), lower dose 0.25 (L), and higher dose 0.5 (H) g/kg feed for 35 days. At the conclusion of the study, 5 birds per group were taken for blood collection. Besides, another 5 birds per group were driven for 80 kilometers (km) and then taken for blood collection. The findings indicated that probiotic exposure had an improvement effect on serum lipid profiles, liver enzymes, oxidative markers and some biochemical parameters of both healthy C and stressed (S) broilers. Both L and H dose of probiotic significantly decreased low density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglyceride (TG), total cholesterol (TC), uric acid (UA), glucose, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), malonaldehyde (MDA) and creatine kinase (CK) while significantly increased high-density lipoprotein (HDL) and total antioxidant capacity (TAC) in comparison with both C and S broilers groups. Besides, stressed broilers treated by lower and higher probiotic doses (LS and Hs, respectively) revealed a marked rise in serum albumin level in comparison with S group while albumin didn't show significant change comparing L and H groups with the C one. Overall, this study approved that administration of probiotics in a higher dose 0.25 g/kg had a better effect in improving health and decrease stress side effects than that in the lower dose 0.25 g/kg feed.

Introduction

Worldwide, and particularly in developing nations, there is a growing trend in the production, consumption, and demand for poultry (Siddharth *et al*. 2024). Numerous effects, such as the rise of a wide range of diseases and bacterial resistance, have coincided with the chicken industry's increased productivity. The careless use of chemotherapeutic medicines as a result of rearing cycle management approaches is partially to blame for these effects (Lutful Kabir, 2009). Globally, poultry producers aim to satisfy consumer preferences for safe and high-quality meat as well as their needs for the welfare of chickens. The poultry business suffers significant financial losses as a result of the pre-slaughter handling and transportation of broiler chickens at marketing age between farms and processing facilities, which puts the birds under stress and has a detrimental role on their welfare, quality and meat yield, in certain situations, the birds may even die (Barbut, 2015; Gou *et al*., 2021). Transport stressors can be divided into three categories: physical, mental and mixed. Physical ones include catching, creating and environmental factors like wind, temperature changes, vibrations of the vehicle, and air flow. These factors can cause physical harm or even death to the birds. Fear, social interaction, pain, and lack of food and water are examples of mental stressors that cause the bird's natural antioxidant capacity to be depleted and expose its cells to dangerous free radicals (Arif *et al*., 2022).

It was proposed that a mix of outside variables, including crowding, movement, and temperature changes, as well as a lack of food and water, could cause transport-induced stress (Gou *et al*., 2021). These stressors affect the animal cells' normal equilibrium by changing their physiological and biochemical status, which lowers the amount and quality of meat (Arif *et al*., 2022). Most researchers believe that the tracts of typical, healthy, non-stressed fowl have an unstable mix of non-beneficial and good bacteria. If balance is present, the poultry functions to its full efficiency, while if stress is exerted, the good flora, notably lactobacilli, has a propensity to drop in quantity and a growth of the non-beneficial species appears to ensue. This phenomenon may be subclinical and lower growth, feed efficiency, and other production indices, or it may predispose to frank disease, such as diarrhea. The gut's beneficial microflora is mostly constant, but some nutritional and environmental factors could affect it. The most important are severe hygiene, antibiotic therapy, and stress (Fuller, 2001; Lutful Kabir, 2009).

The birds may undergo severe stress during transit, which could result in physiological and behavioral abnormalities (Zulkifli, 2003) including intense anxiety, and financial losses for the producers (Vieira *et al*., 2015; Vecerek *et al*., 2016). Muscle physiological alterations brought on by transport stress have an impact on the quality of the birds' carcasses at slaughter (Utomo *et al*., 2019). However, this stressful environment disrupts the normal gut microbiota, which causes opportunistic infections to thrive excessively (Bello, *et al*., 2018). Poultry under ante-mortem stress produce and consume more glucocorticoids and adrenaline (Arif *et al*., 2022).

The poultry industry has become an important part of the economy in many countries. In huge-scale chicken raising operations, infectious problems and environmental degradation are prevalent and can result in considerable financial losses. In the past few years, attempts to avoid and manage disease have led to a major increase in the use of veterinary drugs. However, the usefulness of antimicrobial medications as a preventative intervention has been questioned considering the abundance of data showing the emergence of resistance to antibiotics by dangerous bacteria. Antibiotics may no longer be utilized as stimulants of growth for poultry, and their usage as medicinal agents may have negative side effects. As a result, consumers and manufacturers are searching for alternatives. Some farmers are already employing probiotics instead of antibiotics in an effort to close this gap (Trafalska and Grzybowska, 2004; Griggs and Jacob, 2005; Nava, *et al*. 2005).

Biotechnology's influence on poultry feeding is quite important, as

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the chicken feed industry relies heavily on biotechnology. Nutritionists are constantly working to provide food that is both better and more cost-effective. Good food by itself won't accomplish the goal; better use of it is also necessary. Both dietary modifications and a poor diet can affect the gut's microbiota balance, making chicken more susceptible to digestive disorders. Nutritionists and veterinary specialists have recently paid close attention to the appropriate use of nutrients and the application of probiotics to promote poultry growth (Lutful Kabir, 2009).

Probiotics are known to be "live microorganisms that when given in sufficient quantities impart a health advantage on the host" by the FAO/WHO. To be more specific, probiotics are living, nonpathogenic, harmless bacteria that are beneficial to the host's health when ingested (Hotel and Cordoba, 2001; Sun *et al*., 2024). Probiotics are frequently utilized as an affordable, secure, and practical treatment for a number of illnesses (Quigley, 2011).

Nowadays, a wide variety of species are utilized in probiotic formulations. These include Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Escherichia coli, Bifidobacterium species, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus helveticus, Lactobacillus casei, Lactobacillus lactis, Lactobacillus salivarius, and Lactobacillus plantarum. Besides enhancing gut health (Ooi and Liong, 2010),

probiotics have been shown to provide additional benefits, like bolstering the immunity (Galdeano *et al*., 2007), protection from cancer (Hirayama and Rafter, 2000), antihypertensive role (Yeo and Liong, 2010), antioxidative effects (Songisepp *et al*., 2004), improving mineral absorption (Scholz-Ahrens *et al*., 2007), dermatitis reduction (Weston *et al*., 2005), prevention of vulvovaginal candidiasis in women (Falagas *et al*., 2006), arthritis reduction (Baharav *et al*., 2004) and decreasing allergy (Ouwehand, 2007)

In poultry, probiotics work by (i) altering metabolism by boosting digestive enzyme production, lowering ammonia generation and bacterial production of enzymes; (ii) improving digestion and the consumption of food; (iii) preserving the natural gut flora through resistance and exclusion due to competition; and (iv) boosting immunity (Lutful Kabir, 2009). Although probiotics are generally thought to be nonpathogenic, they may be contagious, particularly in immunocompromised and disabled populations (Land *et al*., 2005). Probiotics' survival rate will drop under environmental stressors, and their physiological and nutritional value will also significantly decline. To enhance their usefulness, probiotics' stress-coping mechanisms must be clarified, and their resilience to environmental variables must be strengthened (Wang *et al*., 2025).

Some probiotic strains showed an antibiotic resistance, raising concerns about the potential for horizontal gene transfer to the host or to pathogenic microorganisms within the gastrointestinal tract (Ooi and Liong, 2010). Given these risks, thorough safe confirmation of using probiotics in industrial and commercial applications is of paramount importance. Therefore, it is essential to optimize both the dosage and duration of use to ensure efficacy and minimize potential adverse effects. Based on this, our research was carried out to assess the appropriate dosage and assess the safety profile of this probiotic strain.

Materials and methods

Ethical approval

Ethics clearance and participation consent The University's criteria for handling experimental animals were followed in every step of this work. The Committee on Research Ethics of the Faculty of Veterinary Medicine at Assiut University in Egypt granted ethical approval (06/2024/0267).

Housing and Birds

In Animal and Poultry Management section, Egypt, 210 male (Ross 708 strain) 1-day old broiler, weighed and were divided into 21 floor pens ,10 birds per 100 cm x 100 cm floor, have a comparable mean body weight. Five centimeters of dry, new wood shaving was utilized as bedding. The temperature was 35°C throughout the bird's first week life and progressively dropped 0.5°C/day till it reached 26°C in the experiment's conclusion. The relative humidity was almost 50%. Starting with 23 hours light and 1 hour darkness (during the first 7 days of age), an alternating light schedule (10 lux) was used. Toward the end of the trial, the light and dark times were gradually reduced to 20 hours and 4 hours, respectively.

Drugs and chemicals

In this investigation, a probiotic (CLOSTAT HC SP Dry, Europe, Kemin, NV; Belgium, Herentals) was used. A distinct, patented strain of Bacillus subtilis PB6 that forms spores was found in CLOSTAT. This strain was obtained from chickens that had survived exposure to Clostridium perfringens in a typical raising environment. Intestine pathogenic colonization increases chicken mortality and illness, which CLOSTAT was created to reduce it.

Experimental Plan

In seven replicates of ten broilers each, the 21 floor pens were distributed into one of three nutritional treatments: a standard meal supplemented by probiotic (Bacillus subtilis PB6) at dose 0 (C), 0.25 (L), and 0.5 (H) g/kg diet. Company's suggestion served as the basis for the CLOSTAT nutritional treatment dosage. When they achieved market weight, the dietary therapy was administered from day 1: 35.

Initially, a tiny amount of the initial diet was combined with the appropriate dosages of probiotics, followed by a bigger amount of the initial diet until the entire quantity of every diet was evenly combined. From day 1: day 14, the birds were taken as starting food; from day 15: day 28, they had grown food; and on day 29: 35, they had finished food. Every pen had clean drinker with water. Five birds from each pen were removed for the collection of blood samples at the conclusion of the experiment (day 35). Besides, another 2 birds per each pen, total 14 per each group, were transported 80 km to the abattoir, then randomly choose 5 birds per each group for blood collection.

Group C: Control untreated group (0 probiotic).
Group L: Treated group with lower probiotic dose (0.25 g/kg feed).
Group H: Treated group with higher probiotic dose (0.5 g/kg feed).
Group S: Stressed untreated group (0 probiotic).
Group Ls: Stressed treated group with lower probiotic dose (0.25 g/kg feed).
Group Hs: Stressed treated group with higher probiotic dose (0.5 g/kg feed).

Collection and preparation of samples

On day 35, birds sedated by sodium pentobarbital (30 mg/ ml) to collect samples. Each bird's heart was punctured to extract five millimeters of blood, and transferred to a tube without anticoagulant, centrifuged 15 min, 3000 rpm to gain serum and reserved at -80°C until the measuring.

Biochemical measurements

Plasma lipid profiles were carried out using a spectrophotometer and the colorimetric method with a commercial kit produced by a Biotechnology Company in Egypt, Cairo as triglyceride (TG) (Catalog number: 314002), high density lipoprotein (HDL) (Catalog number: 266001), low density lipoprotein (LDL) and total cholesterol (TC) (Catalog number: 230002) with calculating the very low-density lipoprotein (VLDL) = TG/5. Moreover, liver enzyme alanine aminotransaminase (ALT) (Catalog number: 260001), aspartate aminotransferase (AST) (Catalog number: 260001), albumin level (Catalog number: 211001), glucose (Catalog num-

ber: 250001), creatine kinase (CK), Catalog number CKSLR 25, purchased from (LAB-CARE DIAGNOSTICS, INDIA), and uric Acid assay kit ab65344 from Abcam company were measured.

Albumin was measured using kits supplied by Diamond Diagnostics (Egypt). A calorimetric kit (Catalog number: TA2513, Biodiagnostic, Egypt, Giza) was used to evaluate total antioxidant capacity (TAC). The thiobarbituric acid response was used to determine the amounts of malondialdehyde (MDA) in accordance with the protocol of Ohkawa *et al*. (1979).

Analysis of statistics

Tukey's post-test was used to determine the groups' significance level after a one-way ANOVA. Prism software (GraphPad Software; version 8.0.1; Inc. San Diego, USA, CA) was used in statistical analyses. At $P < 0.05$, variations will be deemed statistically significant.

Results

Effect of probiotics on broilers serum lipid profiles

S group cleared a significant improvement in serum levels of TC, TG, LDL and VLDL with significant decline of HDL level in comparison with the C group. A significant decline was detected in serum TC, TG, LDL and VLDL while there was significantly increase in HDL in the L and H groups compared to the C group. Also, there were significant decreases in serum TC, TG, LDL and VLDL while there was significantly rise in HDL in the Ls and Hs groups compared to the S group. Differences between L and H groups showed a non-significant change in TC, LDL. Moreover, H group had a significant decrease in TG and VLDL, while showed a significantly increased HDL than that of the L group. In addition, Hs group had a significant decrease in TC, TG, LDL and VLDL with a significantly increased HDL than that of the Ls group (Table 1).

Effect of probiotics on broilers serum biochemical parameters

Serum from S broilers showed a significant enhancement in UA, glucose and CK levels with a significant decline in albumin level in comparison with that of the C broilers. Supplementation with L and H significantly reduced UA, glucose and CK levels in serum while there was a non-significant increase in albumin level when compared with the C group. Additionally, there was a significant decrease in serum UA, glucose and CK in the Ls and Hs groups compared to the S group. Differences between L and H groups showed a non-significant change in albumin, UA, glucose and CK while Hs group had a significant reduction in UA, glucose and CK than that of the LS group. Albumin concentrations showed a non-significant change between S and Ls groups and between Ls and Hs groups while Hs has a significant increase in albumin level than the S group (Table 2).

Effect of probiotics on serum liver enzymes of broilers

S group cleared a significant improvement in serum levels of AST and ALT in comparison with the C group. A significant reduction was observed in serum AST and ALT in H broilers in comparison with that of the C broilers. Moreover, L broilers serum had a significant decline in ALT with a non-significant AST change when compared with the C one. In addition, Ls and Hs showed significant decline in serum levels of ALT and AST in comparison with S group. Comparison between L and H groups showed a non-significant change in AST level while H group had a significant decrease in ALT level than that of the L group. Moreover, comparison between Ls and Hs groups showed a significant decline in ALT and AST (Table 3).

Table 1. The effect of probiotics on broilers serum lipid profiles.

Treatment	C	L	H	S	Ls	Hs	P value
TC (mg/dl)	122.12±2.82^{a}	113.42±2.89^{b}	114.96±3.13^{b}	135.48±2.5^{c}	117.08±2.21^{d}	113.36±4.23^{e}	< 0.0001
TG (mg/dl)	86.6±4.22^{a}	79.6±1.14^{b}	67.8±2.28^{c}	120.4±1.14^{d}	113.4±2.41^{e}	105.8±1.64^{f}	< 0.0001
HDL (mg/dl)	51.6±1.14^{a}	56.6±1.52^{b}	62.0±2.12^{c}	32.6±1.82^{d}	37.8±0.84^{e}	42.2±3.19^{f}	< 0.0001
LDL (mg/dl)	53.2±0.84^{a}	40.94±1.14^{b}	39.4±0.55^{b}	78.8±0.45^{c}	56.6±0.88^{d}	50.0±0.70^{e}	< 0.0001
VLDL (mg/dl)	17.32±0.84^{a}	51.92±0.23^{b}	13.56±0.46^{c}	24.08±0.23^{d}	22.68±0.48^{e}	21.16±0.33^{f}	0.09

Results are expressed as Mean±SD of 5 rats per treatment. Values in the same row followed by different superscripts (a, b, c, d, e, f) are significant ($P < 0.05$). C: control untreated group (0 probiotic); L: treated group with lower probiotic dose (0.25 g/kg feed); H: treated group with higher probiotic dose (0.5 g/kg feed); S: stressed untreated group (0 probiotic); Ls: stressed treated group with lower probiotic dose (0.25 g/kg feed) and Hs: stressed treated group with higher probiotic dose (0.5 g/kg feed).

Table 2. The effect of probiotics on broilers serum biochemical parameters.

Treatment	C	L	H	S	Ls	Hs	P value
Albumin (g/dl)	1.60±0.12^{a}	1.74±0.11^{a}	1.82±0.08^{a}	0.56±0.13^{b}	0.62±0.13$^{b, c}$	0.80±0.1^{c}	< 0.0001
UA (mg/dl)	7.10±0.42^{a}	6.40±0.24^{b}	5.20±0.27^{c}	12.38±0.24^{d}	11.52±0.26^{e}	10.52±0.31^{f}	< 0.0001
Glucose (mg/dl)	241.40±1.34^{a}	237.40±1.140^{b}	230.60±1.95^{c}	268.60±1.14^{d}	264.40±1.14^{e}	251.60±2.07^{f}	< 0.0001
CK (U/L)	145.40±3.65^{a}	133.00±3.46^{b}	107.00±5.70^{c}	191.80±1.48^{d}	183.00±2.83^{e}	171.60±1.52^{f}	< 0.0001

Results are expressed as Mean±SD of 5 rats per treatment. Values in the same row followed by different superscripts (a, b, c, d, e, f) are significant ($P < 0.05$). C: control untreated group (0 probiotic); L: treated group with lower probiotic dose (0.25 g/kg feed); H: treated group with higher probiotic dose (0.5 g/kg feed); S: stressed untreated group (0 probiotic); Ls: stressed treated group with lower probiotic dose (0.25 g/kg feed) and Hs: stressed treated group with higher probiotic dose (0.5 g/kg feed).

Table 3. The effect of probiotics on serum liver enzymes of broilers.

Treatment	C	L	H	S	Ls	Hs	P value
ALT (U/L)	4.60±0.42^{a}	3.30±0.27^{b}	2.00±0.61^{c}	9.00±0.12^{d}	8.06±0.13^{e}	7.52±0.31^{e}	< 0.0001
AST (U/L)	296.60±1.14^{a}	293.80±1.48$^{a, b}$	292.40±1.14^{b}	412.00±2.12^{c}	408.60±2.19^{d}	400.20±0.84^{e}	< 0.0001

Results are expressed as Mean±SD of 5 rats per treatment. Values in the same row followed by different superscripts (a, b, c, d, e) are significant ($P < 0.05$). C: control untreated group (0 probiotic); L: treated group with lower probiotic dose (0.25 g/kg feed); H: treated group with higher probiotic dose (0.5 g/kg feed); S: stressed untreated group (0 probiotic); Ls: stressed treated group with lower probiotic dose (0.25 g/kg feed) and Hs: stressed treated group with higher probiotic dose (0.5 g/kg feed).

Table 4. The effect of probiotics on serum oxidative markers of broilers.

Treatment	C	L	H	S	Ls	Hs	P value
TAC (mmol/L)	1.44±0.09[a]	1.60±0.07[b]	1.82±0.08[c]	0.62±0.08[d]	0.84±0.05[e]	1.020±0.08[f]	< 0.0001
MDA (nmol/ml)	2.96±0.11[a]	2.46±0.27[b]	1.34±0.21[c]	8.14±0.22[d]	7.28±0.19[e]	6.34±0.21[f]	0.78

Results are expressed as Mean±SD of 5 rats per treatment. Values in the same row followed by different superscripts (a, b, c, d, e, f) are significant ($P < 0.05$). C: control untreated group (0 probiotic); L: treated group with lower probiotic dose (0.25 g/kg feed); H: treated group with higher probiotic dose (0.5 g/kg feed); S: stressed untreated group (0 probiotic); Ls: stressed treated group with lower probiotic dose (0.25 g/kg feed) and Hs: stressed treated group with higher probiotic dose (0.5 g/kg feed).

Effect of probiotics on serum oxidative markers of broilers

S serum group cleared that there was a significant decline in TAC while there was a significant rise in MDA level compared to the C group. Moreover, there was a significant enhanced TAC while there was a significant decline in MDA levels of both broilers' serum either L or H groups in comparison with that of the C group. Both Ls and Hs serum showed a significant increase in TAC and a significant decrease in MDA levels compared to S serum group. Also, there was a significant rise in TAC concentration in H and Hs in comparison with L and Ls respectively while there was a significant reduction in MDA in H and Hs in comparison with L and Ls respectively (Table 4).

Discussion

Broilers are subjected to a variety of stress-inducing elements during transportation to the slaughterhouse, including movement, cage density, noise, microclimatic conditions, time, distance, and season (Arif *et al*., 2022). Our results showed that broilers exposed to transportation stress gave a significant enhancement in serum levels of TC, TG, VLDL and LDL with significantly declined HDL level in comparison with the unstressed control broilers. That is in accordance to Erisir *et al*. (2008) who reported that plasma levels of cholesterol, triglyceride, corticosterone, glucose, and AST increased in transported ducklings. Broilers exposed to transportation stress increased TG level in the plasma (Arif *et al*. 2022). Another results indicated significant increases in cholesterol, triglycerides and LDL-C, levels during mental stress (Patterson *et al*., 1993). Also, heat stress improved liver and abdominal adipose relative weight, blood TC and TG levels, liver TC, TG and VLDL content, suggested that this because of the liver's enhanced TG release, as well as the abdominal adipose tissue's improved fatty acid absorption ad fat production (Lan *et al*. 2022).

Probiotics are live microorganisms which, when consumed, encourage the growth of good bacteria in the digestive system and provide other health advantages. Probiotics may be used as substitute nutrients to improve health (Chen *et al*., 2017; Zarezadeh *et al*., 2023). Our finding revealed a significant decline in serum TC, LDL, TG and VLDL while there was significantly increase in HDL in all probiotic treated groups in comparison with the other untreated groups in this research. That agree with (Gadelha and Bezerra, 2019) who approved that added probiotics to diet significantly reclined TC, LDL, and TG and enhanced HDL levels. Previous in vivo research demonstrated that probiotics could improve lipid profiles, such as lowering plasma/serum TG, LDL and TC or raising HDL (Ooi and Liong 2010). The processes by which probiotics control hypocholesterolemia effects and the ideal dose, time of therapy and frequency for various probiotic strains are frequently not adequately covered in studies looking at the effectiveness of probiotics in lowering cholesterol. A number of theories have been put forth, including the following: bile-salt hydrolase of probiotics enzymatically deconjugates bile acids (Lambert *et al*., 2008), probiotics assimilate cholesterol (Pereira and Gibson, 2002), probiotics co-precipitate cholesterol with deconjugated bile (Liong and Shah, 2006), probiotics attach cholesterol to their cell membrane (Liong and Shah, 2005), probiotics incorporate cholesterol into their cellular wall as they grow (Lye HueyShi *et al*., 2010) and probiotics convert cholesterol to coprostanol (Lye *et al*., 2010). Another aspect mentioned in research is the generation of fatty acids (short-chain), which inhibits the liver's production of fatty acids and cholesterol (Begley *et al*., 2006; Ooi and Liong, 2010).

This study demonstrated that serum from transported stressed broilers showed a significant enhancement in UA, glucose and CK levels with a significantly declined albumin level in comparison with that of the unstressed one. Moreover, treatment with probiotic in both lower and higher doses significantly reduced UA, glucose and CK levels in both transported and un-transported broilers. The increased glucose level in case of stress possibly be explained by the fact that the sympathetic adreno-medullary axis and the hypothalamo-hypophysial-adrenal axis are activated when perceived stress causes endocrine changes. Stress causes the release of catecholamines and glucocorticoids, which alter blood sugar levels and cause physiological changes (Scanes, 2016).

By restoring pancreatic islet β-cell function and reducing insulin resistance, probiotics may help maintain glucose homeostasis. First, by encouraging glucagon-like peptide-1 (GLP-1) secretion, probiotics have been demonstrated to alleviate insulin resistance. By lowering body weight and increasing peripheral tissue sensitivity to insulin, GLP-1 is one of the key mechanisms for decreasing insulin resistance (Meier *et al*., 2005; Raun *et al*., 2007; Seghieri *et al*., 2013). Probiotics may improve blood lipids, decrease inflammation, and regulate blood glucose by playing an significant role in the metabolism and overall health state of the body (Sun *et al*., 2024). Furthermore, probiotics' ability to lower blood cholesterol levels may help postpone the onset of insulin resistance. Probiotic consumption may lessen the harm that elevated cholesterol causes to liver tissue cells, which would enhance the expression of genes linked to glycogen secretion and decrease the mRNA expression of important enzymes involved in liver gluconeogenesis (Park *et al*., 2017; Farida *et al*. 2020). This may enhance insulin sensitivity, decrease hepatic gluconeogenesis, and increase the production of liver glycogen (Huang *et al*., 2021).

Broilers that were exposed to stress density and feed withdrawn stress showed significant decrease in blood albumin and UA levels (Nwaigwe *et al*., 2020). A marker of protein catabolism, circulating uric acid rises in response to increased catabolism of proteins or amino acids (Hussnain *et al*., 2020). Fish with high stocking densities had higher serum UA levels because of enhanced protein catabolism and gluconeogenesis (Costa *et al*., 2012). Others showed that when monostrain probiotics were administered to Asian male patients with illnesses, their UA levels decreased (Othman *et al*., 2025). Probiotics like Lactobacillus johnsonii YH1136 reduce blood levels of UA by blocking the activity of hepatic xanthine oxidase (XOD), a crucial enzyme in the synthesis of UA (Zhang *et al*., 2024). A combination of Lactobacillus strains dramatically decreased serum UA levels and enhanced liver activity in case of metabolic-associated fatty liver disorder (Lin *et al*., 2024). Also, probiotics encourage gastrointestinal excretion of UA and increase the expression of UA transporters (J. Cao *et al*., 2023).

Under normal circumstances, myosin heavy chain-associated components lactate dehydrogenase (LDH) and CK are unable to pass through muscle cell membranes (Willoughby *et al*., 2003). Muscle injury has been identified by their elevated serum levels (Foschini and Prestes, 2007). A number of preslaughter stressors, such as climate, distance, transit, and length of lairage, cause animals to become exhausted and release hormones, enzymes, CK, catecholamines and cortisol to the circulatory system, which triggers a number of secondary processes involving energy metabolism, immunological response, breathing, and osmotic control (Chulayo and Muchenje, 2013). In accordance, Baird *et al*. (2012) demon-

strated that giving mice probiotics to their orthotropic-transplanted extensor digitorium longus (EDL) muscle decreased their LDH and CK activity, a sign that muscle regeneration was increasing (). It was found that probiotic supplementation exhibited a remarkable reduction effect on CK concentration overall (Shirkoohi*et al*., 2025).

Our results clarified that transported stressed broilers serum showed significant rise in AST and ALT levels in comparison with that of the unstressed one. In addition, treatment with probiotic significantly declined ALT and AST levels in both transported and un-transported broilers. This is in agree with (Tang *et al*., 2022) who reported that broiler exposed to heat stress enhanced serum AST, hepatic MDA and SOD (superoxide dismutase) showed that heat stress damaged broiler livers and hindered liver growth. Moreover, Erisir *et al*. (2008) recorded that plasma levels of AST increased in transported ducklings.

Overall, the data suggests that the addition of probiotic improved liver function via modulating the intestinal tract and lowering liver enzyme levels (Huang *et al*., 2015;J. Cao *et al*., 2023). Supplementing with prebiotics, probiotics and synbiotics improved TG levels in addition to liver function (AST, alkaline phosphatase and ALT) and hepatic fibrosis. In a non-alcoholic fatty liver disease case (NAFLD), prebiotic, probiotic or synbiotic supplements improved lipid profiles, liver enzymes and inflammatory cytokines (Pan *et al*., 2024). Endotoxin (lipopolysaccharide), one of the enterotoxins released by pathogenic bacteria when the microbiota in the gut is dysbiotic, increases the permeability of the lining of the intestine and causes transfer of bacteria, which can result in endotoxemia and long-term harm to liver cells (Xu *et al*., 2022). Probiotics can reduce the liver's inflammatory reaction by regulating intestinal ecological problems and enhancing the integrity of the intestinal mucosal barrier (C. Cao *et al*., 2023).

Moreover, S serum group showed that there was a significant reduction of TAC while there was a significant increase in MDA level in comparison with the C group. Additionally, there was a significant enhancement in TAC while there was a marked decline in MDA levels in all probiotic treated groups. It is commonly known that MDA, a lipid peroxidation molecule, is a sign of oxidative stress in tissues (Cheng *et al*., 2017). Broilers exposed to transportation stress increased MDA level in plasma (Arif *et al*., 2022). According to other reports, during heat stress, yellow-feather broiler intestine GSH-Px activity reduced, and MDA levels elevated (Lan *et al*., 2020). Also, in breast muscle of broilers exposed to transportation stress TAC decreased (Gou *et al*., 2021). Overall, the antioxidative and oxidative equilibrium was upset when broilers were exposed to transportation stress, and the rise in antioxidant enzyme activity has been thought to be a defense mechanism against oxidative stress.

Probiotics significantly improved TAC, nitric oxide (NO) and glutathione (GSH) while reduce MDA biomarkers in patients with diabetes (Chen *et al*., 2025). The antioxidative and Probiotics' main modes of action include enhancing the diversity of the gut microbiota, boosting the strength of the intestinal wall, and lowering lipopolysaccharide (LPS) translocation to lessen systemic endotoxemia (Basso *et al*., 2016). In the end, these processes result in improved antioxidant defenses and less activation of inflammatory systems. By strengthening the body's antioxidant response against harmful free radicals and oxidative damage, raising GSH and TAC helps to maintain the integrity of cells and improve the function of endothelial cells (Tandon and Tandon, 2024).

Conclusion

According to current study, probiotics may be effectively incorporated into poultry feeds as nutritional aids to support growth, improving general health and protection from transportation stress effects in broilers. Probiotic pre-treatment in broilers-exposed to transportation stress is efficient to restore health by inhibiting lipid peroxidation, suppress oxidative stress and decrease UA, glucose, CK and liver enzymes. To further understand the molecular mechanisms behind probiotics' ability to defend against the negative consequences of transportation stress, more research is strongly advised.

Conflict of interest

The authors have no conflict of interest to declare.

References

Arif, A., Ibrahim, S.F.M., Abouelezz K.F.M., Omar, A.H.M., 2022. Influences of different transport durations on blood biochemical, meat quality, and meat yield of broiler chickens. Archives of Agriculture Sciences Journal 5, 165-179.

Baharav, E., Weinberger, A., Mor, F., Halpern, M., 2004. Lactobacillus GG bacteria ameliorate arthritis in Lewis rats. The Journal of Nutrition 134, 1964-1969.

Baird, M.F., Graham, S.M., Baker, J.S., Bickerstaff, G.F., 2012. Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. Journal of nutrition and Metabolism 1, 960363.

Barbut, S., 2015. The science of poultry and meat processing. https://s3.amazonaws.com/download.poultryandmeatprocessing.com/v01/SciPoultryAndMeatProcessing%20-%20Barbut%20-%20v01.pdf

Basso, F.G., Pansani, T.N., Turrioni, A.P.S., Soares, D.G., de Souza Costa, C.A., Hebling J., 2016. Tumor necrosis factor-α and interleukin (IL)-1β, IL-6, and IL-8 impair in vitro migration and induce apoptosis of gingival fibroblasts and epithelial cells, delaying wound healing. Journal of Periodontology 87, 990-996.

Begley, M., Hill, C., Gahan, C.G.M., 2006. Bile salt hydrolase activity in probiotics. Applied and Environmental Microbiology 72, 1729-1738.

Cao, C., Shi, M., Wang, X., Yao, Y., Zeng, R., 2023. Effects of probiotics on non-alcoholic fatty liver disease: A review of human clinical trials. Frontiers in Nutrition 10, 1155306.

Cao, J., Wang, T., Liu, Y., Zhou, W., Hao,H., Liu, Q., Yin, B., Yi, H., 2023. Lactobacillus fermentum F40-4 ameliorates hyperuricemia by modulating the gut microbiota and alleviating inflammation in mice. Food & Function 14, 3259-3268.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang X., Zhao, L., 2017. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9, 7204.

Chen, X., Yan, L., Yang, J., Xu, C., Yang, L., 2025. The impact of probiotics on oxidative stress and inflammatory markers in patients with diabetes: a meta-research of meta-analysis studies. Frontiers in Nutrition 12, 1552358.

Cheng, K., Song, Z.H., Zheng, X.C., Zhang, H., Zhang, J.F., Zhang, L.L., Zhou, Y.M., Wang, T., 2017. Effects of dietary vitamin E type on the growth performance and antioxidant capacity in cyclophosphamide immunosuppressed broilers. Poultry Science 96, 1159-1166.

Chulayo, A.Y., Muchenje, V., 2013. The effects of pre-slaughter stress and season on the activity of plasma creatine kinase and mutton quality from different sheep breeds slaughtered at a smallholder abattoir. Asian-Australasian journal of Animal Sciences 26, 1762.

Costa, L.S., Pereira, D.F., Bueno L.G.F., Pandorfi, H., 2012. Some aspects of chicken behavior and welfare. Brazilian Journal of Poultry Science 14, 159-164.

Erisir, Z., Poyraz, O., Erisir, M., Onbasilar, E.E., Erdem, E., 2008. The changes of the body weight and some blood parameters of Pekin ducklings dependent on transportation duration. Journal of Animal and Veterinary Advances 7, 1190-1195

Falagas, M.E., Betsi, G.I. Athanasiou, S., 2006. Probiotics for prevention of recurrent vulvovaginal candidiasis: a review. Journal of Antimicrobial Chemotherapy 58, 266-272.

Farida, E., Nuraida, L., Giriwono, P.E., Jenie, B.S.L., 2020. Lactobacillus rhamnosus reduces blood glucose level through downregulation of gluconeogenesis gene expression in streptozotocin-induced diabetic rats. International journal of Food Science 1, 6108575.

Foschini, D., Prestes, J., 2007. Acute hormonal and immune responses after a bi-set strength training. Fitness & Performance Journal 6(1).

Fuller, R., 2001. The chicken gut microflora and probiotic supplements. Poultry Science. 38, 189-196.

Gadelha, C.J.M.U., Bezerra, A.N., 2019. Effects of probiotics on the lipid profile: systematic review. Jornal Vascular Brasileiro 18, e20180124.

Galdeano, C.M., De Moreno De Leblanc, A., Vinderola, G., Bonet, M.E.B., Perdigón, G., 2007. Proposed model: mechanisms of immunomodulation induced by probiotic bacteria. Clinical and Vaccine Immunology 14, 485-492.

Gou, Z., Abouelezz, K.F.M., Fan, Q., Li, L., Lin, X., Wang, Y., Cui, X., Ye, J., Masoud, M.A., Jiang, S., 2021. Physiological effects of transport duration on stress biomarkers and meat quality of medium-growing Yellow broiler chickens. Animal 15, 100079.

Griggs, J.P., Jacob, J.P., 2005. Alternatives to antibiotics for organic poultry production. Journal of Applied Poultry Research. 14, 750-756.

Hirayama, K., Rafter, J., 2000. The role of probiotic bacteria in cancer prevention. Microbes and Infection 2, 681-686.

Hotel, A.C.P., Cordoba, A., 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Prevention 5, 1-10.

Huang, D., Gao, J., Li, C., Nong, C., Huang, W., Zheng, X., Li, S., Peng, Y., 2021. A potential probiotic bacterium for antipsychotic-induced metabolic syndrome: Mechanisms underpinning how Akkermansia muciniphila subtype improves olanzapine-induced glucose homeostasis in mice. Psychopharmacology 238, 2543-2553.

Huang, Z., Mu, C., Chen, Y., Zhu, Z., Chen, C., Lan, L., Xu, Q., Zhao, W., Chen, G., 2015. Effects of dietary probiotic supplementation on LXRα and CYP7α1 gene expression, liver enzyme activities and fat metabolism in ducks. British Poultry Science 56, 218-224.

Hussnain, F., Mahmud, A., Mahmood, S., Jaspal, M.H., 2020. Effect of transportation distance and crating density on preslaughter losses and blood biochemical profile in broilers during hot and humid weather. Turkish Journal of Veterinary & Animal Sciences 44, 418-426.

Lambert, J. M., Bongers, R.S., de Vos, W.M., Kleerebezem, M., 2008. Functional analysis of four bile salt hydrolase and penicillin acylase family members in Lactobacillus plantarum WCFS1. Appl. Environ. Microbiol . 74, 4719-26.

Lan, R., Li, Y., Chang, Q., Zhao, Z., 2020. Dietary chitosan oligosaccharides alleviate heat stress–induced intestinal oxidative stress and inflammatory response in yellow-feather broilers. Poultry Science 99, 6745-6752.

Lan, R., Wang, Y., Wei, L., Wu, F., Yin, F., 2022. Heat stress exposure changed liver lipid metabolism and abdominal fat deposition in broilers. Italian Journal of Animal Science 21, 1326-1333.

Land, M.H., Rouster-Stevens, K., Woods, C.R., Cannon, M.L., Cnota, J., Shetty, A.K., 2005. Lactobacillus sepsis associated with probiotic therapy. Pediatrics 115, 178-181.

Lin, J.H., Lin, C.H., Kuo, Y.W., Liao, C.A., Chen, J.F., Tsai, S.Y., Li, C.M., Hsu, Y.C., Huang, Y.Y., Hsia, K.C., 2024. Probiotic Lactobacillus fermentum TSF331, Lactobacillus reuteri TSR332, and Lactobacillus plantarum TSP05 improved liver function and uric acid management-A pilot study. Plos one. 19, e0307181.

Liong, M.T., Shah, N.P., 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. Journal of Dairy Science 88, 55-66.

Liong, M.T., Shah, N.P., 2006. Effects of a Lactobacillus casei synbiotic on serum lipoprotein, intestinal microflora, and organic acids in rats. Journal of Dairy Science 89, 1390-1399.

Lutful Kabir, S.M., 2009. The role of probiotics in the poultry industry. International Journal of Molecular Sciences 10, 3531-3546.

Lye, H.S., Rusul, G., Liong, M.T., 2010. Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. Journal of Dairy Science 93, 1383-1392.
Lye HueyShi,, Gulam Rusul Rahmat-Ali, Liong MinTze, 2010. Mechanisms of cholesterol removal by lactobacilli under conditions that mimic the human gastrointestinal tract. International Dairy Journal 20, 169-175.
Meier, J.J., Kemmeries, G., Holst, J.J., Nauck, M.A., 2005. Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. Diabetes 54, 2212-2218.
Nava, G.M., Bielke, L.R., Callaway, T.R., Castaneda, M.P., 2005. Probiotic alternatives to reduce gastrointestinal infections: the poultry experience. Animal Health Research Reviews 6, 105-118.
Nwaigwe, C.U., Ihedioha, J.I., Shoyinka, S.V., Nwaigwe, C.O., 2020. Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. Veterinary World 13, 2294.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95, 351-358.
Ooi, L.G., Liong, M.T., 2010. Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. International Journal of Molecular Sciences 11, 2499-2522.
Othman, R.B., Sassi, M.B., Hammamia, S.B., Dziri, C., Zanina, Y., Salem, K.B., Jamoussi, H., 2025. Effect of Probiotics on Uric Acid Levels: Meta-Analysis with Subgroup Analysis and Meta-Regression. Nutrients 17, 2467.
Ouwehand, A.C., (2007). Antiallergic effects of probiotics1. The Journal of nutrition. 137, 794S-797S.
Pan, Y., Yang, Y., Wu, J., Zhou, H., Yang, C., 2024. Efficacy of probiotics, prebiotics, and synbiotics on liver enzymes, lipid profiles, and inflammation in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis of randomized controlled trials. BMC Gastroenterology 24, 283.
Park, S.S., Yang, G., Kim, E., 2017. Lactobacillus acidophilus NS1 reduces phosphoenolpyruvate carboxylase expression by regulating HNF4α transcriptional activity. Korean Journal for Food Science of Animal Resources 37, 529.
Patterson, S.M., Gottdiener, J.S., Hecht, G., Vargot, S., Krantz, D.S., 1993. Effects of acute mental stress on serum lipids: mediating effects of plasma volume. Biopsychosocial Science and Medicine 55, 525-532.
Pereira, D.I.A., Gibson, G.,R., 2002. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Applied and Environmental Microbiology 68, 4689-4693.
Quigley, E.M.M., 2011. Gut microbiota and the role of probiotics in therapy. Current Opinion in Pharmacology 11, 593-603.
Raun, K., von Voss, P., Gotfredsen, C.F., Golozoubova, V., Rolin, B., Knudsen, L.B., 2007. Liraglutide, a long-acting glucagon-like peptide-1 analog, reduces body weight and food intake in obese candy-fed rats, whereas a dipeptidyl peptidase-IV inhibitor, vildagliptin, does not. Diabetes 56, 8-15.
Scanes, C.G., 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poultry Science 95, 2208-2215.
Scholz-Ahrens, K.E., Ade, P., Marten, B., Weber, P., Timm, W., Asil, Y., Glüer, C.C., Schrezenmeir, J., 2007. Prebiotics, Probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure1. The Journal of Nutrition 137, 838S-846S.
Seghieri, M., Rebelos, E., Gastaldelli, A., Astiarraga, B.D., Casolaro, A., Barsotti, E., Pocai, A., Nauck, M., Muscelli, E., Ferrannini, E., 2013. Direct effect of GLP-1 infusion on endogenous glucose production in humans. Diabetologia 56, 156-161.
Shirkoohi, N.M., Mohammadi H., Gallaly, D.Q., Djafarian, K., 2025. The effects of probiotic supplementation on body composition, recovery following exercise-induced muscle damage, and exercise performance: A systematic review and meta-analysis of clinical trials. Physiological Reports 13, e70288.
Siddharth, M., Rokade, J.J., Bhanja, S.K., Tyagi, J.S., Monika, M., Pearlin, B.V., Kumar, A., Gopi, M., 2024. Transportation stress: Impact on behaviour and welfare in meat-type chickens under indian scenario. Heliyon 10.
Songisepp, E., Kullisaar, T., Hütt, P., Elias, P., Brilene, T., Zilmer, M., Mikelsaar, M., 2004. A new probiotic cheese with antioxidative and antimicrobial activity. Journal of Dairy Science 87, 2017-2023.
Sun, C., Liu, Q., Ye, X., Li, R., Meng, M., Han, X., 2024. The Role of Probiotics in Managing Glucose Homeostasis in Adults with Prediabetes: A Systematic Review and Meta-Analysis. Journal of Diabetes Research 2024, 5996218.
Tandon, R., Tandon, A., 2024. Unraveling the multifaceted role of glutathione in Sepsis: A comprehensive review.. Cureus 16.
Tang, L.P., Liu, Y.L., Zhang, J.X., Ding, K.N., Lu, M.H., He, Y.M., 2022. Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. Poultry Science 101, 102085.
Trafalska, E., Grzybowska, K., 2004. Probiotics--an alternative for antibiotics? Wiadomosci lekarskie (Warsaw, Poland: 1960) 57, 491-498.
Bello, U.A., Idrus, Z., Yong Meng, G., Awad, E.A., Soleimani Farjam, A., 2018. Gut microbiota and transportation stress response affected by tryptophan supplementation in broiler chickens. Italian Journal of Animal Science 17, 107-113.
Utomo, M.C.C., Sarjana, T.A., Suprijatna, E., Kismiati, S., Nasoetion, M.H., Atmomarsono, U., Windriasari, E., 2019. The influence of transportation distance during the dry season on broiler chickens' carcas traits. IOP Publishing.
Vecerek, V., Voslarova, E., Conte, F., Vecerkova, L., Bedanova, I., 2016. Negative trends in transport-related mortality rates in broiler chickens. Asian-Australasian Journal of Animal Sciences 29, 1796.
Vieira, F.M.C., Deniz, M., da Silva, I.J.O., Barbosa Filho, J.A.D., Vieira, A.M.C., Gonçalves, F.S., 2015. Pre-slaughter losses of broilers: effect of time period of the day and lairage time in a subtropical climate. Semina: Ciências Agrárias 36, 3887-3895.
Wang, D., Xu, R., Liu, S., Sun, X., Zhang, T., Shi, L., Wang, Y., 2025. Enhancing the application of probiotics in probiotic food products from the perspective of improving stress resistance by regulating cell physiological function: A review. Food Research International 199, 115369.
Weston, S., Halbert, A., Richmond, P., Prescott, S.L., 2005. Effects of probiotics on atopic dermatitis: a randomised controlled trial. Archives of Disease in Childhood 90, 892-897.
Willoughby, D.S., McFarlin, B., Bois, C., 2003. Interleukin-6 expression after repeated bouts of eccentric exercise. International Journal of Sports Medicine 24, 15-21.
Xu, X., Poulsen, K.L., Wu, L., ,S., T. Miyata, Song, Q., Wei, Q., Zhao, C., Lin, C., Yang, J., 2022. Targeted therapeutics and novel signaling pathways in non-alcohol-associated fatty liver/steatohepatitis (NAFL/NASH). Signal Transduction and Targeted Therapy 7, 287.
Yeo, S.K., Liong, M.T., 2010. Angiotensin I-converting enzyme inhibitory activity and bioconversion of isoflavones by probiotics in soymilk supplemented with prebiotics. International Journal of Food Sciences and Nutrition 61, 161-181.
Zarezadeh, M., Musazadeh, V., Ghalichi, F., Kavyani, Z., Nasernia, R., Parang, M., Jamilian, P., Jamilian, P., Fakhr, L., Ostadrahimi, A., 2023. Effects of probiotics supplementation on blood pressure: an umbrella meta-analysis of randomized controlled trials. Nutrition, Metabolism and Cardiovascular Diseases 33, 275-286.
Zhang, X., Jiang, J., Xin, J., Sun, N., Zhao, Z., Gan, B., Jiang, Gong, Y.X., Li, H., Ma, H., 2024. Preventive effect of Lactobacillus johnsonii YH1136 against uric acid accumulation and renal damages. Frontiers in Microbiology 15, 1364857.
Zulkifli, I., 2003. Effects of early age feed restriction and dietary ascorbic acid on heterophil/lymphocyte and tonic immobility reactions of transported broiler chickens. Asian-Australasian Journal of Animal Sciences 16, 1545-1549.