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Probiotic Effects on Behavior, Stress Indicators and Antioxidant Activity of Transported Broiler Chicken

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ABSTRACT

Transportation before slaughtering causes bad effects on chicken welfare and health, but little is known about the effect of probiotic dietary fed in broiler chickens having transport stress. So, this study was designed to detect the effects of two doses of a probiotic (Bacillus subtilis) on some stress indicators, antioxidant activity and fear response of broiler chickens exposed to transportation stress.

Two hundred and ten broiler chicks were distributed among 21 floor pens (10 chicks in each pen), each pen was exposed to one of three diet treatments containing a probiotic at 0 (control), 0.25 (0.25X), and 0.5 (0.5X) g/kg. At 35 days old, birds were exposed to transportation and then blood samples were collected for measuring stress and antioxidant markers.

The serum corticosterone and cholesterol levels were decreased in probiotic fed birds in comparison with controls ($P < 0.05$). Compared to control broiler chickens, 0.5X birds had higher levels of catalase and serum superoxide dismutase ($P < 0.05$). In addition, the 0.5X birds spent less time in the tonic immobility test ($P = 0.0427$). The results suggest that the probiotic supplement may prove to be an important management tool for the broiler industry to diminish the negative effects of transport stress, potentially safeguarding the welfare and health of broiler chickens.

1. INTRODUCTION

Transportation is a process associated with stressors which may decline welfare (Ghareeb and Böhm, 2009). Feed withdrawal, confinement and temperature fluctuations during transportation annoy the normal microflora (Jiang et al., 2020) which leads to increases susceptible pathogens as salmonella to bind and colonize in the epithelium of intestinal causing contamination to carcass during processing (Burkholder et al., 2008). Moreover, weight loss is a the most common problem, and it depends on the microclimate and fasting duration during transportation (Cockram and Dulal, 2018). In addition, birds may hurt from heat stress due to exposure to temperature above 30˚C from 4 weeks of age till marketing (Akşit et al., 2006) during summer season 30 to 36°C (Warriss et al., 2005; El-Deep et al., 2016).

So, the transportation effect not only stopped at slaughter, but affects muscle-meat conversion efficacy and meat quality parameters as texture, color that may affect consumer acceptability and processing functionality of the more processed products. That may cause more losses to the poultry industry (Schwartzkopf-Genswein et al., 2012). Also, transportation causes activation of hypothalamic-pituitary-adrenal axis (HPA) which alter gut microbial components in poultry, increasing animal sensitivity to pathogenic infection that may be due to enhanced corticosteroid hormones production suppressing the immune system (Ayoola et al., 2020).

One of the biggest causes of transportation economic loss is the alternation in blood component like decrease the level of glucose (Zhang et al., 2009; Ulupi et al., 2018), increase heart beats, hormone (corticosterone), metabolites enzymes, electrolytes, weight loss and death (Ghareeb and Böhm, 2009) as

it leads to 40 % of mortalities of broilers on arrival as a response to stress (Bayliss and Hinton, 1990). The variety effect of these factors differs according to the bird range from mild distress and aversion to severe injury and death.

Microbiota is normally established with the host in the animal intestine. Overall, microflora has two types in the digestive tract, beneficial and harmful bacteria. Commensals one is responsible for immune system stimulation, vitamin production and removal of pathogenic bacteria. The second type is harmful pathogenic type of bacteria causing infection, toxin production and intestinal disorders (Jeurissen et al., 2002).

Generally, in chicken gastrointestinal tract there are two kinds of microorganisms. First type is allochthonous bacteria or transitory microflora which is exogenous in nature and is added as a dietary complementary in feed or in drinking water as probiotic dietary supplement (Patterson and Burkholder, 2003). Second is autochthonous or established bacteria that found in the gut by inoculation resulting from normal feeding behaviour and surrounding environment of the bird (Gusils et al., 1999). Some research explored that allochthonous bacteria introduced by probiotics may inhibit the colonization and infection of pathogens microbes in the gastrointestinal tract (Fuller et al., 1998).

Probiotics used in daily nourishment in order to stimulate proliferation of beneficial microorganisms, rather than pathogenic bacteria (Ghasemi et al., 2020; Joya et al., 2020).Moreover, they lowered level of cholesterol (Ghasemi-Sadabadi et al., 2019; Shokaiyan et al., 2019), LDL (low density lipoprotein), triglyceride (Deraz, 2018; Joya et al., 2020), enhanced immune response (Yang and Sheu, 2012), improved carcass yield (Ghasemi-Sadabadi et al., 2019), and antioxidant activity (Bai et al., 2017) while decreased stress markers as corticosterone hormone in animals (Lei et al., 2013). However, conflicting findings have also been stated like probiotics have no significant effect on performance (Abudabos et al., 2013), carcass yield (Ramlucken et al., 2020), antioxidant activity, spleen weight (Hery et al., 2020), corticosterone level (Aluwong et al., 2013; Hery et al., 2020).

Probiotics effects depends upon many factors like, strain, dose, age of bird, administration method, ability of selected strain to survive at environmental temperature, viability, long term storage and this may be responsible for the differences in results (Hong et al., 2005; Aluwong et al., 2013). Further, previous studies didn't find changes in antioxidant status, glucose, cholesterol and fear response of broiler chickens after probiotic addition. So, the objective of this study was to determine the influences of dietary supplementation of a probiotic (Bacillus subtilis) on the stress indicators, antioxidant status, and fear response of Ross 708 broiler chickens exposed to transportation stress. We hypothesized that the dietary probiotic supplement would mitigate the deleterious effects of transportation, resulting in improved health status and fear response.

2. MATERIALS AND METHODS

2.1. Probiotic

A Probiotic (CLOSTAT™ HC SP Dry, Herentals, Kemin, Europe, NV, Belgium) was used in this study. It contained sodium bicarbonate and bacillus subtilis. CLOSTAT™ contained patented, unique spore-forming strain of bacillus subtilis PB6, that was isolated from chickens who had survived at high exposure to clostridium perfringens in the environment. PB6 attacked and killed pathogenic bacteria and clostridia, which may negatively affect the gastrointestinal tract (GIT). PB6 was able to survive in harsh conditions and compete with other ingredients, such as pelleting temperatures and acidity of the stomach. With multiple product delivery options, CLOSTAT™ was the industry leader in reducing intestinal pathogen colonization which leads to sickness in poultry (Teo and Tan, 2007).

2.2. Animals and Housing

All animal procedures and handling were approved by the Animal Care and use committee, Faculty of Veterinary Medicine, Assiut University, Egypt.

Two hundred and ten 1-day-old broiler male chicks (Ross 708 strain; El-wade, Assiut, Egypt) were weighed and allocated at 21 floor pens (100 cm × 100 cm floor pen each 10 birds) with similar average body weight in an environment-controlled room (The Animal and Poultry Behavior and Management Research Unit, Faculty of Veterinary Medicine, Assiut University, Egypt). Dry and fresh wood shaving as bedding was used at 10 cm depth. The bird management was according to the guidelines of (Aviagen, 2018).

Environmental temperature in the first week of life was 35°C then decreased to 26°C (0.5°C/d.) until the experiment end. Humidity and temperatures were measured twice per day using wall mount thermo-hygrometer fixed 30 cm above the litter surface (Mohammed et al., 2021a).

2.3. Experimental Design

Table (1): Components of base diet[1], separated by the growth phase[2].

Ingredient %	Starter (1-14d)	Grower (15-28d)	Finisher (29-42d)
Corn ground	57.66	63.76	66.9
Soybean meal 47.5%	35.27	29.68	26.3
Soybean oil degummed	3	3	3.52
Calcium carbonate	1.41	1.38	1.49
Phosphate monocalcium	1.42	1.02	0.82
L-Lysine	0.11	0.1	0.02
Salt plain	0.48	0.46	0.48
L-Threonine 98%	0.06	0.04	0
DL-Methionine	0.24	0.21	0.12
Poultry turkey starter	0.35	0.35	0.35
Calculated Analysis[3]			
Crude protein %	23.4	22.8	19.2
Poultry ME kcal/kg	3050	3151	3200
Calcium %	0.95	0.85	0.75
Available phosphorus %	0.50	0.44	0.36
Methionine %	0.66	0.59	0.53
Methionine+Cystine %	1.04	0.97	0.86
Lysine %	1.42	1.29	1.09
Threonine %	0.97	0.89	0.74
Na %	0.22	0.20	0.19

[1] The ration formulation was produced according to Aviagen (2018), and the treatments were the regular diets supplemented with 0 (control), 0.25 (0.25X), and 0.5 (0.5X) g kg^{-1}probiotic, respectively.

[2] The diets were formulated by El-salam food mill. (Assiut, Egypt)

[3]Provided per kilogram of diet: vitamin A, 13.233 IU; vitamin D3, 6.636 IU; vitamin E, 44.1 IU; vitamin K, 4.5 mg; thiamine, 2.21 mg; riboflavin, 6.6 mg; pantothenic acid, 24.3 mg; niacin, 88.2 mg; pyridoxine, 3.31 mg; folic acid, 1.10 mg; biotin, 0.33 mg; vitamin B12, 24.8 µg; choline, 669.8 mg; iron from ferrous sulfate, 50.1 mg; copper from copper sulfate, 7.7 mg; manganese from manganese oxide, 125.1 mg; zinc from zinc oxide, 125.1 mg; iodine from ethylene diamine dihydroidide, 2.10 mg; selenium from sodium selenite, 0.30 mg.

All precautions for dealing with laboratory animals were taken into consideration and the Ethics Committee of Assiut University (experiment No. 17200789). The 21 floor pens were randomly divided to 1 of 3 dietary treatments in 7 replicates of 10 broilers per replicate: a regular diet mixed with the probiotic, B. subtilis PB6 (CLOSTAT™; Europe, Kemin, NV; Herentals, Belgium) at 0 (control), 0.25 (0.25X), and 0.5 (0.5X) g/kg feed. The concentrations of CLOSTAT dietary treatments were used according to the company's recommendation. The dietary treatment was from day 1 to day 35 until they reached the market weight. A small amount of the basal diet was first mixed with the probiotic amounts, and then with a larger amount of the basal diet until the total amount of the respective diets were well homogenized. The birds were fed with starter diet from day 1 to 14, followed by grower diet from 15 to 28 days of age, and finally a finisher diet from 29 to 35 days. Water was provided in sterile clean drinkers at all pens (Mohammed et al., 2019). The treatments were started at day 1 of age (Table 1).

Transport stress: At 35 days old, 105 (35 birds per treatment) birds were subjected to catching, handling, crating in plastic boxes, loading and transported for a journey of 80 km (90 min approximately). After the birds had arrived at our laboratory, they were housed in battery cages (5 birds/cage) (28°C) and subjected to physiological and behavioural measurements.

2.4. Data collection

2.4.1. Behavioural test (Tonic immobility test):

At day 35 (after transport stress), a total of 42 birds (14 birds per group) tonic immobility duration were tested at the first day of arrival. TI was done in a different room having the same conditions as the bird room. The bird was situated on its back in a U-shaped cradle. The bird was then restrained on its sternum for 5 seconds with one hand, holding the head and neck by the other hand. Hand pressure was gradually removed so that if the chick still moved, another induction period was started, until the movement stopped. Stopwatch was beginning after removal of the hands pressure. The experimenter then left, moving out of sight of the bird. If the bird righted itself in less than 10 second, the restraining procedure was done again. TI duration was considered 0 s if TI was not induced after three trials, while the birds were removed from the cradle after 600 s if no attempt to right themselves was made (Mohammed et al., 2021b).

2.4.2. Blood samples collection:

Seven birds were taken from each group to investigate the stress indicators (corticosterone, glucose, and cholesterol) and antioxidant activity (super oxide dismutase and catalase enzymes). The birds were slaughtered according to the traditional Halal Islamic Method (Shahdan et al., 2016): cutting the jugular veins, bled for 120 second, and then semi-scalded at 54°C for 30 seconds. Blood samples were collected during the bird's exsanguinations as following:

Serum was collected by taken three mL of blood from each bird in anticoagulant test tube. The tubes were kept at the room temperature for 30 minutes then stored for 60-90 minutes at a refrigerator and then centrifuged 10 minutes for 3000 r.p.m and by using micropipette the serum was transferred to another Epindoorf`s tube and kept at -80º C, until analysis.

Serum corticosterone (ng/ml) was measured by using the commercial Assay Max ™ corticosterone ELISA kits (St. Charles, MO, United States).

Serum glucose (mg/dl), Cholesterol (mg/dl), superoxide dismutase (SOD) and catalase enzymes were performed by spectrophotometer, colorimetric method using a commercial kit manufactured by Egyptian company for biotechnology, Cairo, Egypt.

2.5. Statistical analysis:

The experimental design was performed in a randomized block design with the dietary supplement as the fixed effects and pen (n = 7/treatment) considered as the experimental unit. The data was analysed by one-way ANOVA (SAS Institute Inc., Cary, NC). The Tukey-Kramer test was used to compare the means when a significant difference was detected. Statically difference was set $P \leq 0.05$; and the results were reported as mean ± SEM.

3. RESULTS

The means of the tonic immobility test of transported and non-transported broiler chickens supplemented with probiotics are presented in Table 2. Compared to control broiler chickens, the 0.5X birds spent less time in the tonic immobility test (P = 0.0427). However, there were no significant changes in tonic immobility test duration in the non-transported birds (P = 0.9906).

The probiotic effects on serum corticosterone, glucose and cholesterol of broiler chickens exposed to transport stress are presented in Table 3. Serum corticosterone concentration was decreased in the dose 0.5X birds in comparison with control and other treatment (P = 0.0078). Also, serum cholesterol levels were decreased in the probiotic fed birds in comparison with control, the 0.5X birds had the highest decrease (P = 0.0001). However, the probiotic supplementation did not affect the serum glucose level regardless of its dose (P = 0.7369).

The probiotic effect on serum concentrations of SOD and catalase of broiler chickens exposed to transport stress are presented in Table 4. 0.5X birds had higher levels of catalase enzyme (P = 0.054) while the level of the serum SOD was increased in the probiotic fed birds in comparison with controls (P = 0.0265).

Table (2): Effect of a probiotic on tonic immobility test of transported and non-transported broiler chickens.

Treatment	Duration of non-transported broilers (per sec)	Duration of transported broilers (per sec)
Control	120.57	166.64^{b}
0.25X	127.00	142.34^{ab}
0.5X	117.71	37.02^{a}
SEM	**48.84**	**35.45**
P value	**0.9906**	**0.0427**

^{a,b} Means ± SEM in the same column with different superscripts differ significantly ($P < 0.05$).

Table (3): Effect of a probiotic on stress markers (serum corticosterone, glucose and cholesterol) of broiler chickens exposed to transport stress.

Treatment	Corticosterone (ng/ml)	Glucose (mg/dl)	Cholesterol (mg/dl)
Control	2.227^{a}	249^{a}	182.14^{a}
0.25X	2.175^{a}	257.3^{a}	169^{b}
0.5X	1.90^{b}	258.50^{a}	154.75^{c}
SEM	**0.069**	**9.297**	**2.897**
P value	**0.0078**	**0.7369**	**0.0001**

^{a,b,c} Means ± SEM in the same column with different superscripts differ significantly ($P < 0.05$).

Table (4): Effect of a probiotic on antioxidant status (serum superoxide dismutase and catalase) on broiler chickens exposed to transport stress.

Treatment	Superoxide dismutase (U/mg)	Catalase (U/mg)
Control	24.75^{b}	0.27^{b}
0.25X	33.10^{a}	0.28^{ab}
0.5X	33.35^{a}	0.29^{a}
SEM	**2.19**	**0.004**
P value	**0.0265**	**0.0542**

[a,b] Means ± SEM in the same column with different superscripts differ significantly ($P < 0.05$).

4. DISCUSSION

For every animal species, the intestinal microbiota functions as a fictitious endocrine organ that secretes a variety of bioactive compounds that are essential for the regulation of the brain's stress response and associated behavioural health. Many studies have recorded that there is a strong bond between animal social behaviors and gastrointestinal microbiota, as microbiota affects hosts behaviors (Jiang et al., 2020). Also, the intestinal microbiota has a critical role in immune-stimulants, antioxidant activity and regulating the metabolism in broiler chickens (Rehman et al., 2008). According to recent research, food additives like probiotics can improve immunological stimulation and prevent or reduce mental illnesses in chickens by regulating the microbiota-gut-brain axis (Abudabos et al., 2016; Cheng et al., 2019).

Preslaughter transport stress causes a major economic loss in broiler chickens and poultry enterprises affecting their health and welfare (Gou et al., 2021). Animals and poultry exposed to pre-slaughter transport stress show enhanced secretion of glucocorticoids and epinephrine (Arikan et al., 2017), which increase changes in the behavioral and physiological status of the bird's.

Intestinal bacteria are important for host metabolism, nourishment and immunity. The population of intestinal bacteria is affected by many factors, such as animal sex, age, diet, stressors and feed additives (Sohail et al., 2013). In normal healthy chickens, the intestinal bacteria stay stable, whereas environmental stress happened, as poor nutrition, overcrowding, infection, human handling, cold weather, extreme hot and transportation, weakens the normal intestinal profile (Sohail et al., 2013). The environmental stress has become a major topic of interest in animal agriculture industry, especially due to concerns and public awareness. The harmful effects of transport stress induce lipid peroxidation, endocrine disorders, suppressed immunity, microbial dysbiosis, intestinal damage and changes animal behaviour (Fluck et al., 1997; Burkholder et al., 2008; Sohail et al., 2010; Farag and Alagawany, 2018).

Corticosterone, glucose and cholesterol levels have been used as stress markers in many animals' species, including chickens (Puvadolpirod and Thaxton, 2000). In our findings, corticosterone concentration was decreased in the 0.5X birds in comparison with other groups exposed to transport stress. These results agree with Price et al. (2018) who recorded that, dietary supplementation of probiotic (yeast fermentation from Original XPC®) reduced blood corticosterone (stress indictor) in broiler chicken compared to the controls under heat stress conditions (35°C from 28 to 42 days). Similarly, Nelson et al. (2018) reported that, dietary probiotic (yeast fermentation from Original Avicare and XPC) supplementation to broiler chickens raised on reused litter, and under heat stress with water/feed withdrawal showed lowered corticosterone concentration compared with the control.

In contrast, Meimandipour et al. (2010) found that, addition of lactobacillus probiotics on broiler chicks feed significantly enhanced blood corticosterone level at 14 and 28 days of age compared with controls. While Cengiz et al. (2015) said that probiotic dietary addition (Primalac® contained a minimum of 109 cfu/g live Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium thermophiles and Enterococcus faecium) didn't show effect on corticosterone of broiler chickens blood raised under high stocking density (20 birds/m^2). This may be explained by probiotic bacteria's effect on microbiota and intestinal health. A safe and balanced microbial community can contribute to normalizing adrenal activity (Gareau et al., 2007; Sohail et al., 2012).

Puvadolpirod and Thaxton (2000) reported that triglycerides and cholesterol increased in broiler chicken under stress. In addition, Sahin et al. (2005) demonstrated that plasma glucose and cholesterol of growing Japanese quails were significantly increased as a response to heat stress (34°C for 8 hr. daily), in

comparison with the control group (22°C). In the current research, cholesterol levels were decreased in the probiotic fed birds in comparison with controls after transport stress with dose effect, 0.5X birds had the highest decrease. These findings agree with Sohail et al. (2010) who found a decreased cholesterol blood level in heat stressed broiler chickens supplied with dietary lactobacillus probiotic in comparison with that of the control.

Also, dietary supplementation of bacillus licheniformis and bacillus subtilis to broiler chickens exposed to feed restriction (5h/d.) decreased blood cholesterol levels (Abdel-Hafeez et al., 2017). Contrary to our results, Hussein et al. (2020) discovered that, broiler chickens fed with bacillus subtilis DSMZ (Gallipro®) or bacillus subtilis PB6 (Clostat™) and inoculated with clostridium perfringes had no change in blood triglyceride and cholesterol levels. The low serum cholesterol concentration in the probiotic-supplemented groups may be attributed to either lower cholesterol digestion or cortisol levels by lactobacilli bacteria (Buck and Gilliland, 1994).

Stress significantly raises blood glucose levels as a response to an increase in glucocorticoids, which leads to stimulating gluconeogenesis, like in the metabolism of muscular protein (Tawfeek et al., 2014). However, the current results clarified that probiotic supplementation did not change the glucose levels of broiler chickens after transport stress. Similarly, dietary supplementation of bacillus subtilis did not affect blood glucose levels in broilers (Abdel-Hafeez et al., 2017).

In contrast, Abdelqader et al. (2020) found that, dietary supplementation of bacillus subtilis had the ability to maintain blood glucose parameters in a pattern like thermoneutral chicken under thermal stress conditions (30°C). Also, Latipudin et al. (2018) discovered that, dietary supplementation of a probiotic lactobacillus acidophilus, lactobacillus plantarum, lactobacillus plantarum, lactobacillus acidophilus, trichosporon beigelii, Cryptococcus humicolus, cryptococcus humicolus, trichosporon beigelii) maintained broiler chickens blood glucose levels after transportation compared to controls.

Moreover, supplementation with 0.12 gm /kg of bacillus subtilis PB6 (Clostat™) as a probiotic in broilers feed and exposed to infection by clostridium perfringes showed significantly increased blood glucose level in comparison with the positive control birds (Hussein et al., 2020). Absence of the differences in the blood glucose level depended on the dose of the bacillus subtilis based probiotic that may be not enough to induce a significant effect on blood glucose level.

Chickens' tonic immobility (TI) is the most used behavioral test to detect fear in poultry (Soroko and Zaborski., 2020; Mohammed et al., 2021b). Broiler chickens subjected to stress showed enhanced tonic immobility duration (Bedanova et al., 2007). In this study, the 0.5X birds spent less time in the tonic immobility test in comparison with controls. Same results were demonstrated by Parois et al. (2017) who mentioned that probiotic (Pediococcus acidilactici) in the quail diet declined the immobility duration in comparison with the control.

In the other hand, Ghareeb et al. (2008) mentioned that dietary addition of lactobacillus sp. probiotic (1 x 10 cfu/kg) in broiler fed followed by transportation for 80 km journey (90 min approximately) didn't make any significant change on tonic immobility reactions (TI). In addition, Yildirim et al. (2020) demonstrated no effect on tonic immobility in quails suppled with P. acidilactici probiotics under the negative effect of 2% tryptophan. Declined tonic immobility duration by probiotic could be due to its role in increasing release and synthesis of many neurotransmitters, neuroactive factors and neuromodulators, by which microbiota indirectly and directly transmit signals to the brain, via the vagal afferents, the enteric nervous system, and/or the bloodstream. Among the neuroactive factors pathways, the central serotonergic system which plays an important role in emotional coordination in animals and humans, can be disrupted by fear stress. Various beneficial bacteria of probiotics can enhance release and synthesis of serotonin through tryptophan metabolism regulation in the GIT (Soroko and Zaborski., 2020; Yildirim et al., 2020; Mohammed et al., 2021b).

Stress stimulates ROS (reactive oxygen species) releasing by inhibiting the electron transport apparatus present in the mitochondrial membrane (Mujahid et al., 2005). This plays an important role in regulating transcription factors as Nrf 2 (Na et al., 2008) that coordinates the antioxidant status (Li and Kong, 2009; Nguyen et al., 2009), by controlling many antioxidants products expression such as catalase, superoxide dismutase and glutathione peroxidase.

In the current study, 0.5X birds had higher levels of serum catalase enzyme in comparison with controls and 0.25X birds exposed to transport stress at d 35. While the level of SOD was increased in the 0.5X birds and controls in comparison with 0.25X birds under transport stress at 35 d of age. These findings supported the findings of Gong et al. (2018) who demonstrated that dietary fed with probiotic (Bacillus subtilis Bacillus licheniformis or natto or Bacillus cereus, 108 cfu/kg) increased hepatic

superoxide dismutase and catalase in broiler chickens raised under normal situation.

Otherwise, Cramer et al. (2018) found the addition of 250 ppm of Sporulin® as probiotic supplementation, which contains 3 bacillus subtilis strains, can stimulate oxidative deterioration in broilers exposed to heat stress (32°C for 10 h/day), as bacillus subtilis based probiotic had the ability to enhance glutathione peroxidase (GPx) and SOD activity when compared to the heat stressed control group, but did not affect the catalase activity.

In contrast, Abudabos et al. (2016) said that Clostat™ (Bacillus subtilis PB6) supplementation as a probiotic in broilers infected with salmonella Typhimurium had no significant effect on serum hydrogen peroxide, superoxide dismutase and total antioxidant capacity.

The beneficial effects of probiotics on antioxidant activity returned to their ability to inhibit excessive ROS release and lipid peroxidation by increasing antioxidant capacity in broilers (Aluwong et al., 2013; Abudabos, et al., 2016; Bai et al., 2018). Through inducing antioxidant defense system enzymes that detoxify and produce singlet oxygen (O2) and free radicals, they also activate and translocate nuclear factors to overcome oxidative stress (Seifried et al., 2007). To the best of our knowledge, the present study is the first attempt to unravel SOD and catalase activity in response to dietary addition with probiotics in broiler chickens to cope with transportation stress. The current research approved that the dietary probiotic supplement improves fear response, related stress response and antioxidant status of broiler chickens exposed to transportation stress.

5. CONCLUSION

In the current study, addition of probiotic declined broiler fear response that indicated by tonic immobility test outcomes. In addition, the probiotic effects on fearfulness were recorded by reduced stress markers like serum corticosterone and cholesterol levels and improved antioxidant status like catalase and superoxide dismutase. Overall, our findings indicate that dietary probiotic supplementation may be a useful tool for changing harmful effects of pre slaughter transportation on broiler chickens.

Conflict of interest:

The authors declare no conflict of interest

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