PROBIOTIC SUPPLEMENTATION IMPROVES BEHAVIOUR, BODY GROWTH AND FEATHER CONDITION IN "BROILER CHICKEN" WHEN SUBJECTED TO HEAT STRESS

Ahmed A. Mohammed, Madeha H. Ahmed and Fatma A. Mahmoud[1*]
Department of Animal and Poultry Behavior and Management, Faculty of Veterinary Medicine
Assiut University -71526, Egypt

Received on: 17.04.2021
Accepted on: 05.06.2021

ABSTRACT

This study assessed the implication of dietary probiotic supplementation over body growth, behavior, and feather condition of the broiler chicken when subjected to heat stress. A study has been conducted where a 384 male broiler chicken (Ross-708) of one-day older has been considered and assigned one dietary treatment among the three other treatments (eight replicates of 16 birds/ treatment) involving a probiotic on the condition of 0.5 g kg^{-1} (Highest), 0.25 g kg^{-1} (Lowest), and 0 (Control) of the basal diet. There is a concept of thermal stress which has come into the picture everyday (the time is 08:00 to 17:00 and the temperature is 32°C) on days 15 to 42. The impact of the treatment on the account of behavioral activities is continuously monitored by conducting the direct examination and sampling of the immediate scan. Following behavioral examination, the birds' feather was scored (1 to 5). Categorically, the all 384 birds which are under examination have found some abnormality in the feather of the individuals. Probiotics reduced heat stress in broilers by improving preening behavior. At experimental doses, the score of the feather was not satisfactory for probiotics (P is greater than 0.05), but primary feather abnormalities were reduced. The probiotic group also had higher body weights on day 42 (P<0.05). The efficiency factor of European production and the European broiler index has been elevated at the time of analysis as was the unit return index at the end of the study (P is less than 0.05). The correspondence weights of the liver and spleen in the three dietary treatment groups were significantly different on the day of 42. The supplement of probiotics is a useful management tool for diminishing the drastic effects of heat exhibits in broiler industries, potentially supporting broiler chickens' welfare and performance, particularly in hot climates.

Key words: Broilers, probiotic, heat stress, behavior, feather condition, growth performance

Introduction

The poultry industry is growing rapidly to provide food security and meeting nutritional needs. In the hot regions of the world, improved performance increases heat stress (HS) and impacts broiler performance. For broilers, the best and efficient temperature would be 18-22°C when it comes to the thermoneutral or performance area (Charles, 2002). Ambient temperatures outside of the thermoneutral zone may result in HS, which for poultry is an often times detailed natural stressor (Lara and Rostagno, 2013). This is the only reason where the region of subtropical and tropical is the most suitable for nurturing and raising the poultry production and seasonal effects under moderate climatic conditions (Lin *et al*., 2006). The broiler chickens which are mainly for the purpose of meat are more inclined to the HS due to the selection for quick growth and the increased conversion of feed (Soleimani *et al*., 2011). HS is more focused when it comes to lower growth rate, an impaired immune system, behavioral changes, and reduced welfare (Mack *et al*., 2013). Reducing feed efficiency and broiler growth is among the most significant aspects which are interlinked to the economic deprivation on the ground of HS. There are numerous studies which has been shown the interlinked and dependency between the HS and growth. According to Baziz *et al*. (1996), every 1ºC rise in ambient temperature reduced feed consumption by 3.6%. Sohail *et al*. (2012) at the time of production phase ending documented a reduced weight of broiler chicken (32.6 per cent) with an improved rate of feed conversion (+ 25.6%). Broiler chickens modify their behavior for aligning the body heat to the level of thermoneutral which can alleviate HS. The intake of the feed is decreased when it comes to the time of "HS conditions", and in a different context the behavior rrelevant to wing elevation, sitting, and panting are increased to dispel the overloaded heat (Mack *et al*., 2013; Sohail *et al*., 2013). "Wing spreading", *i.e*. raising wings outwards prompts heat transmission to the external climate (Gerken *et al*., 2006). Such behavioral change proves that HS-exposed birds feel anxious; thereby showing that it adversely affects their welfare. HS stimulates the "hypothalamic-pituitary-adrenal axis (HPA)", and along with that it also damaging effects over the performance of broiler and the activities related to the behavior. HS alters the microbial community when it comes to the poultry gut (Yu *et al*., 2012), leading to the expansion of harmful pathogens, namely *Escherichia coli* (*E. coli*) and *Salmonella spp.* (Barekatain *et al*., 2020). In addition to that the mortality risk will also increase (Quinteiro-Filho *et al*., 2012). The probiotics can be referred as the living microbils which have a beneficial effect on the host by stabilizing intestinal flora (Fuller, 1989). The efficiency of feed and increased growth in the broiler chickens has been assorted after the supplementation of nutritional probiotic (Marimuthu *et al*., 2020), despite the prevailing of conflict result (Sugiharto *et al*., 2017; Sohail *et al*., 2015). The host benefits from prebiotics, which helps the colon bacteria to enhance quickly so that they can increase the effectiveness (Healey *et al*., 2017). Probiotics strengthen lactobacillus implantation and durability in the area of the intestinal (Ray *et al*., 2020). It regulates the biological and behavioral operations in the pathways of local and systematic which mean the axis of

[1*]Corresponding author present address: Department of Animal Behavior and Husbandry, Faculty of Veterinary Medicine, Sohag University, 8524, Egypt.

"microbiota-gut-brain" (Gareau, 2014) and the axis of "microbiota-gut-immune" (Rooks and Garrett, 2016). In addition, supplementing broilers with anti-stressors in their diet leads to a modulation in feather scores and abnormal locations of primary feathers. Birds can try losing more heat during heat stress by convection through the exposure of more areas of unfeathered skin (Fathi *et al*., 2017). The alternatives in the concentration of probiotics and dosage are responsible for the conflicting findings. Therefore, this study investigated that the influence of the supplementation of dietary probiotic (2 dosages of *Bacillus subtilis*) when it comes to the behavioral description, performance, and the feather condition as well of Ross 708, heat-stressed chickens (broiler). The probiotic was hypothesized to weaken the impacts over theheat-stressed broilers.

Materials and Methods

The all methodologies that has been utilized for this specific study has been accepted by the "Animal Care and Use Committee of the Faculty of Veterinary Medicine, Assiut University", that located in Assiut (Egypt).

Probiotics

Sporulin® is a well-defined, poultry-specific, probiotic product that combines three isolated live spores of *B. subtilis* strains to promote beneficial gut microflora (AM0904, NP122, and B2; Sporulin®, Novus International Inc.).

Animals and Housing

In all, 384 male broiler chicks among the strain of Ross 708 were weighed and assigned to one of the 24-floor pens (100 ×100 centimeter/pen) along with the same average weight within a room which is fully temperature controlled. In this room, the chicks are maintained at the temperature of 34°C till day 7 and they are maintained at the temperature of 30°C when it comes to day 8 to 14, and accordingly the treatment of HS has been delivered. It was established at the temperature of 32°C for continuously 9 hours (08:00 A.M –17:00 P.M) in everyday when the experiment period was going on at the "Animal and Poultry Behavior and Management Research Unit, Faculty of Veterinary Medicine, Assiut University". The concerned management of the broilers has maintained the guidelines of Ross Company. Humeral lighting schedule of the treatment was in the stabilize form at "30 lux for 23L: 1D" up to 3 days, then 10 lux up to 42 days as long as 20L:4D (Mahmoud *et al*., 2015).

Dietary treatments

Twenty-four pens were assigned into the 3 nutritional treatments randomly along with 8 replicas (16 birds/pen). The teams are assigned with the several experiments which are enclosed here in this segment -

(Control), basal diet only; basal diet +0.25 gkg^{-1} probiotics (Lowest); basal diet + 0.5 gkg^{-1} probiotics (Highest). The respective Sporulin® amounts were combined with the basal diets homogeneously. The birds were allowed to eat a "starter diet" when they are at the age of 1 to 14 days, the "grower diet" has given to the birds who belong to the domain of 15-28 days, and in the last phase, the finisher diet has been given to the age of 29-42 days. Table 1 demonstrates the structure and composition of the commercial broiler basal diets (Mohammed *et al*., 2018). The "plastic water" controller which is economically available that is associated with the automotive water line was attached which helps to sustain the pens by supplying the water *ad libitum* (Mohammed *et al*., 2018).

Behavioral observations

Six broiler chickens were randomly selected per pen (48 in total per treatment) and labelled with a blue livestock marker on the back. The birds' preening behavior was observed (slightly pecking or scraping of its own feather) by an instantaneous scanning method (Altmann, 1974) in the time interval of two time on everyday basis 10:00 and 11:00, and between 14:00 and 15:00, three times a week between 15 and 42 days of age. In the equivalent day, data were assorted for behavior when it comes to gathering the data and information related to production that helps to prevent normal activity disturbance. The focal broiler chickens were observed at 1.5 m from outside the pen to prevent a disturbance of normal behavioral variety. The information has seen as a segment of "observed behaviors" in the context of total possible numbers (Kristensen *et al*., 2007).

Feather condition

Feather score

Once 42 days of the experiment had been completed, approximately 5 birds were given the feather score for the back, wing, breast, and tail which get the rank of 1 to five (Lai *et al*., 2010).

The position of primary feather (unstructured shape of the wing)

After completing the one week on the experiment, it was noticed that there is a shift in the context of a primary feather direction. This is happened due to the metacarpus and phalanx axisis perpendicular to the vertical axis.

Numerous chickens have been found with the unstructured wing and that is also counted in Fig 1. This kind of abnormality in the birds' feather looks like "helicopter flanges" due to syndrome of malabsorption, an infectious disease marked by retarded growth and scarcity of skin pigmentation that has observed in broilers chicken whose age is under 1 to 3 weeks (Rebel, 2006). The feather abnormality was further investigated at 42 days of age in all birds.

The percentage of abnormal feather positions per pen was calculated as:

$$\text{Abnormal feather positions (\%—pen)} = \frac{\text{Number of birds with abnormal feather position/pen}}{\text{Total number of birds/pen}}$$

Growth performance

The performance parameters evaluated were weight of body, the "average daily weight-gain", "feed conversion ratio", "mortality", and "economic growth efficiency" for calculating the "European Production Efficiency Factor (EPEF)" and European Broiler Index (EBI)" (Van *et al*., 2003). At the end of each week of the "initial stage (1-14 days)", the grower stage (15-28 days), and the finishing stage (29-42 days), we calculated the body weight, "body weight gain (BWG)", "feed conversion ratio (FCR)", "the average daily gain (ADG)", and intake of feed. The rate of mortality has recorded on a daily basis and the viability percentage was

calculated using the data obtained. The following formulae were used for the analysis of performance indicators:
"BWG (gperperiod)" = "BW (g) at the time of period ending - BW (g) of the first day"; (2)
"ADG (g/chick/d)" = "BWG/number of days in the period of growth"; (3)
"FCR (kg feed/kg gain)" = "cumulative feed intake (kg)/total gain of the weight (kg)"; (4)
"Viability (%)" = "the chicks are alive till the end of experiment duration (%)"; (5)

$$\text{European broiler index} = \frac{\text{Vability \% x ADG}}{\text{FCR x 10}}$$ (Van *et al*., 2003) (6)

$$\text{European production efficiency factor} = \frac{\text{Body Weight (in kg)xViability}}{\text{Age (day) x FCR}}$$ (Van *et al*., 2003) (7)

Unit Return Index (Samarakoon and Samarasingle, 2012):
URI = [(ABW x SD x L x PLW) – (AFC x FCR x ABW) – (MPC) – (PPC)]/Age in days, (8)

Where ABW = average body weight (kg), AFC = average feed cost (Rs/kg), SD = the stocking density (kg/m^2), FCR= the feed conversion ratio, L = livability (%), MPC = medication cost per bird (Rs), PLW = price of live weight (Rs/kg), PPC = price per chick (Rs). The Unit Return Index was calculated after the accomplishment of 42nd day in the experimental period.

Internal organs

In 42nd day, two birds were chosen at random from each replication and then euthanized by cervical dislocation (16 birds/ treatment). The liver and spleen were collected and weighed separately, and then calculated as a percentage of the chicks' total body weight (Blasco *et al*., 2010).

Statistical analysis

The program of "Statistical Analysis" Version 9.1 (SAS Ins., Cary, NC, United States of America) was appointed to analyze the data. Individual broilers were considered an experimental unit in analyzing blood parameters. Pens were considered for the analysis of behavior and growth performance. The data have been reported as mean ± standard error (SE).

Results

The impact of probiotic supplementation on the behavior of broiler chicken

Fig. 2 illustrates the correlation between the probiotic treatment and behavioral movement. The probiotics treated birds in both cases which include 0.25 g kg^{-1} (lowest), and 0.5 g kg^{-1} (highest) probiotic concentrations, the percentage of preening activities was highest (P<0.05) contrasted with the control group.

The condition of feather

The implication of multiple concentrations of probiotic on the broiler chick feather score raised under HS is shown in Fig. 3. The probiotic supplementation had a massive influence on the thigh and tail feather coverage in both high and low probiotic-supplemented groups in contrast to control (P<0.05). The percentage feather

Table 1: Basal diet elements,[1] in the three segregated growth phases.

Ingredients (%)	Basal diets		
	Starter period (1-14 days)	Grower period (15-28 days)	Finisher period (29-42 days)
Corn	52.0	52.3	62.8
Soybean meal,48 % CP	40.0	39.1	29.7
Soy oil	3.59	4.97	4.11
Sodium chloride	0.51	0.46	0.43
DL Methionine	0.30	0.24	0.23
L-Lysine HCL	0.13	- - -	0.07
Threonine	0.06	- - -	- - -
Limestone	1.29	1.15	1.12
Monocalcium phosphate	1.75	1.48	1.17
Vitamin/mineralpremix[2]	0.35	0.35	0.35
Calculated analyses			
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 Mohammed *et al*. (2018)

[2]Provided the amount in diet (per kilogram): 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 ìg; 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 diaminedihydroidide- 2.10 mg; Selenium from sodium selenite- 0.30 mg.

coverage on the wings and back was not affected (P>0.05).

Poultry diagnostic laboratory results made it clear that the six birds examined were free from any disease. Moreover, an effect of probiotic supplementation was found on the abnormal wing shape of chicks reared under high temperature (Fig. 4). The outcome of this experiment directed that the percentage of abnormal wing shapes has decreased by probiotic treatment (P<0.05). The group of control reported the highest percentage of primary feather abnormalities, on the other side, the lowest percentage was captured and the lowest probiotic treated broilers.

Growth achievements of the broiler chicken

There are some differences in body weight across all treatments after ending the experimental activities (P<0.05) (Fig. 5). The data and information have gleaned (Average Daily Gain, Body Weight and "Feed Conversion Ratio" along with the livability) were assimilated to calculate the "European Broiler Index (EBI)", and the "European Production Efficiency Factor (EPEF)" that is mentioned in Fig. 6. Higher statistic for "EBI" and "EPEF" are recorded in the probiotic-treated broilers (Low and High) in the first phase (1-7days), and the second phase (7-14 days) of the starter period (P<0.05), while values for chickens in the grower phase (15-28 days) were similar (P is greater than 0.05). Differences in the two probiotic-supplemented groups and the control were significant for EBI and EPEF (P<0.05) in the finisher phase (28-42 d). The Unit Return Index for the high probiotic treatment was higher than the low probiotic treatment and control group (P<0.05) (Fig. 7). BW positively affected the assessment of economic efficiency in the EPEF and EBI formulae. There are higher values in both indices EBI and EPEF in the probiotic treated groups indicating better performance than the control. In other countries of the world, EPEF is appointed as an entity for evaluating growth performance in broilers (Van *et al*. 2003). The factors that include BWG, FCR, and livability are considered prevalent steps for evaluating the broiler's performance in the context of EPEF.

Carcass characteristics

The implication of probiotic dietary supplementation on certain carcass traits just after the end of the experimental period has been shown in Table 2. Results indicate that the major positive changes in the liver and spleen were improved (P is less than 0.05) when it comes to the "highest probiotic treatment" than the "lowest probiotic and control one".

Discussion

The implication of "probiotic supplementation" on the context of broilers behavior

The broiler chickens demonstrate various behaviors to enhance thermoregulation and decrease the HS effects, including an elevated respiratory percentage, the spread of wing, and open-beak-breathing (panting) (Lin *et al*., 2015). The final result portrays that in contrast to the implication of a daily control diet, probiotics supplementation has elevated preening activities of broiler chickens under HS. In support, Aguihe *et al*. (2020) stated that the increase in preening activities at the time of nourishment with probiotics during high stocking density stress. In poultry, preening is referred to as the actions which are subjected to comfort (Hinde, 1970), and probiotics decreased HS-associated discomfort. The behavioral implication in chickens (broiler) when fed probiotics revealed that they experience less stress when it comes to the exertion of higher temperature as compared with one having regular diet. Probiotics assist dispel heat through the skin. Further research will investigate these relationships to determine how probiotics mitigate multiple effects of HS.

The implication of probiotic supplementation of broilers in the context of feather form

Feathers play an impending role in thermoregulation; their insulating properties impair heat dissipation. Heat-stressed birds can radiate off excess heatof the entire body for keeping the "standard thermal-homeostasis" (Lai *et al*., 2010). In this study, the feather score did not differ between the "probiotic treated groups" and the "control group" in the feathers of the wings and back but showed a significant difference in the tail and thigh feather scores. Feathering scores are favored, mainly around the tails and thighs, because of some additional supply of nutrition through probiotic supplementation (Moreira *et al*., 2006).

High doses of probiotic supplementation reduced primary feather abnormalities compared with the low probiotic treatment and control birds. Our findings indicate that the upright position of the primary feathers was intended to expose more un-feathered skin area, which helps to allow the bird to lose more heat through convection. Pathological diagnoses confirmed this suggestion, as there were no pathological changes in the postmortem examination (Mahmoud *et al*., 2015).

The implication of probiotic supplements on the growth achievement of broiler chicken

Under HS, birds decrease the eating habit along with movement that means for reducing body heat production (Sohail *et al*., 2013). This decreases metabolism, has an uprising on the ground of "BW" and "BW gain", and in addition to that, it also increases the "conversion ratio" of feed (Sohail *et al*., 2012). The result of this experiment has disclosed that the probiotics would help to increase the production because the BW in the probiotic-supplemented birds was higher than birds eating the basal diet under HS conditions at 42 days. Probiotic supplementation in broiler rations increases feed intake and enhances intestinal absorption and disease resistance by improving digestion and nutrient absorption by boosting the numerous bacteria inside the intestine. Similar to our results, Jahromi *et al*. (2015) determines that the appointed production improvementin chickens are adherent with the synbiotics or the probiotics when the condition is under major stress. This is despite the fact that numerous scholars have declared that there is no change when it comes to production under the extreme heat condition (Sohail *et al*., 2012; 2013), or the condition of thermoneutral (Salehimanes *et al*., 2016). There are some variations when it comes to implication of probiotics and synbiotics in the previous studies that identified the differences in the bacterial stain and their strength in the context of diet, as concluded by Ajuwon (2016). These findings support increased preening and elevated wing position in probiotic-fed birds; along with increased body weight, this indicates that probiotics enhance the broiler's capacity to withstand the effects of HS (Mahmoud *et al*., 2015).

Table 2: The implication of various level of probiotic over the relative weight of broiler chicken internal organs when subjected to heat stress.

Treatment Organs	Dietary treatment			
	Control	Low	High	P-value
Liver	43.62±2.06^{b}	45.21±2.06^{b}	54.26±2.06^{a}	0.003
Spleen	1.98±2.06^{b}	2.10±2.06^{b}	2.68±2.06^{a}	0.0008

The data are demonstrated as the "means ± standard error of the mean (SEM)". Means inside a same row where no comparable "superscripts" differ extensively ($p<0.05$).

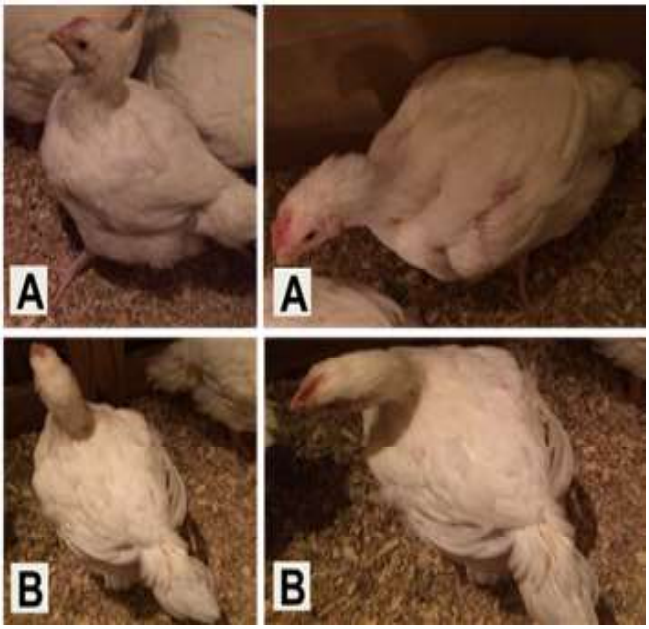

Fig. 1: Photos of broiler chicken exhibiting abnormal feather behavior (helicopter wings).
Based on the figure above, photo (A) represents the normal wing and (B) is an illustration of an abnormal wing.

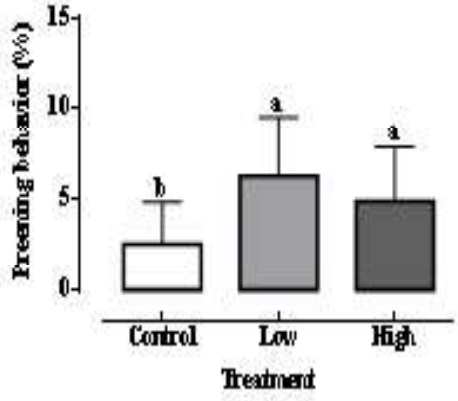

Fig. 2: The implication of dietary probiotic concentrations on preening behavior (%) of the chickens (broilers) when subjected to heat stress. In this segment data represent the "means ± standard error (SE)". The means having "dissimilar superscripts" differ significantly (P is less than 0.05).

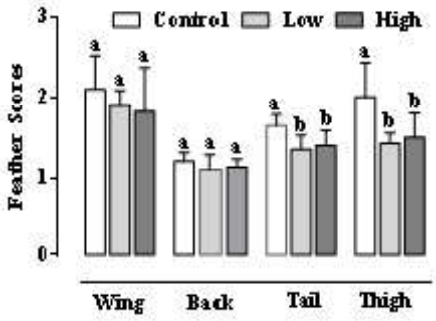

Fig. 3: Feather scores of chicken (broiler) in heat stress with or without supplementation of dietary probiotics. The data address the means ± standard error (SE). Means with dissimilar superscripts differ significantly ($P<0.05$).

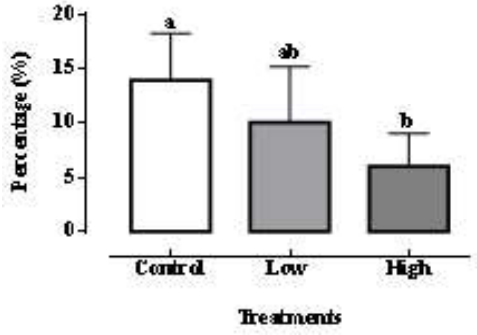

Fig. 4: The percentage of broiler chickens showing abnormal feather behavior (helicopter wings) was different in the groups supplemented with dietary probiotics. The data represent the means ± standard error (SE). Means with dissimilar superscripts differ significantly ($P<0.05$).

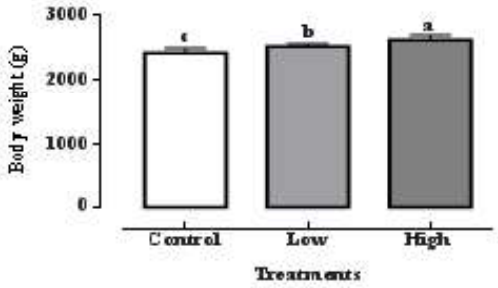

Fig. 5: Shows the implication of dietary probiotic concentrations over the body weight (g) of the broiler (chicken)under heat stress at 42 days of age. The data represent the means ± standard error (SE). The Means with dissimilar superscripts vary significantly ($P<0.05$).

The growth economic efficiency was evaluated by calculating the "European production Efficiency Factor (EPEF)" and the "European Broiler Index (EBI)" (Van, 2003). The rising of the numbers of the two indices (EBI and EPEF) in probiotic treated broilers (at 7, 14, and 42 d), can be attributed to significant differences in BW and ADG (Van, 2003), indicating that performance is better in probiotic treated birds. The "EPEF" is naturally utilizedin various countries as a tool for measuring a growth performance of broiler chickens (Van, 2003). The factors concentrated in the EPEF (BWG, FCR, and livability) are referred to as the Universal process for evaluating the broiler performance. The results were consistent Kokoszyñski *et al*. (2019), who noted that supplementation with probiotics had a

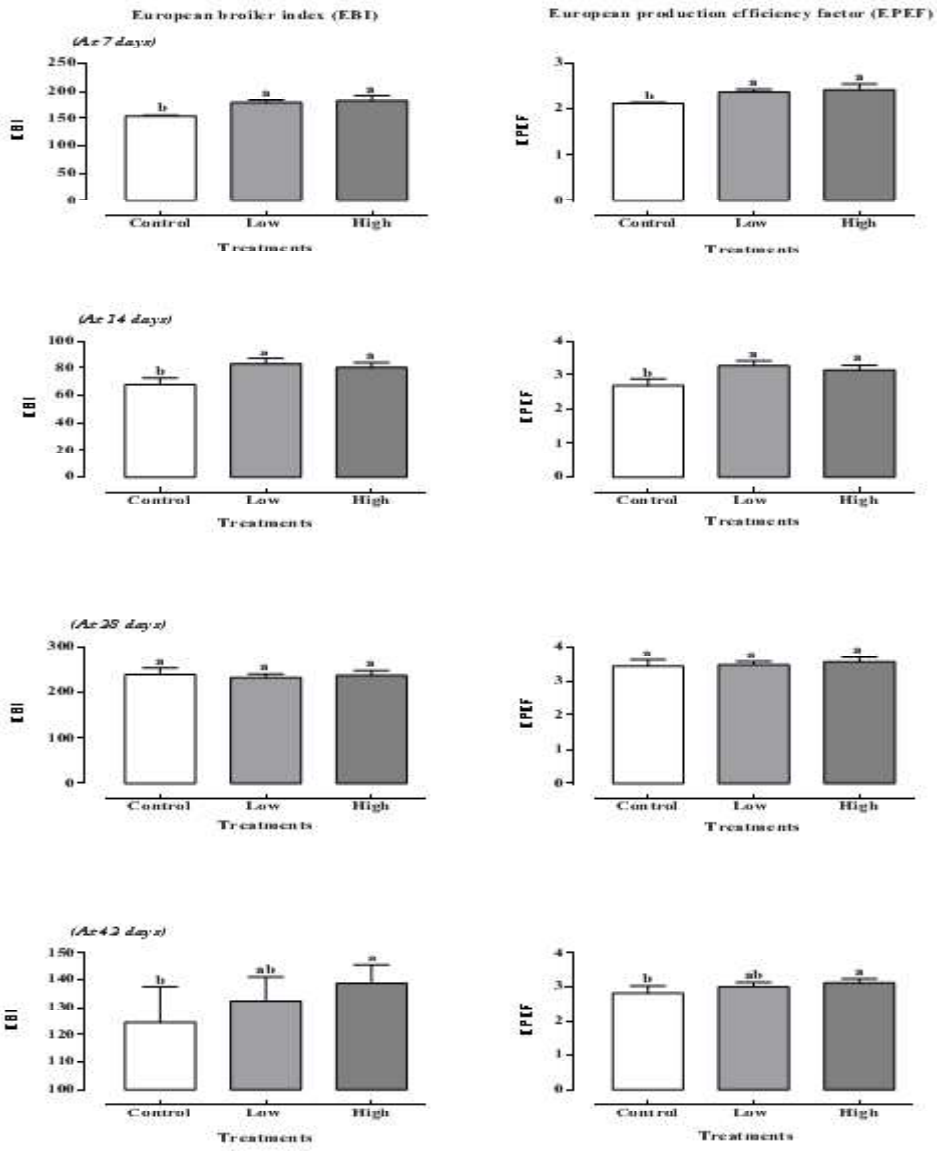

Fig. 6: Showing the implication of nutritional probiotic concentrations in the performance framework ("European Broiler Index" &"European Production Efficiency Factor") of chicken (broiler)on the circumstances of heat stress. The data represent the means ± standard error (SE). The means with the various superscripts varied significantly (P<0.05).

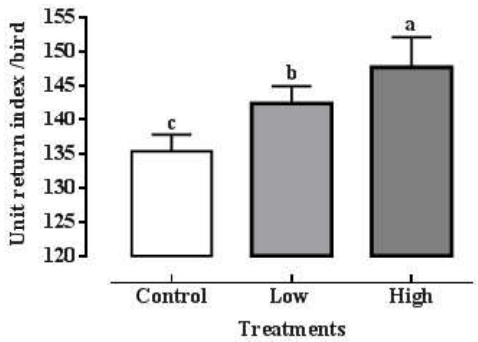

Fig. 7: Show the consequence of dietary probiotic concentrations on performance criterion (Unit return index; Samarakoon and Samarasinghe, 2012) of broiler chicken under heat stress. The data represent the means ± standard error (SE). Means with dissimilar superscripts differed significantly (P<0.05).

tremendous impact on the performance of broilers, including the EPEF. In contrast, Jørgensen *et al*. (2020) confirmed that feeding broiler chickens with probiotics had a negligible impact on the economic performance of the broiler, including the EBI.

The unit return index (URI) provides an overall idea of technical performance and maximizes the use of the floor in the houses for the purpose of broiler activities. Thereby, the URI has asserted the evaluation of the optimum period of growth and incentive payments. This research has presented the crucial data which can increase in URI for the high probiotic treated birds compared to the low probiotic and control treatments, which concurs with Stêczny and Kokoszyñski (2020), who demonstrated that a good yield and higher return from the probiotic-supplemented diet could contribute to a higher poultry return. This could be due to the high-probiotic treatment significantly increasing average body weight compared with the low-probiotic treatment and control groups.

The implication of probiotic supplementation on carcass traits

Feeding probiotics with the specifications of 0.5 g kg^{-1} (Highest) and 0.25 g kg^{-1} (Lowest) of broiler chicken in the present study significantly affect relative weights of spleen and liver in their age of 42nd day, possibly because of the probiotic effects on improvement of health status. Supporting this suggestion, Smialek, Burchardt and Koncicki (2018) demonstrated significant effects on some carcass traits (such as liver weight) by introducing probiotics into the broiler diet. Furthermore, different levels of antibody output and increased immunity are responsible for the main impact of probiotics on spleen weight (Agboola *et al*., 2016). He *et al*. (2020) reported that probiotic administration did not affect considerably the relative mass of the spleen and liver in broilers.

Conclusions

In the current experiment, probiotics had an obvious effect on improving broiler behavioral patterns following HS. Simultaneously, probiotic supplementation improved feather condition and reduced primary feather abnormalities in broilers. Overall, the findings indicate that supplementing the broiler diet with probiotics helps improve production profiles under HS conditions. Furthermore, after accomplishment of the broiler cycle, probiotic supplementation results in higher increases in BW. The performance obtained in probiotic-fed birds was better than in the control group, as shown by the predominant of two indices named EBI and EPEF. Dietary probiotics at a level of 0.5g kg^{-1} are recommended as an alternative growth promoter to antibiotic and along with the antioxidant accompaniment in the context of diets for the broilers to reduce dramatically the stress created from the heat.

Acknowledgments

We, fortunately, perceive the entire researchers and the staff members belong to "Animal and Poultry Behavior and Management", and the members of "Faculty of Veterinary medicine" in the university of Assiut. Furthermore, we want to thank every student of the graduate level at Assiut University, who has provided their benefaction extensively all through the examination's advancement and information gathering.

Reference

Agboola A, Omidiwura B and Iyayi E (2016) Influence of supplemental levels of probiotic on growth response, intestinal microbiota and carcass characteristics of broilers. *Am. J. Expt. Agr.* **12**:1-7.https://doi.org/10.9734/AJEA/2016/25082

Aguihe P, Kehinde A, Halidu S and *et al.* (2020) Effect of multienzymes probiotic supplementation in fermented shea butter cake based diets on the carcass traits and the biochemistry of broiler chickens. *Agri. Sci. Technol*. **12**: 227-233. http://dx.doi.org//10.15547/ast.2020.03.

Ajuwon K (2016) Toward a better understanding of mechanisms of probiotics and prebiotics action in poultry species. J. Applied Poult. Res. **25**: 277-283. https://doi.org/10.3382/japr/pfv074

Altmann J (1974) Observational study of behavior: Sampling methods. *Behaviour*. **49**: 227-266. https://doi.org/10.1163/156853974X00534

Barekatain R, Howarth GS, Willson NL, Cadogan D and Wilkinson S (2020) Excreta biomarkers in response to different gut barrier dysfunction models and probiotic supplementation in broiler chickens. *Plos One.* **15**(8): e0237505. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0237505.

Baziz HA, Geraert P, Padilha J and Guillaumin S (1996) Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. *Poult. Sci.* **75**: 505-513. https://doi.org/10.3382/ps.0750505.

Blasco A, Ouhayoun B and Masoero G (2010) Harmonization of criteria and terminology in rabbit meat research. Revised Proposal. *World Rabbit Sci*. http://doi:org/10.4995/wrs.1996.278.

Charles DR (2002) Responses to the thermal environment. In: poultry environment problems, a guide to solutions (Charles, D.A. and Walker, A.W. Eds.), Nottingham University Press, Nottingham, United Kingdom. pp. 1-16.

Fathi, M. M., Ebeid, T. A., Al-Homidan, I., Soliman, N. K., & Abou-Emera, O. K. (2017) Influence of probiotic supplementation on immune response in broilers raised under hot climate. *British Poult. Sci.* **58**(5): 512-516.

Fuller R (1989) Probiotic in man and animal. *J. Appl. Bacteriol*. **66**: 365-378. https://doi.org/10.1111/j.1365-2672.1989.tb05105.x

Gareau MG (2014) Microbiota-Gut-Brain axis and cognitive function. Advances in Experimental Medicine and Biology Microbial Endocrinology: *The Microbiota-Gut-Brain Axis in Hlth Dis.* 357-371. https://doi.org/10.1007/978-1-4939-0897-4_16.

Gerken M, Afnan R and Dorl J (2006) Adaptive behaviour in chickens in relation to thermoregulation. *Arch. Gefluglk*. **70**: 199-207. https://doi.org/CorpusID:20144304.

He S, Yin Q, Xiong Y, *et al.* (2020) Effects of dietary fumaric acid on the growth performance, immune response, relative weight and antioxidant status of immune organs in broilers exposed to chronic heat stress. *Czech. J. Anim. Sci.* **65**: 104-113. https://doi.org/10.17221/13/2020-CJAS.

Healey G, Murphy R, Butts C, *et al.* (2017) Variability in gut microbiota response to an inulin-type fructan prebiotic within an in vitro three-stage continuous colonic model system. *Bioactive Carbohydrates and Dietary Fibre*. **11**: 26-37. https://doi.org/10.1016/j.bcdf.2017.07.001.

Hinde, RA (1970) Animal Behaviour: A Synthesis of ethology and comparative psychology. 2nd ed. McGraw-Hill, New York, NY. Breed MD (2017) 1953 the chasm between ethology and comparative psychology. *Conceptual Breakthroughs in Ethology and Animal Behavior* 63-66. https://sci-hub.doi/10.1016/b978-0-12-809265-1.00020-4.

Jahromi MF, Altaher YW, Shokryazdan P, *et al.* (2015) Dietary supplementation of a mixture of Lactobacillus strains enhances performance of broiler chickens raised under heat stress conditions. *Int. J. Biometeorol.* **60**:1099–1110. https://doi: 10.1007/s00484-015-1103-x.

Jørgensen SL, Poulsen LL, Bisgaard M and Christensen H (2020) Evaluation of a new probiotic concept for broilers. https://doi.org/10.1101/2020.02.25.964460.

Kokoszyñski D, Wasilewski R, Stêczny K, Kotowicz M, Hrnèar C and Arpášová H (2019) Carcass composition and selected meat quality traits of Pekin ducks from genetic resources flocks. *Poult. Sci*. **98**(7): 3029-3039. https://www.sciencedirect.com/science/article/pii/S0032579119302342.

Kristensen HH, Prescott NB, Perry GC, *et al.* (2007) The behaviour of broiler chickens in different light sources and illuminances. *Appl. Anim. Behaviour Sci.* **103**: 75-89. https://doi.org/10.1016/j.applanim.2006.04.017.

Lai PW, Liang JB, Hsia LC, *et al.* (2010) Effects of varying dietary zinc levels and environmental temperatures on the growth performance, feathering score and feather mineral concentrations of broiler chicks. *Asian-Aust. J. Anim. Sci.* **23**: 937-945. https://doi.org/10.5713/ajas.2010.90495.

Lara L and Rostagno M (2013) Impact of Heat Stress on Poultry

Production. *Anim.* **3**: 356-369. https://doi.org/10.3390/ani3020356.

Lin H, Jiao H, Buyse J and Decuypere E (2006) Strategies for preventing heat stress in poultry. *Worlds Poult. Sci.J.* **62**: 71-86. https://doi.org/10.1079/WPS200585.

Mack L, Felver-Gant J, Dennis R and Cheng H (2013) Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poult. Sci.* **92**: 285-294. https://doi.org/10.3382/ps.2012-02589.

Marimuthu S, Selvam R, Kaninathan A and Souza P (2020) Effect of dietary supplementation of phytogenic feed additive on performance traits, serum neopterin, and cutaneous basophil hypersensitivity response in heat-induced stress model of broiler chickens. *J. Adv. Vet. Anim. Res.* **7**: 141-147.https://doi.org/10.5455/javar.2020.g403.

Mohammed A, Jacobs J, Murugesan G and Cheng H (2018) Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. *Poult. Sci.* **97**: 1101-1108. https://doi.org/10.3382/ps/pex421.

Moreira J, Mendes A, Garcia R, *et al.* (2006) Evaluation of strain, dietary energy level and stocking density on broiler feathering. *Revista Brasileira de Ciência Avícola*. **8**: 15-22. https://doi.org/10.1590/S1516-635X2006000100002.

Quinteiro-Filho WM, Rodrigues MV, Ribeiro A, *et al.* (2012) Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: Role of acute hypothalamic-pituitary-adrenal axis activation1. *J. Anim. Sci.* **90**: 1986-1994. https://doi.org/10.2527/jas.2011-3949.

Ray SM, Ghule S, Muthukumar S, *et al.* (2020) Effects of dietary supplementation of a single-and a multi-strain probiotic on growth performance and intestinal histomorphology of commercial broiler chickens. *Int. J. Poult. Sci*. **19**: 363-371. https://doi.org/10.3923/ijps.2020.363.371.

Rebel J, Balk F, Post J, *et al.* (2006) Malabsorption syndrome in broilers. *Worlds Poult. Sci. J.* **62**: 17-30. https://doi.org/10.1079/WPS200481.

Rooks MG and Garrett WS (2016) Gut microbiota, metabolites and host immunity. *Nature Reviews Immunol.* **16**: 341-352. https://doi.org/10.1038/nri.2016.42.

Salehimanesh A, Mohammadi M and Mehr MR-A (2016) Effect of dietary probiotic, prebiotic and synbiotic supplementation on performance, immune responses, intestinal morphology and bacterial populations in broilers. *J. Anim. Physiol. Anim. Nutr*. **100**: 694-700. https://doi.org/10.1111/jpn.12431.

Samarakoon S and Samarasinghe K (2012) Strategies to improve the cost effectiveness of broiler production. *Trop. Agri. Res*. **23**: 338. https://doi.org/10.4038/tar.v23i4.4869.

Smialek M, Burchardt, S and Koncicki A (2018) The influence of probiotic supplementation in broiler chickens on population and carcass contamination with Campylobacter spp.-Field study. *Res. Vet. Sci.* **118**: 312-316. https://www.sciencedirect.com/science/article/pii/S0034528817301455.

Sohail M, Hume M and Byrd J (2012) Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poult. Sci*. **91**: 2235-2240. https://doi.org/10.3382/ps.2012-02182.

Sohail M, Ijaz A and Younus M (2013) Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. *J. Applied Poult. Res*. **22**: 485-491. https://doi.org/10.3382/japr.2012-00682.

Sohail MU, Hume ME, Byrd JA, *et al* (2015) Molecular analysis of the caecal and tracheal microbiome of heat-stressed broilers supplemented with prebiotic and probiotic. *Avian Pathol.* **44**: 67-74. https://doi.org/10.1080/03079457.2015.1004622

Soleimani A, Zulkifli I, Omar A and Raha A (2011) Physiological responses of 3 chicken breeds to acute heat stress. *Poult. Sci.* **90**:1435–1440. https://doi.org/10.3382/ps.2011-01381

Sugiharto S, Yudiarti T, Isroli I, *et al.* (2017) Dietary supplementation of probiotics in poultry exposed to heat stress - a review. *Annals of Anim. Sci.* **17**: 591-604. https://doi.org/10.1515/aoas-2016-0062.

Van I, *et al.* (2003) Growth and broilers industrialization. Ed. Ceres, Bucharest. pp. 235-236.

Yu LCH, Wang JT, Wei SC and Ni YH (2012) Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology. *World J. Gastrointest. Pathophysiol.* **3**: 27-43. http://doi.org/10.4291/wjgp.v3.i1.27.