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Impact of a Probiotic (PROBAC Plus®) Supplementation on Behaviors and Biochemical Parameters of Broiler Chicken Exposed to Heat Stress

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Abstract: Global warming is an increasing environmental challenge especially in tropical countries. Exposure to high ambient temperatures becomes detrimental to birds survival after change from pre- homeotherrmic to homeotherrms. The aim of this study was to determine how a probiotic supplementation (PROBAC plus® at 1×10^{-3} g feed) influences performance, behavior and some of biochemical parameters of broilers exposed to mild heat stress (34±1°C and RH: 60 to 70 %) from 22 to 42-dold. A total of 120 unsexed 1-d-old broiler chicks (Ross 308) were firstly randomly divided into 2 groups. The first group was control broilers (fed with basal diet) and the second was fed with basal diet supplemented with PROBAC plus® at 1×10^{-3} g feed) from d 1 to d 42-old. At d 22 these 2 groups were randomly divided into 4 groups in a 2 x 2 factorial experimental design (probiotic × high ambient temperature) that continued from 22 to 42-old. Behaviors of birds had been recorded from 22 to 42 d of age. The obtained results showed that heat stressed birds have reduced performance, comfort behaviors and movements activities while the drinking behavior was increased. Heat stressed-birds showed alterations in serum biochemical parameters and higher heterophil/lymphocyte (H/L) ratio and corticosterone concentration than other groups. Addition of PROBAC plus® to diets enhanced performance and improved behavior of birds reared with or without stress. Probiotic addition to diet of heat stressed-birds significantly reduced H/L ratio and corticosterone hormone and improved some serum biochemical parameters. The results suggested that PROBAC plus® probiotic can be used as anti-stress feed additive to counteract the adverse effects of heat stress in addition to its efficacy as growth promoter when supplemented in diets of broilers from d1 to d 42.

Key words: PROBAC plus® · Heat Stress · Performance · Behavior · Serum Biochemical Parameters · Broiler Chicken

INTRODUCTION

Broiler chickens after 3 weeks of age become homoeothermic; they can maintain their central body temperature when kept at room temperature. When prevailing temperatures rise to 32°C (The comfort zone from 22°C to 27°C), they experience heat stress [1]. The negative effect of heat stress on growth rate and production is primarily due to reduced feed intake [2]. High ambient temperatures could also reduce the thyroid activity, plasma triiodothyronine (T_3) and thyroxin (T_4) in poultry [3, 4]. In addition, heat stress also increases heterophils / lymphocytes (H/L) ratio [5] and corticosterone level [6]. Poultry scientists are working to provide solutions for poultry producers to alleviate the significant problems such as heat stress that could results in major economic losses [7, 8].

Probiotics as a live microbial feed supplement which improve the host animal intestinal microbial balance [9] were able to affect animal performance and welfare, particularly through the modulation of the gut microbiota [10] improving resistance to bacterial colonization and enhancing host mucosal immunity; thus could result in a reduced pathogen load and an improved health status of the animals [11]. Therefore, this study was performed to investigate the effect of diet supplementation with PROBAC plus® on performance, behavior, carcass characteristics and serum hormones and biochemical parameters of broiler chickens reared under heat stress.

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Table 1: Experimental Design and Groups

Groups	C	HS	HS/Pr	Pr
Feed (From d 1 to d 42 of age)	Basal	Basal	Basal/PROBAC plus®	Basal/PROBAC plus®
Ambient temperature (From d 22 to d 42 of age)	22°C	34±1°C	34±1°C	22°C

MATERIALS AND METHODS

Experimental Birds: Broilers (Ross 308) were obtained from a commercial hatchery in El-Mansoura and reared in Faculty of Veterinary Medicine farm, South Valley University, Qena, Egypt. Chicks were housed, fed and vaccinated as described previously [12]. Temperature was set initially at 35°C and gradually reduced at a rate of 1°C each other day till reach 22°C at the 4th week as described. For the two groups of heat stress, the temperature was adjusted at 34±1°C under RH (60 to 70 %) from 22 to 42-d of age as described earlier [13]. Probiotic groups were fed a commercial probiotic mixture (PROBAC plus®, Animal Health Care company, Reg. No: M.O.A.:9948, Cairo, Egypt) at 1×10^{-3} g feed from 1 to 42-d of age.

Experimental Design: A total of 120, 1-d-old -unsexed broiler chicks were divided into 4 groups; control (C), heat stress (HS) and addition of probiotic to heat stressed-birds (HS/Pr) and non-stressed group (Pr) (Table 1).

Behavioral Observation: Behaviors were recorded from 22 to 42-d of age. The behaviors of 5 birds / group were recorded by direct observation using a modified focal sampling technique [14]. The behavioral patterns were categorized as ingestive, resting and comfort behaviors as described previously [12]. In brief; each group was observed once a day and 2 days/week at 13:00-13:30.

Broiler Performance: Feed intake (FI), feed conversion ratio (FCR) and body weight gain (BWG) were determined on a weekly basis.

Sampling and Carcass Characteristics: Carcass and eviscerated carcass weights were determined for 10 birds from each group at 42-d-old. The relative weights of heart, empty gizzard, liver and spleen were also calculated.

Serum Heterophil / Lymphocyte (H/L) Ratio and Hormones: From 5-bird/group, the H/L ratio was counted [15] and in serum samples T_3, T_4 and corticosterone hormones were estimated by stat fax-2100 (Awareness technology, INC, USA) using ELISA kits. Serum biochemicals were estimated by digital- VIS/ultraviolet spectrophotometer (723C Visible Spectrophotometer, Shanghai Phenix Optical Scientific Instrument Co., Ltd, China). Total serum proteins and albumin (g/dl) were assayed by a colorimetric method using a commercial kit as previously described [12].

Statistical Analysis: SPSS (Statistical Package for the Social Sciences) version 16 for window was used. One way analysis of variance (ANOVA) was performed followed by least significant difference (LSD). A P-value of <0.05 was considered significant.

RESULTS

Effect of PROBAC plus® on Performance of Broiler Chicken Exposed to Heat Stress: The feed intake (FI) and feed conversion ratio (FCR) of birds exposed to heat stress and fed on diet with or without PROBAC Plus® supplementation are presented in Table 2. Broilers reared under heat stress (Group 2: HS) showed a numerically decrease in FI at 3rd , 4th , 5th and 6th weeks compared with birds kept at room temperature (Table 2). Addition of probiotic to diet (at 1×10^{-3} g feed) of heat stressed birds from d 1 to d 42 (Group 3: HSPr) didn't counteract the effect of heat stress at the 1st week of age (P = 0.926) and the FI of that group was decreased compared to control birds at 2nd, 3rd, 4th and 5th weeks, while at 6th week FI didn't increase than control but it was higher than HS group (Table 2). Addition of probiotic to the diet of broilers reared without stress application (Group 4: Pr), didn't affect FI at 1st, 2nd, 5th and 6th weeks compared with their control birds (Table 2). Broilers exposed to high ambient temperature showed a significantly increased feed conversion ratio (FCR) (at 5th and 6th weeks old) compared with those kept at room temperature (P<0.05, Table 2). Inclusion of a probiotic to the diet of heat stressed birds from d 1 to d 42 (Group 3: HSPr) decreased the FCR at 2nd, 3rd and 6th weeks of age compared to control birds (P < 0.05, Table 2). Interestingly, addition of probiotic to diet of broilers reared without stress significantly decreased the FCR (P<0.05) compared with control birds (Table 2).

Exposure of broilers to a high ambient temperature (34± 1°C) from 22 to 42-d-old (Group 2: HS) reduced their body weight (BW) at the 5th and 6th weeks compared with birds kept at room temperature (Table 3). Inclusion of a probiotic to the diet (at 1×10^{-3} g feed) of heat stressed birds from d 1 to d 42 (Group 3: HSPr) counteracted the effect of heat stress at the 6^{th} week of age and the body weight of that group was comparable to control (P = 0.119, Table 3). Interestingly, addition of probiotic to the diet of broilers reared without stress application (Group 4: Pr), increased the BW at 4^{th}, 5^{th} and 6^{th} weeks compared with their control partners (P<0.05, Table 3). Further, heat stress progressively decreased the body weight gain (BWG) at 5^{th} and 6^{th} week-old and daily weight gain (DWG) compared with control. Further, HSPr had higher final BWG (at the 6^{th} week) than control group. Additionally, probiotic inclusion in diet of broilers reared without heat stress increased BWG at 4^{th} and 6^{th} weeks compared with control (P<0.05, Table 3).

Effect of PROBAC plus® on Behavior of Broiler Chicken Exposed to Heat Stress: The behaviors of birds exposed to heat stress and fed on diet with or without PROBAC Plus® supplementation are presented in Tables 4 and 5. Ingestive behavior of the heat stressed birds at day 22, 29, 33 and 40 was numerically decreased compared with control but at day 26 it was similar to control (Tables 4 and 5). At day 36, HS birds have higher ingestive behavior than control (Table 4). Movement activities of broilers of HS group at days 22 and 36 were higher than control birds, but at days 26 and 33 movement activities for HS birds were similar to control birds. In contrast, at day 29 and 40 movement activities were similar for HS and control birds. Comfort behaviors of HS birds at day 22, 29 and 40 were lower than control. In contrast to these results, HS group had higher comfort behaviors than control birds at day 26 and 36, while at day 33, HS birds have comfort behaviors similar to control.

At day 22, decreased ingestive behavior was found in HSPr and Pr birds compared with control birds. Similar results were recorded at day 26. While at day 29, the ingestive behavior was decreased for HSPr and Pr birds compared with control birds. At day 33, Pr group had lower ingestive behavior than control while no differences were recorded between control and HSPr groups. At day 36, increased ingestive behaviors compared with control birds were found in HSPr and Pr groups. At day 40, the ingestive behavior was not different between groups (Tables 4 and 5).

Resting behavior at day 22 was not different between HSPr and control birds but lower in Pr birds compared with control birds. However, at day 26, resting behavior was decreased in HSPr birds compared to Pr and control birds. At day 29, resting behavior HSPr was decreased compared with control birds. At day 33 and 36 no change in resting behavior was found among groups. However, at day 40 resting behavior was decreased in HSPr and Pr groups compared with control birds.

Movement activities at days 22 and 26 didn't differ between birds fed with probiotic either reared with or without stress. HSPr and Pr groups showed decreased movements activities compared with control birds at day 29. However, increased movement activities of HSPr group compared with control birds at day 33 while there was no difference between Pr and control birds. At day 36, HSPr and Pr groups had increased movement activities compared with control birds. However, at day 40 movement activities were decreased for HSPr birds compared with control birds while there was no difference between Pr and control birds.

At day 22 comfort behaviors increased in HSPr and Pr birds compared with control birds. In contrast, it was decreased compared to control day 26. At day 29, comfort behaviors were increased in Pr group and decreased in HSPr group compared with control group. At day 33, comfort behaviors increased for HSPr and Pr birds compared with control birds. While such behaviors were different among groups at day 36, however, at day 40, comfort behaviors were increased in Pr and decreased in HSPr compared with control birds.

Effect of PROBAC Plus® on Carcass Characteristics of Broiler Chicken Exposed to Heat Stress: Broilers of HS group had significantly lower carcass weight and eviscerated carcass weight compared with control broilers (P<0.05, Table 6). HSPr birds had significantly increased carcass and eviscerated carcass weights compared with control broilers. Broilers of Pr group had increased carcass weight and eviscerated carcass weight compared with control broilers. Neither absolute nor relative weights of spleen were affected by treatments (Table 6).

Heat stressed birds showed a decreased liver weight compared with control and HSPr birds. Pr group had higher liver weight compared with control birds (P = 0.01). But the relative liver weight didn't differ for control and HS birds (Table 6). HS and HSPr birds showed reduced absolute and relative weights of the heart compared with control birds (P<0.05). Also Pr broilers didn't differ from control partners. HS broilers had an increased absolute and relative weights of gizzard compared with control birds (P<0.05), but HSPr birds showed decreased absolute and relative weights of gizzard to be comparable with control. Pr broilers had an increased the absolute and reduced the relative weights of gizzard compared with control (Table 6).

Table 2: Impacts of PROBAC plus® on feed intake (FI) and feed conversion (FC) of broilers reared at room and high ambient temperatures

Parameters	C	H	HPr	Pr	P value
Feed intake					
1st week	206 ±8.88	208±6.96	207±9.27	204±13.06	0.926
2nd week	466±8.75^{a}	462±7.78^{a}	399±7.00^{b}	454±6.78^{a}	0.041
3rd week	931±8.28^{a}	860±7.97^{b}	827±6.89^{c}	863±5.79^{b}	0.001
4thweek	928±13.78^{a}	863±9.67^{b}	832±10.37^{c}	888±9.93^{d}	0.001
5thweek	982±11.55^{a}	819±9.192^{b}	799±8.46^{c}	986±8.031^{a}	0.001
6thweek	1187±71.73^{a}	729±9.19^{b}	835±12.2^{c}	1165±10.98^{a}	0.001
Feed conversion					
1st week	1.11±0.110	1.09±0.100	1.07±0.100	1.08±0.100	0.935
2nd week	2.09±0.100^{a}	2.17±0.101^{a}	1.75±0.141^{b}	2.00±0.12^{a}	0.032
3rd week	2.37±0.103^{a}	2.09±0.100^{b}	2.05±0.068^{b}	2.22±0.101^{a}	0.003
4thweek	1.48±0.100^{a}	1.45±0.142^{ab}	1.37±0.141^{ab}	1.28±0.100^{b}	0.082
5thweek	1.99±0.173^{a}	2.37±0.100^{b}	2.11±0.178^{a}	1.85±0.100^{a}	0.044
6thweek	4.36±0.500^{a}	9.04±1.842^{b}	1.75±0.141^{c}	1.81±0.100^{c}	0.001

$^{a, b, c, d}$ Means (±SD) within the same row with different superscripts are significantly different (ANOVA followed by LSD test, P < 0.05, *n* = 30). Abbreviations; C = Control, HS= Heat stress (33±1 °C) from 22 to 42-d-old, Pr = PROPAC plus® at 1× 10^{-3} feed from d1 to d 42 and HSPr = Heat stress/ PROPAC plus®.

Table 3: Impacts of PROBAC plus® on feed intake (FI) and body weight gain (BWG) of broilers reared at room and high ambient temperatures

Parameters	C	H	HPr	Pr	P value
Initial body weight	55±1^{a}	59±1^{b}	57±1^{ab}	54±1^{a}	0.001
Final body weight	2240±49^{a}	1883±31^{b}	2332±28^{a}	2719±54^{c}	0.001
Daily weight gain	52±3.3^{a}	43 ±2.8^{b}	54±3.7^{a}	63±4.2^{c}	0.001
BW (g)					
1st week	235±7 ^{a}	240±5 ^{a}	243±4 ^{a}	239±5 ^{a}	0.171
2nd week	458±11 ^{a}	450±10 ^{b}	471±7 ^{c}	466±9 ^{c}	0.014
3rd week	850 ±21^{a}	863±16^{ab}	873±14^{bc}	854±16^{ac}	0.042
4thweek	1476±29^{a}	1457±24^{a}	1478±22^{a}	1544±24^{b}	0.001
5thweek	1967±41^{a}	1802±28^{b}	1856±33^{b}	2076±42^{c}	0.001
6thweek	2240±49^{a}	1883±31^{b}	2332±28^{a}	2719±54^{c}	0.001
BWG (g)					
1st week	181±6.59 ^{a}	181±6 ^{a}	186±4 ^{a}	186±5 ^{a}	0.928
2nd week	223±14 ^{a}	210±11^{a}	228±45 ^{a}	227±10 ^{a}	0.089
3rd week	392±25^{a}	413±18^{b}	402±18^{ab}	388±19^{a}	0.090
4thweek	717±29^{a}	664±24^{b}	676±22^{b}	760±24^{c}	0.001
5thweek	491±50^{a}	345±10^{b}	378±14^{c}	532±45^{d}	0.001
6thweek	260±64^{a}	145±37^{b}	544±44^{c}	676±62^{d}	0.001

$^{a, b, c, d}$ Means (±SD) within the same row with different superscripts are significantly different (ANOVA followed by LSD test, P<0.05, *n = 30*). Abbreviations; C = Control, HS= Heat stress (33±1 °C) from 22 to 42-d-old, Pr = PROPAC plus® at 1× 10-3 feed from d 1 to d 42 and HSPr = Heat stress/ PROPAC plus®.

Table 4: Impacts of PROBAC plus® supplementation on frequency of behavioral patterns (No /30min) at afternoon for of broilers reared at room and high ambient temperatures

	Day 22					Day 26					Day 29				
Treatments	C	HS	HSPr	Pr	*P* Value	C	HS	HSPr	Pr	*P* value	C	HS	HSPr	Pr	*P* value
Feeding	30±4.5^{a}	10±2.3^{b}	14±1.9^{bc}	16±2.7^{c}	0.004	30±5.3^{a}	22±3.4^{b}	20±2.7^{b}	6±1.8^{c}	0.001	22±3.8^{a}	8±1.6^{b}	8±2.1^{b}	18±2.8^{a}	0.001
Drinking	30±4.8^{a}	28±2.8^{a}	28±3.8^{a}	20±3.5^{b}	0. 004	14±2.5^{a}	20±2.9^{b}	16±2.3^{ab}	24±3.4^{c}	0.002	20±3.4^{a}	8±1.4^{b}	12±2.6^{b}	18±2.7^{a}	0.001
Crouching	24±3.8^{a}	42±5.3^{b}	26±3.1^{a}	22±2.7^{a}	0.001	34±4.1^{a}	32±3.7^{a}	28±2.8^{b}	28±2.4^{b}	0.025	36±3.4^{a}	16±2.6^{b}	12±1.9^{b}	42±4.4^{c}	0.001
Huddling	14±2.8^{a}	14±3.1^{a}	12±2.4^{a}	8±1.7^{b}	0.006	10±1.9^{a}	14±2.8^{b}	10±1.4^{a}	16±2.3^{b}	0.007	4±1.7^{a}	8±2.3^{b}	2±0.7^{a}	4±1.2^{a}	0.002
Standing	14±2.3^{a}	34±5.1^{b}	20±3.2^{b}	18±2.7^{a}	0.001	26±3.5^{a}	32±3.9^{ac}	44±5.7^{b}	34±4.6^{c}	0.001	40±4.6^{a}	20±2.6^{b}	14±1.3^{c}	38±3.8^{a}	0.001
Walking	24±3.2^{a}	18±2.6^{b}	18±2.9^{b}	14±1.6^{b}	0.001	38±5.1^{a}	30±3.2^{b}	24±2.4^{c}	10±1.8^{d}	0.001	46±4.7^{a}	14±2.3^{b}	6±1.1^{c}	36±4.2^{d}	0.001
Running	0	2±0.6^{a}	0	6±1.8^{b}	0.002	2±0.9^{a}	2±0.4^{a}	0	4±1.3^{b}	0.001	0	2±0.9	0	4±1.8	0.001
Stretching *	18±2.6^{a}	20±3.1^{a}	28±4.2^{b}	0	0.002	10±1.8^{a}	14±3.1^{ac}	20±3.7^{bc}	16±2.2^{c}	0.004	18±3.2^{a}	4±1.1^{b}	2±0.8^{c}	16±2.8^{a}	0.001
Shaking	0	6±1.8^{a}	2±0.6^{b}	0	0.001	4±1.2^{a}	2±0.5^{b}	2±0.8^{b}	4±2.1^{a}	0.028	4±1.9^{a}	2±1.1^{b}	0	4±2.2^{a}	0.022
Preening	56±6.7^{a}	34±3.8^{b}	34±4.2^{bc}	44±5.3^{c}	0.001	32±3.2^{a}	42±3.6^{b}	54±6.2^{c}	40±4.2^{b}	0.001	32±4.8^{a}	24±3.6^{b}	18±2.3^{b}	64±6.4^{c}	0.001

$^{a, b, c, d}$ Means (±SD) within the same row with different superscripts are significantly different (ANOVA followed by LSD test, P<0.05, *n = 5*). * stretching of leg and / or wing. Abbreviations; C = Control, HS= Heat stress (34±1 °C) from 22 to 42-d-old, Pr = PROPAC plus® at 1× 10^{-3} feed and HSPr = Heat stress/ PROPAC plus®.

Table 5: Impacts of PROBAC plus® supplementation on frequency of behavioral patterns (No /30min) at afternoon for of broilers reared at room and high ambient temperatures

Time of day	Day 33					Day 36					Day 40				
Treatments	C	HS	HSPr	Pr	P value	C	HS	HSPr	Pr	P value	C	HS	HSPr	Pr	P value
Feeding	15±3.5^{a}	6±2.3^{b}	9±2.8^{b}	6±1.8^{b}	0.002	6±1.4^{a}	9±2.1^{ac}	24±3.5^{b}	12±2.7^{c}	0.001	12±2.6^{a}	4±1.1^{b}	7±1.8^{b}	14±3.2^{a}	0.001
Drinking	15±3.3^{a}	15±2.8^{a}	12±3.1^{b}	9±1.5^{b}	0.009	15±2.6^{a}	27±4.3^{b}	12±2.2^{a}	18±3.1^{a}	0.001	13±4.2^{a}	12±2.1^{a}	13±2.7^{a}	12±3.7^{a}	0.919
Crouching	15±2.8^{a}	18±3.1^{ab}	24±4.1^{b}	21±3.2^{b}	0.038	6±1.1^{a}	3±0.4^{b}	6±1.9^{a}	9±2.4^{c}	0.003	23±5.1^{a}	17±3.1^{b}	12±2.3^{b}	12±1.9^{b}	0.001
Huddling	6±1.7^{a}	9±2.2^{a}	3±0.8^{b}	6±1.2^{a}	0.005	12±2.3^{a}	24±3.7^{b}	24±2.9^{b}	27±4.1^{b}	0.001	14±3.6^{a}	8±2.7^{b}	5±1.2^{b}	8±1.8^{b}	0.003
Standing	12±2.3^{a}	21±3.7^{b}	15±2.9^{a}	9±1.9^{a}	0.001	3±0.6^{a}	6±1.2^{a}	15±2.8^{b}	18±3.8^{b}	0.001	18±3.4^{a}	8±1.7^{bc}	9±2.1^{c}	15±2.8^{a}	0.001
Walking	9±1.8^{a}	3±1.4^{b}	6±2.2^{a}	9±2.7^{a}	0.008	0	0	0	0	0	12±3.2^{a}	4±1.1^{b}	5±1.4^{b}	12±2.9^{a}	0.001
Running	0	0	9±3.4	0	0.001	0	9±1.7	0	9±2.3	0.001	0	0	0	7±1.6	0.001
Stretching *	9±1.8^{a}	9±2.1^{a}	15±2.6^{b}	12±2.3^{ab}	0.001	0	0	0	3±0.6	0.001	7±1.6	0	0	8±2.1	0.002
Shaking	0	0	3±0.7	0	0.001	30±4.3^{a}	27±3.6^{a}	24±2.9^{a}	18±2.6^{b}	0.003	0	0	0	2±0.6	0.001
Preening	18±3.1^{a}	21±2.8^{a}	42±5.9^{b}	24±3.5^{a}	0.001	6±1.4^{a}	9±2.1^{ac}	24±3.5^{b}	12±2.7^{c}	0.001	7±2.1^{a}	4±1.2^{a}	5±1.6^{a}	24±4.7^{b}	0.001

$^{a, b, c, d}$ Means (±SD) within the same row with different superscripts are significantly different (ANOVA followed by LSD test, P<0.05, *n* = *5*). * stretching of leg and / or wing. Abbreviations; C = Control, HS= Heat stress (34±1 °C) from 22 to 42-d-old, Pr = PROPAC plus® at 1× 10^{-3} feed and HSPr = Heat stress/ PROPAC plus®.

Table 6: Impacts of PROBAC plus® on carcass and organ weights (g) of broilers reared at room and high ambient temperatures

Parameters	C	H	HPr	Pr	P value
Carcass weight	2054.5±44.71^{a}	1748.5±35.12^{b}	2165.5±24.39^{c}	2523.5±50.27^{d}	0.001
Eviscerated weight	1717.0±44.02^{a}	1450.0±28.62^{b}	1825.0±23.10^{c}	2115.0±42.57^{d}	0.001
Spleen	2.80±0.17^{a}	2.42±0.34^{b}	2.81±0.25^{a}	3.26±0.23^{c}	0.001
Heart	10.36±0.6^{a}	7.76±0.37^{b}	9.51±0.40^{ab}	12.48±0.56^{c}	0.001
Liver	37.15±1.09^{a}	31.72±1.94^{b}	38.45±1.34^{a}	46.77±1.61^{d}	0.001
Gizzard	39.30±1.55^{a}	32.63±2.09^{b}	35.50±1.71^{ab}	41.59±2.09^{c}	0.001
Relative weights					
Spleen %	0.13±0.08	0.12±0.017	0.11±0.006	0.12±0.007	0.897
Heart %	0.47±0.01	0.43±0.01	0.40±0.04	0.46±0.01	0.771
Liver %	1.66±0.04	1.67±0.08	1.67±0.05	1.71±0.03	0.516
Gizzard %	1.77±0.10^{a}	1.73±0.06^{ab}	1.52±0.06^{b}	1.52±0.07^{b}	0.001

$^{a, b, c, d}$ Means (±SD) within the same row with different superscripts are significantly different (ANOVA followed by LSD test, P < 0.05, *n* = 30). Abbreviations; C = control, H = High stocking density and Pr = PROPAC plus® at 1× 10^{-3} feed from d1 to d 42 and Hpr = High stocking density/ PROPAC plus®.

Table 7: Impacts of PROBAC plus® on Stress parameters of broilers reared at room and high ambient temperatures

Parameters	C	H	HPr	Pr	P value
H / L Ratio	0.20±0.03^{a}	0.70±0.09^{b}	0.51±0.09^{c}	0.22±0.03^{a}	0.001
Cortisol (mg/dl)	0.88±0.09^{a}	1.37±0.13^{b}	1.06±0.05^{a}	0.66±0.05^{c}	0.001
T_3 (ng/ml)	1.38±0.32^{ac}	1.57±0.17^{ab}	1.57±0.07^{ab}	1.28±0.28^{c}	0.057
T_4 ((µg/dl)	2.20±0.16^{a}	3.43±0.03^{b}	2.87±0.15^{c}	2.81±0.11^{c}	0.001

$^{a, b, c, d}$ Means (±SD) within the same row with different superscripts are significantly different (ANOVA followed by LSD test, P < 0.05, *n* = 30). Abbreviations; C = control, H = High stocking density and Pr = PROPAC plus® at 1× 10^{-3} feed from d1 to d 42 and Hpr = High stocking density/ PROPAC plus®.

Table 8: Impacts of PROBAC plus® on serum biochemical parameters of broiler reared at room and high ambient temperatures

Parameters	C	H	HPr	Pr	P value
Total Protein (g/dl)	2.50±0.14^{a}	1.31±0.02^{b}	1.78±0.05^{c}	2.83±0.14	0.001
Albumin (g/dl)	1.21±0.09^{a}	1.19±0.03^{a}	1.21±0.03^{a}	1.42±0.03^{b}	0.001
Globulin (g/dl)	1.29±0.07^{a}	0.11±0.02^{b}	0.57±0.07^{c}	1.41±0.14^{a}	0.001
A/G ratio	0.94±0.06^{a}	1.91±0.17^{b}	1.34±0.43^{c}	1.05±0.13^{a}	0.001
Cholesterol (mg/dl)	112.03±1.14^{a}	148.58±0.92^{b}	112.71±2.77^{a}	99.20±2.7^{c}	0.001
Creatinine (mg/dl)	0.26±0.06^{a}	0.26±0.04^{a}	0.44±0.05^{b}	0.32±0.05^{ab}	0.05
Urea. (g/dl)	3.60±0.92^{a}	2.40±0.67^{b}	4.20±0.20^{c}	3.80±0.37^{ac}	0.001
AST (mg/dl)	193.00±7.20^{a}	243.80±13.89^{b}	276.40±20.84^{c}	258.60±26.17^{d}	0.001
ALT (IU/dl)	19.20±0.86^{a}	22.40±2.03^{b}	17.60±0.92^{a}	23.20±3.33^{b}	0.001
Glucose (mg/dl)	136.60±18.93^{a}	167.80±18. 38^{b}	144.20±11.51^{a}	119.80±14.96^{c}	0.001

^{a,b,c,d}Means (±SD) within the same row with different superscripts are significantly different (ANOVA followed by LSD test, P < 0.05, *n* = 30). Abbreviations; C = control, H = High stocking density and Pr = PROPAC plus® at 1× 10^{-3} feed from d1 to d 42 of age, and Hpr = High stocking density/ PROPAC plus®.

Effect of PROBAC plus® Supplementation on Serum Hormones of Broiler Chicken Exposed to Heat Stress: Broilers of HS group showed a significantly increased H/L ratio (at 6th weeks old) compared with those kept at room temperature (P<0.05, Table 7) and HSPr broilers had lower H/L ratio compared with HS birds (P < 0.05) but still higher than control birds (P<0.01). However, Pr broilers didn't differ from control birds (P = 0.88, Table 7).

Heat stressed broilers have a significantly increased serum corticosterone compared with control birds but addition of probiotic to the diet of heat stressed broilers significantly decreased corticosterone concentration compared with heat stressed broilers without probiotic feeding (P<0.05) and comparable to the control birds. Addition of probiotic to diet of birds reared without heat stress decreased significantly (P = 0.028) corticosterone concentration (0.66 ± 0.05 mg/dl) compared with control (Table 7).

On other hand, there were no differences in serum levels of T_3 in all groups (Table 7). Whereas HS broilers had higher T_4 compared with control birds (P<0.01) and HSPr had significantly decreased T_4 level compared to HS (P<0.05) but higher than control group. Pr broilers had increased T_4 compared with control broilers (P<0.01, Table 7).

Effect of PROBAC Plus® Supplementation on Serum Biochemical Parameters of Broiler Chicken Exposed to Heat Stress: Broilers of HS group showed a decreased total protein (g/dl) compared with control birds (P<0.01), HSPr broilers had significantly higher protein compared with HS and lower than control birds (P < 0.05). Interestingly, addition of probiotic to diet of broilers reared without stress significantly increased total protein (P<0.05) compared with control birds (Table 8). Serum albumin of broilers of HS and HSPr groups didn't differ from control birds. While that of Pr group showed a significant increase in serum albumin compared with control birds (P < 0.05, Table 8).

HS birds have a decreased serum globulin compared with control and HSPr birds had significantly increased globulin compared with HS birds (P<0.05) but globulin concentration still significantly lower than control (P<0.01). Broilers of Pr group had non-significantly higher globulin level compared with control (P = 0.379, Table 8). Albumin/Globulin ratio (A/G) ratio of HS birds was significant higher than HSPr and control birds (P<0.01). Broilers of Pr group had similar A/G to control birds.

Broilers of HS group showed a significantly increased serum cholesterol compared with control birds (P<0.05) while HSPr birds had normal cholesterol level compared with control birds. Further, Pr broilers had significantly decreased cholesterol compared with control birds (P<0.01, Table 8). HS birds had a similar creatinine concentration to control birds but Pr group had higher creatinine than control and HS groups (P<0.05, Table 8). Broilers of HS had a similar urea concentration of control birds and HSPr birds increased urea concentration compared with HS birds but was not significantly different from control nor Pr group (P = 0.468, Table 8).

Serum aspartate transferase (AST) of HS birds didn't differ from control birds while HSPr birds had increased AST level compared with control (P<0.01) but not compared with HS birds. Pr group had significantly increased AST compared with control (P<0.05, Table 8). In turn, serum alanine-transaminase (ALT) didn't differ between experimental groups. Similarly, serum glucose didn't show significant differences between treatments (P = 0.218, Table 8).

DISCUSSION

Rearing of broilers under heat stress is the main problem facing poultry industry especially in tropical countries such as Egypt. The results of the current study showed that exposure of broiler chicken to heat stress at 4^{th} weeks of age (34±1°C) adversely affected the performance, behavior and welfare parameters in terms of lower feed intake and reduced daily weight gain and final body weight. In addition to its alteration of welfare and health of broiler chicken in this study, heat stress had a high adverse effect on birds' performance.

It was reported that increased environmental temperature stimulates the peripheral thermal receptors to transmit suppressive nerve impulse to the appetite center in the hypothalamus causing a decrease in feed consumption [16]. Another explanation is that, body weight decreases during heat stress due to expenditure of higher energy to maintain their body temperature in addition to their lowered feed intake [17]. In the current study, the association between impaired productivity and altered welfare status of birds was recorded. The reduced performance of stressed birds may result from the stressors adverse effect on behavior and welfare of broilers. In focus the reduction of feeding behavior during heat stress could be due to reduced appetite [18, 19]. Not only feeding behavior was affected but also drinking behavior was increased during heat stress in a trial to reduce the adverse effect of high temperatures. This is in line with previous studies [20]. Resting behavior was increased during high temperature to suppress the physiological stress responses. This observation was also recorded in a recent study [21]. In contrast, the movement activities of stressed birds were reduced to minimum as a physiological response in case of heat stress [22]. Furthermore, comfort behaviors in the current experiment were clearly reduced during stress. This result is in line with Andrews *et al.* [18] but not in line with Spinu *et al.* [23] . These alterations in the behavioral patterns of stressed broilers can adversely affect the production as well as the health of birds.

In the present study, carcass traits and organ weights as important health indicators were significantly changed due to stress. In focus, the reduced carcass weight and eviscerated carcass weight were recorded for heat stressed birds. The poor carcass characteristics may be due to a reduced feed intake and impaired utilization of nutrients [24, 25]. The liver, being one of the most vital organs of the body, constitutes the lifeline system of birds. This organ also plays major roles in the detoxification, digestion, metabolism and utilization of feed nutrients as a center of a number of digestive, metabolic and productive activities, this vital immune organ was showing atrophy due to heat stress [24]. Thus the reduced liver weight sue to exposure to high ambient temperature in this study this can be explained as cardiovascular adjustment in response to heat including vasodilatation in the cutaneous vascular bed and vasoconstriction in hepatosplanchnic vascular area [25] leading to fatty and parenchymatous degeneration of the hepatocyte that was manifested by shrinkage in the liver size [17]. Similar to the previous report of Kucuk *et al.* [24] and Keambou *et al.* [26] gizzard and heart weights were decreased by heat stress.

Heat stress resulted in important physiological stress responses in terms of higher H/L ratio and release of corticosterone (Table 7). The reason behind this can be the alteration of immunity of birds [27]. Furthermore, heat stress increased the serum corticosterone concentration due to alteration of the activity of the neuroendocrine system of poultry, resulting in an activation of the hypothalamic-pituitary-adrenal axis [28]. Similar results were previously reported [29]. In the current study, the health status of birds was estimated by measurement of clinical serum biochemical parameters. Heat stress suppressed the concentration of circulating total protein and globulins (Table 8), suggesting that higher temperature alters the protein metabolism. An association between higher total protein, globulin and corticosterone was noted for heat stressed broilers in this study. It is known that corticosterone suppresses the synthesis of body proteins [27]. Moreover, cholesterol was also increased in the blood of heat stressed birds as a result of higher blood corticosterone [6, 27]. Furthermore, heat stress increased serum concentration of T_4. It was found that when chickens are exposed to warm temperature, T_4 was inactivated by conversion into reverse T_3 (r-T3). The circulating serum T_4 level depends on both the rate of secretion and the rate of utilization of T_4 [30]. Such a decrease in T_4 utilization could possibly explain the higher circulating T_4 levels found at 34±1°C in the present study. Also the elevated level of total T_4 of heat-exposed birds was reported by Moss and Balnave [31] and could be related to a reduction in its utilization. Another explanation is that the reduction in feed intake observed at the high temperature may be a contributing factor in determining the higher T_4 levels. In general, all these adverse effects were the main causes of reducing the performance of broilers.

PROBAC plus® beneficial effects may be related to maintaining normal intestinal micro flora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activities and by promoting digestion and appetite or feeding behavior [19, 32]. As well as, probiotic supplementation overcome the negative impacts of these stressors on resting, movement activities and comfort behaviors as stressed birds fed with probiotic increased their activities and increased the frequency of preening and stretching of leg and / or wing [19]. So the improving of behavior of stressed birds fed with probiotic could lead to an increased productivity of broilers.

It is known that, the role of probiotic in overcoming the adverse effect of stress resulted in an improved health status of broilers. Particularly, carcass weight and eviscerated carcass weight in HSPr birds were comparable to the control birds. Similar results were already recorded [19, 33]. As well as, probiotic feeding to stressed birds resulted in an increased heart weight. This finding is similar to a previous study [34]. In this study, probiotic supplementation decreased H/L ratio for heat stressed broilers may be due to stimulation of immune system and reduction of inflammatory reaction of chicks [35]. These findings are in agreement with earlier studies [27, 33]. As well as probiotic has the efficacy in decreasing the corticosterone level of heat stressed birds as probiotic bacteria may indirectly stimulate the afferent neurons through a cytokine neurohumoral route, causing a reduction in the levels of circulating corticosterone and adrenocorticotropic hormone [34]. Another interpretation that may be these supplements influence the gut health and microbiome, it can be postulated that a healthy and balanced microbial community may have helped normalize adrenal gland activity. Thus POBAC Plus ® supplementation could improve welfare status of birds in terms of reducing stress induced rise in H/L ratio and corticosterone hormone.

Improving performance of broiler chickens fed probiotic is mainly due to maintaining of beneficial microbial population [36, 37], improving feed intake and nutrient digestibility [32], altering bacterial metabolism [38] and reducing of cell turnover of the intestinal epithelium [39]. Similarly, Edens *et al.* [40] concluded that administration of *Lactobacillus reuteri* resulted in an increased villus height, indicating that probiotics are potentially able to enhance nutrient absorption and thereby improve growth performance and feed efficiency. As well as, *Saccharomyces cerevisiae* as probiotic yeast can improve nutrient absorption that may lead to improvement of performance of broilers [41]. The role of PROBAC Plus® supplementation as a growth promoter in broiler industry may be due to enhancing the behavior and welfare of birds. Broilers supplemented with probiotic tend to rest more which may lead to higher body weight. These findings are in agreement with Hocking *et al.* [42] and Webster [43] . As well as, the comfort and movement behaviors were increased for birds fed with probiotic than its control partner. Similar results were already recorded before [19]. Not only performance and behavior of birds were improved due to probiotic feeding but also carcass traits. Weight of carcass and eviscerated carcass weight were increased due to probiotic feeding. This result is similar to the results observed by Roshanfekr and Mamooee [44] and Aluwong *et al.* [45]. In focus, higher heart, liver and gizzard weights were recorded in current study. These results were recorded earlier [45].

Indeed, the feeding of probiotic was able to improve the serum biochemicals in terms of increasing the serum total protein and serum globulin and albumin indicating that probiotic can significantly stimulate protein metabolism in broiler chickens. The reasons behind this may be attributed to the probiotic effect as stimulator for the synthesis of liver proteins [27, 33& 46] resulting in higher liver protein and plasma protein synthesis. As a direct positive effect of probiotic feeding, serum cholesterol was significantly decreased due to a reduced absorption and/or a decreased synthesis of cholesterol in the gastrointestinal tract [36, 47]. It was speculated that *Lactobacillus acidophilus* reduced the serum cholesterol by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors of cholesterol synthesis [12]. Another explanation of this result was that probiotic microorganisms inhibited hydroxymethyl-glutaryl-coenzyme A; an enzyme involved in the cholesterol synthesis pathway thereby decreased cholesterol synthesis [48].

Furthermore, PROBAC Plus® supplementation improved T_4 of stressed birds due to enhancing the activity of corticotrophin releasing factor (CRF), which is known to stimulate thyrotropin secretion and hence, T_4 secretion [37]. These observations are in line with Khan *et al.*[49] and Motlagh *et al.*[50]. Finally, probiotic addition to the diet of heat stressed broilers (34±1°C) showed a great efficacy to enhance and counteract the adverse effects of heat stress and is beneficial as anti-stress feed additive.

CONCLUSIONS

It can be concluded that exposure of broilers to high ambient temperature (34±1°C) at 4th weeks old led to heat stress that caused obvious negative effects on the growth performance and behavior of birds as well increased H/L ratio and serum corticosterone level. Furthermore, addition of probiotic partly removed the adverse effect of heat stress, improved birds' performance, enhanced behavioral activities of birds and corrected some serum biochemicals and corticosterone hormone concentration. Thus PROBAC plus® was beneficial in counteracting the adverse effects of heat stress in broiler chickens.

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