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ENHANCING TOLERANCE OF BROILER CHICKENS TO HEAT STRESS BY SUPPLEMENTATION WITH VITAMIN E, VITAMIN C AND/OR PROBIOTICS

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Abstract

Heat stress is one of the major challenges which the poultry industry faces during summer in tropical and subtropical regions. This study was conducted to evaluate the impact of Vitamin (Vit) E, C and/or probiotics on improving tolerance of broiler chickens to chronic heat stress (CHS). A total of 294, 1-day-old Cobb-500 broiler chicks were allocated into seven treatment groups; Thermoneutral group was raised under a thermoneutral condition during 25–42 d of age. The other six groups were raised for three successive days per week at 36±2ºC and 75–85% relative humidity for 7 h daily: heat stressed group, Vit E (100 mg/kg diet), Vit C (200 mg/kg diet), Vit C+Vit E, probiotics (*Saccharomyces cerevisiae* and *Lactobacillus acidophilus* at 2 g/kg diet) and Vit C+Vit E+probiotics. Exposure to CHS decreased body weight gain (BWG), feed intake (FI), and abdominal fat. It had adverse impact on feed conversion ratio (FCR), packed cell volume (PCV), monocyte, basophil, total protein, and phagocytic activity while increased plasma cholesterol and aspartate aminotransferase (AST) compared to the thermoneutral group. Vit E, Vit C or probiotics alone decreased the adverse effects of CHS on growth rate throughout the experimental period. Vit C and E were equally potent during the experimental period, but were less effective than the combination of both vitamins. Vit E increased the dressing percentage and abdominal fat as compared to the thermoneutral group, but decreased AST while increasing basophil, monocyte and globulin compared to the heat stressed group. In addition, serum albumin and AST of Vit E were lower compared to Vit C, but cholesterol was higher. Vit E increased red blood cells and white blood cells, but plasma cholesterol was increased compared with the heat stressed group. Vit C increased PCV, lymphocytes, monocyte, basophil and albumin and decreased neutrophil. Both vitamins without/with probiotic induced a further increase in basophil, serum total protein, and albumin. It could be concluded that supplementation of Vit E, Vit C, probiotics, and different combinations reduced some of the adverse effects of CHS, and Vit E+Vit C+probiotics was the most effective for economic traits followed by Vit E+Vit C or probiotics.

Key words: vitamin E, vitamin C, probiotics, heat stress, hematology, broilers

In tropics and subtropics, one of the major challenges facing the poultry industry is the adverse effects of CHS on growth, physiological and economic traits of chickens (Attia et al., 2009 a and b; Varasteh et al., 2015) and meat quality (Attia et al., 2011). Several nutritional remedies have been recommended to relieve the detrimental effects of CHS, e.g. Vitamin (Vit) C and E and adjusting dietary levels of energy, protein and/or amino acids (Daghir, 2008; Attia et al., 2011; Zeferino et al., 2016; Attia and Hassan, 2017).

Vitamins such as Vit E (tocopherols) are fat-soluble and possess antioxidant properties to protect the membranes of the cell against oxidation (Traber and Atkinson, 2007). Niu et al. (2009) reported that body weight gain (BWG) was effectively maintained by 100 mg/kg Vit E supplementation under the CHS, whereas CHS severely reduced growth and immune parameters of broilers; however, interestingly the immune response of broilers was improved by dietary Vit E supplementation under CHS. Moreover, Rajput et al. (2009) found that broilers fed a diet supplemented with 300 mg Vit E had greater BWG than the control group. Shelf life may also be improved by Vit E supplementation due to delayed oxidative stress and lipid peroxidation (Attia et al., 2006)

Vit C (ascorbic acid) is a water soluble and another natural antioxidant to protect the animals under heat stressors conditions and also had an activity against oxidation (Lin et al., 2005). Mckee and Harrison (1995) observed that Vit C, particularly at 150 ppm, enhanced performance of broiler chicks exposed to multiple concurrent environmental stressors. In addition, Vit C at 40–200 ppm improved growth parameters of broiler chicks exposed to multiple environmental stressors (Vathana et al., 2002; Attia et al., 2009 b, 2011).

Sahin et al. (2002) reported that Vit C and E had synergetic effects for reducing the adverse effect of CHS in chickens. However, Ipek et al. (2007) reported that Vit E, Vit C, and Vit E+C supplementation equally increased lymphocytes numbers and white blood cells (WBCs) and decreased heterophil numbers and the heterophil/lymphocyte ratio ($P<0.05$) in quails. Thus, Vit C or E alone is sufficient to alleviate CHS related problems in quails.

Probiotics are living microorganisms representing a category of the normal intestinal microflora of the animals and chickens and may reduce the harmful impact of CHS (Kizerwetter-Swida and Binek, 2016). They promote growth rate and protect against pathogenic gram-negative bacteria that inhabit the intestine, through competitive exclusion and by lowering the intestinal pH (Kabir, 2009; Kizerwetter-Swida and Binek, 2016). Probiotics such as yeast provide some essential nutrients such as vitamin B complex and many amino acids and enhance digestion by production of many enzymes (Kabir, 2009). It has been shown that high lactobacilli and low pH levels reduce colonization of Salmonella in the crop (Hinton et al., 2000; Kizerwetter-Swida and Binek, 2016).

Blood metabolites and hematology are important indicators of chickens' physiological and health status, and tolerance to heat stress under heat stress condition (Attia et al., 2011; Attia and Hassan, 2017; Bovera et al., 2007; Ehran and Bölükbaş, 2016). Few studies have been conducted to comprehensively evaluate the roles of Vit E, Vit C and probiotics alone or in various combinations of these additives on

growth performance, carcass characteristics, immune status and blood chemistry in broilers under CHS. However, these additives have their own modes of action and thus synergetic influence by such combination treatments may strengthen broilers' ability to cope with heat stress. Thus, the objective of this study was to investigate the effect of Vit E, Vit C, and probiotics alone or in combination on improving broiler chicken tolerance to CHS.

Material and methods

Chickens and experimental design

A total of 294, 1-day-old Cobb-500 broiler chicks as hatched (male: female = 1:1) were wing banded and randomly allocated to seven treatment groups with seven replicates (6 chicks per replicate) in battery brooders. The birds were raised under a 23 h light: 1 h dark lighting program. Thermoneutral group was raised under a thermoneutral condition in a semi-opened house at 25±3ºC and 55% of relative humidity (RH) during 25–42 d of age. Other six treatment groups were raised under a chronic heat stress (CHS) condition which was applied for three successive days per week at 36±2ºC and 75–85% relative humidity (RH) for 7 h daily from 10:00 to 17:00 pm (Attia et al., 2009 a, 2011; Attia and Hassan, 2017): heat stressed group, Vit E (100 mg/kg diet, α-Tocopherols), Vit C (200 mg /kg diet as ascorbic acid), Vit C+Vit E, probiotics (*Saccharomyces cerevisiae* and *Lactobacillus acidophilus* at 2 g/kg diet as recommended by a manufacturer: China Way Corp., Taichung, Taiwan) and Vit C+Vit E+probiotics. Birds were fed corn-soybean meal diets during 1–14, 15–30, and 31–42 d of age as presented in Table 1. Feed intakes (FI), body weights gain (BWG), and feed conversion ratio (FCR) were measured at 14, 30 and 42 d of age during the experimental period. Water and feed were provided *ad libitum.*

Apparent nutrient digestibility

At 42 d, five chickens/treatment were randomly selected from randomly selected 5 pens per treatment (1 bird per pen) and used for nutrient digestibility measurements using a total excreta collection method. The birds were fasted for 24 h and then were fed their corresponding experimental diets for 72 h. The excreta were collected and separately stored for each replicate. The feed was weighed and dried in a forced air oven at 70ºC for 36 h. Samples were then ground and placed in screw-top glass jars until analyses. Fecal nitrogen was separated from urinary nitrogen in excreta samples according to the procedure described by Jakobsen et al. (1960). Dry matter, nitrogen, fat and crude fiber contents of the excreta and feed were measured according to AOAC (1995) and expressed on a dry matter basis. The digestibility of nutrients was calculated using (input-output (retained)/input)*100 data for each nutrient (Attia et al., 2012).

Table 1. Ingredients and chemical composition of the experimental diets

Ingredients (g/kg)	Starter diet (0–14 days)	Grower diet (15–30 days)	Finisher diet (31–42 days)
Yellow corn	550.0	617.9	682.0
Soybean meal	324.0	260.0	212.0
Corn gluten meal	55.0	54.0	40.0
Limestone	11.0	11.2	11.0
Dicalcium phosphate	17.5	15.0	13.0
Vitamin+mineral premix[1]	3.0	3.0	3.0
NaCl	3.0	3.0	3.0
DL-Methionine	3.1	3.0	2.0
L-Lysine (HCL)	3.4	2.9	3.5
Vegetable oils	30.0	30.0	30.5
Total	1000	1000	1000
Analyzed[2] and calculated[3] values (g/kg)			
Dry matter[2]	868	874	869
ME MJ/kg[3]	12.69	13.03	13.28
Crude protein[2]	221	193.4	178.0
Methionine[3]	6.9	6.0	5.1
Sulphur amino acids[3]	1.06	9.4	8.2
Lysine[3]	13.4	11.8	10.6
SAA/lysine ratio	79	80	77
Calcium[3]	9.1	8.5	7.9
Available phosphorus[3]	4.6	4.1	3.6
Crude fat[3]	52.1	61.3	71.9
Crude fibre[2]	47.0	43.3	47.5
Ash[2]	52.5	55.3	5.74

[1]Vitamin+mineral premix provides per kilogram of the diet: vitamin A (retinyl acetate) – 24 mg, vitamin E (dl-α-tocopheryl acetate) – 20 mg, menadione – 2.3 mg, Vitamin D_3 (cholecalciferol) – 0.05 mg, riboflavin – 5.5 mg, calcium pantothenate – 12 mg, nicotinic acid – 50 mg, choline chloride – 600 mg, vitamin B_{12} – 10 mg, vitamin B_6 – 3 mg, thiamine – 3 mg, folic acid – 1 mg, d-biotin – 0.50 mg. Trace mineral (milligrams per kilogram of diet): Mn – 80, Zn – 60, Fe – 35, Cu – 8, Se – 0.60.

Carcass characteristics and meat composition

At 42 d of age, eight chickens (4 males and 4 females; 1 male or 1 female from each pen except for 7th pen; we selected 1 male and 1 female in 7th pen) were randomly selected from each treatment to represent all treatment replicates and slaughtered according to Islamic method (Attia et al., 2016) for carcass characteristics as weight percentages to over pre-slaughter live weight: Dressing, abdominal fat weight, liver, gizzard, heart, and pancreas and intestinal. Samples of 50% of breast meat + 50% of thigh meat were weighed (8 per treatment) and placed in an electric drying oven at 70°C for 24 h until they reached a constant weight. The dried meat was finely ground through a grinder with a sieve (1-mm²) and then thoroughly mixed. The air-dried samples were stored into airtight glass container for further analyses. Dry matter, protein, ether extract and ash were determined according to AOAC (1995).

Meat characteristics

Physical characteristics of mixtures of breast and thigh meat (8 samples per treatment of the slaughter chickens to represent all treatment replicates) were studied. Random selection procedure was the same as above (Carcass characteristics and meat composition section). The ability of meat to hold water (WHC) as well as meat tenderness were determined according to the method of Volvoinskaia and Kelman (1962). Color intensity (optical density) was measured as described earlier (Husani et al., 1950).

Hematological, immunological and biochemical constituents

Eight blood samples per treatment to represent all treatment replicates were collected from wing vein in heparinized tubes at 42 d of age for determination of biochemical constituents. In order to obtain serum, blood was collected in non-heparinized tubes.

Blood hemoglobin (Hgb) and packed cell volume (PCV) were determined (Eilers, 1967). Red blood cells (RBCs) (Hepler, 1966), white blood cells (WBCs) and fractions of WBCs (Lucas and Jamroz, 1961) were counted. Phagocyte index and activity were determined according to Leijh et al. (1986). A hemagglutination inhibition test for Newcastle disease virus (NDV) was quantitated using a microtitration haemagglutination technique (Kai et al., 1988). Blood biochemical analyses were determined using spectrophotometer by commercial diagnostic kits (Diamond, Cairo, Egypt). Serum total protein was measured by Weichselbaum's methodology (1946). Albumin concentration was determined according to the method of Doumas et al. (1977). Globulin concentration was estimated by calculating difference between total protein and albumin. Liver leakage enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed according to the method of Reitman and Frankel (1957). Plasma glucose concentration (mg/100 ml) was measured by the method of Trinder (1969). Plasma cholesterol was determined according to the method of Watson (1960). The alkaline phosphatase (ALP) was assayed in blood plasma by the method of Kind and King (1954).

Statistical evaluation

Data were statistically analyzed using one way ANOVA of SAS® (SAS Institute, 1985; Cary, NC, USA). All percentage data were transformed with Arc sin prior to statistical analysis. Means separation was performed using Duncan's multiple range test and differences were declared at $P<0.05$.

Results

The results for the effect of heat stress and different feed additives on growth performance of broilers, digestibility of nutrients and mortality rate are shown in Table 2. Growth, feed intake and FCR of broilers during 1–42 d periods were significantly affected by treatments. There were significant effects of heat stress and feed additives on digestibility of dry matter and crude protein. The heat stressed group (CHS-treatment) had lower BWG and FI and higher FCR compared to the thermoneutral group (TN) ($P<0.0001$). Vit E, Vit C, Vit E+C, Probiotics, and the combination groups showed improvement on BWG compared to the heat stressed group during

1–42 d periods. The combination group (Probiotic + Vit E + Vit C) had the highest BWG among the chronic heat stress (CHS) groups. The Vit E and probioitc groups consumed less feed than the thermoneutral group, but the other groups had similar feed intake to the thermoneutral and the heat stressed groups. For FCR, Vit E, Vit C, Vit E+C, probiotic, and the combination groups significantly improved FCR compared to the heat stressed group. However, Vit E+C, probiotic and Vit E+C+probiotic were the most effective treatments among the treatments. There was no difference in mortality among the treatments.

The heat stressed group reduced crude protein digestibility compared to the thermoneutral group ($P<0.05$). Probiotic treatment improved dry matter digestibility compared to the heat stressed group. There were no differences in ether extract, crude fiber and crude ash digestibility among the treatments ($P>0.05$).

Dressing percentage and abdominal fat are presented in Table 3. The heat stressed group reduced dressing percentage compared to the thermoneutral group and Vit E group ($P<0.05$). Vit E and the combination groups had higher abdominal fat % as compared to the heat stressed group. However, there were no signifcant effects of different treatments on other carcass traits and chemical or physical parameters of meat (Table 3).

Hgb, PCV, phagocyte activity and most of WBC fractions except for eosinophils were significantly affected by heat stress and feed additives treatments, however, the effect of treatments on RBCs and WBCs were not significant (Table 4). Probiotic and the combination groups exhibited higher Hgb than Vit E+C. The heat stressed group reduced PCV as compared to the thermoneutral group, whereas Vit C, probiotic and the combination groups showed significantly increased PCV as compared to the heat stressed group. The lymphocytes % of Vit C was higher than that of the heat stressed group ($P<0.05$). The heat stressed group had lower monocyte and basophile % as compared to the thermoneutral group. Vit E+C increased monocyte number as compared to the heat stressed group ($P<0.05$), whereas Vit E, Vit C, Vit E+C, and Vit E+C+probiotics groups increased basophile count as compared to the heat stressed group. Vit C reduced heterophil % and heterophil/lymphocyte ratio as compared to the heat stressed group. The impacts of heat stress and dietary treatments on blood plasma, total protein, albumin, globulin, liver enzymes (AST, ALT and alkaline phosphatase), plasma glucose and cholesterol are presented in Table 5. The heat stress group showed significantly lower total protein than the thermoneutral group ($P=0.0001$), whereas Probiotic and combination treatments had significantly higher total protein as compared to the heat stress group. However, there were no significant differences among the heat stress, Vit E, Vit C, and Vit E+C groups. For albumin levels, the heat stress group had significantly lower albumin level than the thermoneutral group and Vit C, Vit E, Vit E+C, Probiotic, and Vit E+C+probiotics groups ($P=0.0001$). For globulin and alkaline phosphate levels, there were no significant differences between the heat stress and the thermoneutral groups. The heat stress group had significantly lower blood glucose level as compared to the thermoneutral group ($P=0.0001$), whereas Vit E+C, probiotic, and Vit E+C+probiotics increased glucose levels as compared to the heat stress group ($P=0.0001$). However, the heat stress group increased AST as compared to the thermoneutral group, Vit E, Vit C, Vit E+C, probiotic, and Vit E+C+probiotic ($P=0.0001$).

Table 2. Growth performance, nutrient digestibility and number of death of broiler chickens as affected by chronic heat stress and addition of vitamins and probiotics (Mean±SD)

Criteria	TN*	CHS†	Vitamin E	Vitamin C	Vitamin E+C	Probiotic	Probiotic + Vitamin E + Vitamin C	P-value
Body weight gain (g)								
1–42 day	1830 a±56.0	1623 d±60.0	1670 c±55.5	1684 c±37.0	1730 b±34.5	1736 b±28.0	1794 ab±63.0	0.0001
Feed intake (g/period)								
1–42 day	3284 a±58.0	3091 b±58.0	3087 b ±82.3	3141 ab±76.8	3133 ab±75.9	3089 b±73.2	3123 ab±52.4	0.0001
Feed conversion ratio (g feed/g gain/period)								
1–42 day	1.79 bc±0.03	1.90 a ±0.02	1.85 ab±0.06	1.87 ab ±0.04	1.81 bc ±0.04	1.78 bc±0.05	1.74 c ±0.07	0.001
Digestibility of nutrients (%)								
dry matter	89.9 ab±0.87	88.9 b±0.96	88.3 b±1.39	89.2 ab±0.83	88.7 b±0.58	90.2 a±0.49	89.1 ab±0.49	0.002
crude protein	84.9 a±1.13	82.9 b ±0.93	83.5 ab±1.91	83.1 ab±1.74	81.1 b±2.76	83.5 ab±0.85	81.5 b±1.23	0.001
ether extract	83.9±1.15	84.0±1.06	83.9±0.59	84.0±0.63	83.2±1.22	84.1±0.74	83.3±0.82	NS
crude fiber	34.1±1.82	32.2 ±0.92	33.6±2.62	32.1±2.80	29.6±3.74	33.2±2.78	3.35±75.6	NS
crude ash	38.7±2.71	40.0 ±4.41	40.5 ±4.36	39.2±3.90	33.9±7.75	40.9±2.31	34.7±4.45	NS
Mortality rate (%)								
1–42 day	4.8±0.48	7.1±0.62	4.8±0.48	4.8±0.48	4.8±0.48	7.1±0.62	4.8±0.54	NS

a, b, c, d – means on the same row followed by a different letter are significantly different ($P<0.05$).
NS = not significant. N = 5.
*TN = Thermoneutral group; †CHS = Chronic heat stress group.

Table 3. Carcass and meat quality characteristics of broiler chickens as affected by chronic heat stress and addition of vitamins and probiotics (Mean±SD)

Trait (%)	TN*	CHS†	Vitamin E	Vitamin C	Vitamin E+C	Probiotic	Probiotic + Vitamin E + Vitamin C	P-value
Dressing percentage and the percentages of internal organs (%)								
dressing percentage (%)	73.3 a±3.80	71.2 b±3.80	73.7 a±2.70	71.9b± 1.50	71.4b ±2.2	70.2 b ±3.30	70.7b±3.60	0.001
The percentage of internal organs[1]								
abdominal fat	0.78 ab±0.30	0.50 b±0.30	0.97 a±0.49	0.55 b±0.36	0.55 b±0.32	0.83 ab±0.52	0.90 a±0.49	0.003
liver	2.47±0.38	2.47±0.38	2.40±0.32	2.66±0.61	2.60±0.36	2.51±0.42	2.53±1.04	NS
gizzard	1.71±0.32	1.71±0.32	1.75±0.27	1.71±0.26	1.78±0.33	1.83±0.28	1.55±0.30	NS
heart	0.53±0.13	0.53±0.13	0.51±0.04	0.54±0.07	0.54±0.08	0.52±0.04	0.46±0.08	NS
pancreas	0.25±0.04	0.25±0.04	0.24±0.04	0.20±0.05	0.22±0.04	0.21±0.02	0.21±0.07	NS
intestinal weight	4.63±0.89	4.63±0.89	4.62±0.54	4.61±0.79	4.27±0.32	4.67±0.56	4.37±0.91	NS
Chemical composition of meat (%)								
dry matter	24.1±0.17	25.1±0.13	25.0±0.10	25.0±0.26	24.9±0.27	25.1±0.34	25.1±0.28	NS
protein	19.1±0.23	18.9±0.16	18.8±0.37	18.7±0.29	18.8±0.45	18.8±0.34	18.9±0.43	NS
lipids	4.87±0.189	5.01±0.29	4.90±0.28	5.10±0.35	4.90±0.29	5.02±0.28	4.96±0.25	NS
ash	1.12±0.04	1.18±0.03	1.18±0.05	1.19±0.05	1.20±0.04	1.19±0.02	1.20±0.04	NS
Physical characteristics of meat								
pH	6.66±0.12	6.70±0.10	6.70±1.10	6.60±0.12	6.60±0.15	6.70±0.09	6.60±0.17	NS
color (optical density)	0.213±0.02	0.212±0.02	0.221±0.03	0.211±0.03	0.214±0.02	0.199±0.02	0.203±0.02	NS
tenderness (cm^2/0.3 g)	2.83±0.17	2.83±0.17	2.90±0.20	2.80±0.20	2.90±0.15	2.80±0.24	2.86±0.20	NS
WHC[1] (cm^2/0.3 g)	4.95±0.24	4.85±0.21	4.92±0.21	4.86±0.22	4.85±0.23	4.84±0.12	4.85±0.20	NS

a, b, c, d – means on the same row followed by a different letter are significantly different ($P<0.05$).
NS = not significant. N = 8.
[1] The percentage of internal organ weights to pre-slaughter live weight.
WHC = water holding capacity.
*TN = Thermoneutral group; †CHS = Chronic heat stress group.

Table 4. Hematological and immunological parameters of broiler chickens as affected by chronic heat stress and addition of vitamins and probiotics (Mean±SD)

Trait[1]	TN*	CHS†	Vitamin E	Vitamin C	Vitamin E+C	Probiotic	Probiotic + Vitamin E + Vitamin C	P-value
Red blood cell characteristics								
RBCs (10^6/mm^3)	2.15±0.06	2.11±0.06	2.23±0.16	2.02±0.13	2.21±0.24	2.03±0.16	2.27±0.2	NS
Hgb (g/Dl)	9.5 a±0.83	8.5 ab ±0.93	8.6 ab±0.74	9.2 ab±0.27	8.1 b±0.54	9.6 a±0.54	9.5 a±0.9	0.01
PCV (%)	28.9 a±1.98	24.9 b±1.74	26.5 ab±0.9	27.9 a±0.65	26.8 ab±0.8	28.0 a±0.35	28.3 a ±2.7	0.008
White blood cells characteristics								
WBCs (10^3/mm^3)	22.5±0.85	21.5±0.61	22.2±1.3	21±0.35	22.7±1.2	22.4±1.1	22.6±1.02	NS
lymphocytes (%)	48.4 ab±0.85	46.4 bc±0.55	47.4 ab±0.93	49.1 a±0.5	47.7 ab±1.8	48.6 ab±1.1	45.2 c ±1.8	0.001
monocyte (%)	1.7 a±0.35	1.0 b ±0.35	1.4 ab ±0.42	1.4 ab ±0.42	1.8 a±0.3	1.2 ab ±0.3	1.6 ab ±0.4	0.03
basophil (%)	8.6 a±0.27	7.2 c±0.27	8.3 ab ±0.27	8.1 ab±0.42	8.0 ab ±0.7	7.7 bc ±0.8	8.7 a±0.3	0.002
eosinophil (%)	9.3±1.3	9.3±1.3	9.2±0.57	9.2±0.83	9.6±0.54	9.4±1.1	9.3±0.8	NS
heterophil (%)	32.0 ab±1.89	36.1 a±0.89	33.7 ab±0.57	32.2 b±1.48	32.9 ab±2.63	33.1 ab±2.24	35.2 ab±2.20	0.02
H/L ratio	66.1 b±2.15	77.8 a±1.57	71.1 ab±0.74	65.6 b±2.85	68.9 ab±1.37	68.1 ab±2.04	77.9 a±1.15	0.01
Antibody titer for Newcastle disease virus ($\log^2$/day)								
20 day of age	4.2±0.83	3.2±0.83	3.4±0.92	3.6±0.54	4.2±0.44	4.4±0.5	4.0±1.0	NS
42 day of age	3.4±0.54	3.4±0.54	3.0±0.73	3.6±0.54	2.8±1.1	4.0±0.77	3.2±0.82	NS
Phagocytes (%)								
activity	21.4 a±0.41	20.4 ab±0.41	21.3 a±0.27	19.9 b±0.65	19.3 b±0.83	19.7 b±0.8	20.0 b±1.1	0.004
index	1.71±0.16	1.72±0.16	1.61±0.2	1.81±0.04	1.63±0.16	1.73±0.3	1.68±0.2	NS
Lymphoid organs (%)								
spleen	0.151±0.08	0.178±0.08	0.139±0.06	0.151±0.04	0.149±0.05	0.136±0.03	0.141±0.05	NS
bursa of fabricius	0.087±0.06	0.117±0.06	0.071±0.04	0.116±0.05	0.101±0.03	0.112±0.05	0.113±0.04	NS

a, b, c, d – means on the same row followed by a different letter are significantly different (P<0.05). NS = not significant. N = 8.
[1]RBCs = red blood cells; Hgb = hemoglobin; PCV = packed cell volume; WBCs = white blood cells; H/L ratio=heterophile/lymphocyte ratio.
*TN = Thermoneutral group; †CHS = Chronic heat stress group.

Table 5. Biochemical blood parameters of broiler chickens as affected chronic heat stress and addition of vitamins and probiotics (Mean±SD)

Trait	TN*	CHS†	Vitamin E	Vitamin C	Vitamin E+C	Probiotic	Probiotic+ Vitamin E + Vitamin C	P-value
Total protein (mg/dL)	5.30 a±0.25	4.40 b±0.15	4.40 b±0.30	4.70 b±0.08	4.70 b±0.19	5.10 a±0.25	5.20 a±0.15	0.0001
Albumin (mg/dL)	3.25 a±0.15	2.30 c±0.13	2.20 c±0.15	2.80 b±0.19	3.00 b±0.20	3.30 a±0.20	3.30 a±0.11	0.0001
Globulin (mg/dL)	2.05 ab±0.25	2.10 ab±0.27	2.20 a±0.27	1.90 ab±0.22	1.70 b±0.25	1.80 b±0.20	1.90 ab±0.20	0.01
Glucose (mg/dL)	88.8 a±0.71	82.8 c±0.84	82.6 c±2.10	84.2 bc±0.83	86.4 ab±2.10	86.4 ab±0.50	87.8 a±1.30	0.0001
Alkaline phosphate (Iu/dL)	16.5 bc±1.23	18.0 ab±1.87	19.4 a±1.34	19.4 a±1.34	16.0 bc±1.22	16.4 bc±0.50	15.4 c±1.30	0.0001
Cholesterol (mg/dL)	178 c±4.40	198 ab±4.40	194 b±3.50	205 a±4.60	205 a±3.04	203 a±3.10	202 a±3.60	0.0006
AST (U/L)	62.4 c±1.31	72.4 a±1.51	66.6 bc±2.70	70.8 a±0.83	64.2 c±0.44	64.4 c±2.20	68.0 b±1.6	0.0001
ALT (U/L)	60.4 b±1.31	61.4 b±1.14	62.6 b±1.70	62.4 b±1.14	63.4 b±1.70	65.8 a±0.80	62.2 b±0.80	0.0003

a, b, c, d – means on the same row followed by a different letter are significantly different ($P<0.05$). AST = aspartate aminotransferase. ALT = alanine aminotransferase.
*TN = Thermoneutral group; †CHS = Chronic heat stress group. N=8.

Discussion

The chronic heat stress group (CHS group) reduced BWG (–11.3%) during 1–42 d of age compared to the thermoneutral group (TN). The heat stressed group also reduced protein digestibility compared to the thermoneutral group. Moreover, FI of the heat stressed group was decreased as compared to the thermoneutral group, suggesting that FI is the major cause for growth retardation under the CHS (Attia et al., 2017). This could be explained by 52.2% decrease in BWG of the CHS group. These findings are in agreement with other related studies. Attia et al. (2011) indicated that 63% reduction in growth of fast-growing broilers at high ambient temperature was due to the decrease in FI. CHS influences the peripheral thermal receptors which transmit nerve impulses that suppress the activity of the appetite center in the hypothalamus causing the FI reduction (Marai et al., 2007). Therefore, less nutrients and molecules become available for enzymatic activities and syntheses, hormone production and heat generation. According to NRC (1994) guidelines, broiler FI is depressed (–1.5%) for each rise of 1°C above the ambient temperature zone (10 to 35°C), which explains the adverse effects on BWG under CHS.

In the present study, broilers exposed to CHS during 25–42 d of age increased FCR compared to the thermoneutral group, suggesting that heavier and older chickens are more susceptible to CHS. Our results are coincided with those reported by Faria Filho et al. (2006) where the direct effects of high environmental temperatures account for 39% of growth reduction and for 100% of poor FCR of heat-exposed broilers.

It was found that CHS decreased dressing percentage (–2.9%) and numerically abdominal fat (–35.9%), whereas other carcass and organ traits as well as meat quality traits were not influenced by CHS in the current study. The decrease in dressing percentage (Table 4) might be attributed to the decrease in protein digestibility and feed intake reduction for tissue growth. Similar to the present findings, Attia et al. (2011) indicated that CHS decreased dressing, liver and giblets but did not affect other carcass and organs traits. Moreover, Shoukry (2001) found that high environmental temperature had no effects on body organ and tissue weights and composition, including carcass, gizzard and heart weights as well as dressing percentage of broiler chickens. On the other hand, it has been reported that broiler chickens exposed to 32°C for 2 wk (from 4 to 6-wk old) had higher abdominal fat %, whereas they had lower absolute and relative weights of breast muscle as compared to the control group at 22°C (Temim et al., 2000).

Heat stress caused significant adverse effects on PCV (–14.1%), monocyte (–41.2%), basophil (–16.3%), total protein (–17%), albumin (–29.2%) and glucose (–6.8%) compared to the thermoneutral group, but increased heterophil/lymphocyte ratio (+17.7), serum cholesterol (+11.2%) and AST (+16%). The decrease in PCV, monocyte, basophil, total protein, albumin and glucose and the increase in heterophil/lymphocyte ratio, cholesterol and AST are clear evidence for the adverse effect of CHS on hematological and immunological traits. Similarly, Attia et al. (2011) reported that CHS impaired hematological and immunological traits of fast- and slow-growing meat type chickens.

In the current study, Vit E, C, or probiotics decreased the effect of CHS on growth performance throughout the experimental period. Especially, the combination of probiotics, Vit E, and Vit C considerably increased BWG and FI and improved FCR compared to the heat stressed group. On the other hand, Vit C and E were equally potent during the experimental period, but were less effective than the combination of probiotics, Vit E and Vit C. Other researchers (Sahin et al., 2003; Ciftci et al., 2005) have reported that dietary supplementation of Vit C and E in chickens increased productivity of chickens although there was inconsistency in the results among the studies. In this regard, Sahin et al. (2002) noted that a combination of these two vitamins offers a good management tool and hence higher livability at high temperatures. However, Ipek et al. (2007) concluded that Vit C or Vit E supplementation alone is adequate to alleviate CHS problems in quails. Similarly, Sahin et al. (2003) found that supplementation of 200–250 ppm of Vit C improved DM, OM, CP and EE digestibility of Japanese quails exposed to a high temperature (33°C).

Probiotics were more efficient especially during 1–42 d of age (7%) than Vit E or C in the present study. Similar results were found with the combination of both vitamins on BWG and FCR. Kalavathy et al. (2008) also reported that probiotic (*Lactobacillus* spp.) improved BW, BWG and FCR and reduced mortality of broilers. Digestibility coefficients of DM of groups supplemented with probiotics might have contributed to the increase in growth performance of these groups in the current study. Stronger effects of the combination of vitamins and probiotics on BWG and FCR could be explained by the synergistic effect of probiotics (in gut functioning) and both vitamins (in antioxidant property).

In the current study, Vit E increased dressing and abdominal fat, but did not affect other carcass criteria, organs, and meat quality traits. Romboli et al. (1997) also reported that no difference in chemical composition of breast muscles was observed in chickens fed different levels of α-tocopheryl acetate (20 or 200 mg/kg feed).

Vit C alone increased PCV (+12.0%), lymphocytes (+5.8%), monocytes (40%), basophils (+12.5%) and albumin (+21.7%) and reduced heterophils (–10.8%) and heterophil/lymphocyte ratio (–15.7) compared to the heat stressed group in the present study, indicating the role of Vit C in improving chickens' tolerance to CHS. The increase in PCV by Vit C supplementation is in agreement with the results of Attia et al. (2011). Similarly, Sahota et al. (1994) found that 50 and 100 mg Vit C/kg diet increased PCV of chickens exposed to CHS.

Vit E decreased AST (–8.0%) as compared to the heat stressed group (CHS). On the other hand, serum albumin (–27.3%), cholesterol (–5.4%) and AST (–5.9%) were lower in this group as compared to Vit C group in the present study. These findings indicate an improvement in liver functions and an increase in immune status due to Vit E supplementation. Vit E ($P<0.05$) increased RBC and WBC but did not affect PCV, serum AST, total protein, albumin, globulin, total lipids and triglycerides. On the other hand, serum cholesterol was higher (+8.93%; $P<0.05$) than that of the control. Similar results were reported by other researchers (El-Sebai, 2000; Allen and Fetterer, 2002). In the current study, supplementation of both vitamins did not surpass the effect of Vit E or C alone except for alkaline phosphatase which was decreased to the level of thermoneutral group (TN).

Probiotics had similar effect to Vit E or C and their combination. However, probiotics had stronger effects on serum total protein, albumin and ALT. A combination of both vitamins and probiotics had similar effects to probiotics alone except for lymphocytes, AST and ALT. In addition, Tollba et al. (2004) also showed that probiotics (lactobacillus, pediococcus, Lacto Sacc and Yea Sacc) under thermoneutral and heat stress conditions increased plasma protein, albumin and globulin fractions, glucose and thyroid hormone (T3), whereas it decreased plasma cholesterol and total lipids but did not affect creatinine, ALT and AST. Supplementation of Vit E increased phagocytes activity to the level of the thermoneutral group which showed higher phagocytes activity compared to Vit E, Vit C, a combination of Vit E and Vit C, or a combination of Vit E, Vit C and probiotics.

Conclusion

CHS decreased performance and physiological response of broiler chicks, whereas supplementation of Vit E, Vit C, probiotics, and their different combinations reduced the adverse effects of CHS. However, mechanisms by which these additives improve growth performance, carcass yield, and blood parameters should be elucidated in order to develop more effective strategies for broiler chickens under CHS.

References

Allen P.C., Fetterer R.H. (2002). Interaction of dietary vitamin E with *Eimeria maxima* infections in chickens. Poultry Sci., 81: 41–48.

Association of Official Analytical Chemists, AOAC (1995). Official Methods of Analysis, 15th ed., Association of Official Analytical Chemists, Washington, DC, USA.

Attia Y.A., Hassan S.S. (2017). Broiler tolerance to heat stress at various dietary protein/energy levels. Europ. Poultry Sci., 81, DOI: 10.1399/eps.2017.171.

Attia A.I., Hassan I.I., El-Zaiat A.A., Abd El-Maksoud A.A. (2003). Effect of dietary oil and ascorbic acid on the performance of broiler chicks under Egyptian summer conditions. Egypt. J. Nutr. Feed., 6: 3–4.

Attia Y.A., Böhmer B.M., Roth-Maier D.A. (2006). Responses of broiler chicks raised under constant relatively high ambient temperature to enzymes, amino acid supplementations, or diet density. Arch. Geflugelkd., 70: 80–91.

Attia Y.A., Hassan R.A., Qota M.A. (2009 a). Recovery from adverse effects of heat stress on slow-growing chicks in the tropics 1: Effect of ascorbic acid and different levels of betaine. Tropic. Anim. Health Prod., 41: 807–818.

Attia Y.A., Hussein A.S., Tag El-Din A.E., Qota E.M., El-Sudany A.M. (2009 b). Improving productive and reproductive performance of dual purpose crossbred hens in the tropics by lecithin supplementation. Tropic. Anim. Health Prod., 41: 461–475.

Attia Y.A, Hassan R.A., Tag El-Din A.E., Abou-Shehema B.M. (2011). Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow-growing chicks exposed to chronic heat stress. Anim. Phys. Anim. Nutr., 95: 744–755.

Attia Y.A., El-Tahawy W.S., Abd El-Hamid A.E., Hassan S.S., Antonino Nizza, El-Kelaway M.I. (2012). Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Ital. J. Anim. Sci., 11:e56:303:308.

Attia Y.A., Bovera F., Abd El-Hamid A.E., Tag El-Din A.E., Al-Harthi M.A., El-Shafy A.S. (2016). Effect of zinc bacitracin and phytase on growth performance, nutrient digestibility, carcass and meat traits of broilers. J. Anim. Phys. Anim. Nutr., 100: 485–491.

Bovera F., Moniello G., De Riu N., Di Meo C., Pinna W., Nizza A. (2007). Effect of diet on the metabolic profile of ostriches (*Struthio camelus* var. domesticus). Trop. Anim. Health Prod., 39: 265–270.

Ciftci M., Nihat E.O., Guler T. (2005). Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. Rev. Med. Vet. – Toulouse, 156: 107–111.

Daghir N. (2008). Nutrient requirements of poultry at high temperature. In: Poultry Production in Hot Climates, Daghir N.J. (ed.). 2nd ed., CAB Publishing.

Doumas B.T., Watson D., Biggs H.G. (1977). Albumin standards and the measurement of blood albumin with bromocisol green. Clin. Chim. Acta, 7: 87–96.

Ehran M.K., Bölükbaş S.C. (2016). Effects of feeding diets supplemented with vitamin E and vitamin C on performance, egg quality and stereological and structural analysis of the liver of laying hens exposed to heat stress. Ital. J. Anim. Sci., 10:4, e58, doi:10.4081/ijas.2011.e58

Eilers R.I. (1967). Notification of final adaptation of an international method and standard solution for hemoglobinometry, specifications for preparation of standard solution. Am. J. Clin. Pathol., 47: 212–214.

El-Sebai A. (2000). Influence of selenium and vitamin E as antioxidants on immune system and some physiological aspects in broiler chicks. Egypt. Poultry Sci. J., 20: 1065–1082.

Faria Filho D.E., Rosa P.S., Vieira B.S., Macari M., Furlan R.L. (2006). Protein levels and environmental temperature effects on carcass characteristics, performance, and nitrogen excretion of broiler chickens from 7 to 21 days of age. Brazil. J. Poultry Sci., 7: 247–253.

Hepler O.E. (1966). Manual of clinical laboratory methods. Thomas, Springfield, Illinois.

Hinton A., Buhr R.J., Ingram K.D. (2000). Reduction of Salmonella in the crop of broiler chickens subjected to feed withdrawal. Poultry Sci., 79: 1566–1570.

Husani S.A., Deatherage F.B., Kunlkle L.E. (1950). Studies on meat.11: Observations on relation of biochemical factors to change in tenderness. Feed Technol., 4: 366–369.

Ipek H., Avci M., Yerturk M., Iriadam M., Aydilek N. (2007). Effects of ascorbic acid and vitamin E on performance and hematological parameters of Japanese quails under high ambient temperature in Sanliurfa. Arch. Geflugelkd., 71: 130–134.

Jakobsen P.E., Gertov K., Nilsen S.H. (1960). Digestibility experiments with poultry (in Danish). Beret. Forsögslab. Kobenhavn, 322: 1–43.

Kabir S.M.L (2009). The role of probiotics in the poultry industry. Int. J. Mol. Sci., 10: 3531–3546.

Kai O.H., Nagase N., Ishikawa M., Suzuki K., Sato K. (1988). Effects of propylthiouracial PTU on the immunological status of the chickens. Dev. Comp. Immunol., 12: 145–149.

Kalavathy R., Abdullah N., Jalaludin S., Wong C.M.V.L., Ho Y.W. (2008). Effect of lactobacillus cultures and oxytetracycline on the growth performance and serum lipids of chickens. Int. J. Poultry Sci., 7: 385–389.

Kind P.R.N., King E.J. (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol., 7: 322–326.

Kizerwetter-Swida M., Binek M. (2016). Assessment of potentially probiotic properties of Lactobacillus strains isolated from chickens. Pol. J. Vet. Sci., 19: 15–20.

Leijh P.C., Van Furth R., Van Zwet T.L. (1986). *In vitro* determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes. In: Handbook of Experimental Immunology, Weir M.D., Herzenberg L.A., Blackwell C. (eds). Oxford, Blackwell Scientific Publications, pp. 1–21.

Lin Y.F., Change S.J., Yang J.R., Lee Y.P., Hsu A.L. (2005). Effects of supplemental vitamin E during the mature period on the reproduction performance of Taiwan native chicken cockerels. Brit. Poultry Sci., 46: 366–373.

Lucas A.M., Jamroz C. (1961). Atlas of Avian Hematology. Agriculture Monograph. 25, USDA, Washington, DC, 271 pp.

Marai I.F.M., El-Darawany A.A., Fadiel A., Abdel-Hafez M.A.M. (2007). Physiological traits as affected by heat stress in sheep – a review. Small Rumin. Res., 71: 1–12.

McKee J.S., Harrison P.C. (1995). Effects of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stresses. Poultry Sci., 74: 1772–1785.

National Research Council (NRC) (1994). Nutrient Requirements of Poultry. 9th ed. Nat. Acad. Press., Washington DC, USA.

Niu Z.Y., Liu F.Z., Yan Q.L., Li W.C. (2009). Effects of different levels of vitamin E on growth performance and immune responses of broiler chickens under heat stress. Poultry Sci., 88: 2101–2107.

Rajput A.B., Kolte B.R., Shisodiya J.M., Chandankhede J.M., Chahande J.M. (2009). Effect of vitamin A, vitamin C, vitamin E and Levamisole on performance of broilers. Vet. World, 2: 225–227.

Reitman S., Frankel S. (1957). Calorimetric method for the determination of blood aminotransferase enzymatic activities. Am. J. Clin. Pathol., 28: 56–63.

Romboli I., Russo C., Zanobini S. (1997). Effect of dietary vitamin E on chemical composition and meat colour in heat stressed muscovy duck. Poult. Meat Qual., 5: 205–211.

Sahin K., Kucuk O., Sahin N., Sari M. (2002). Effects of vitamin C and vitamin E on lipid peroxidation status, serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (34 degrees C). Int. J. Vit. Nutr. Res., 72: 91–100.

Sahin K., Sahin N., Onderci M., Gursu M.F., Lssi M. (2003). Vitamin C and E can alleviate negative effects of heat stress in Japanese quails. J. Food Agr. Environ., 1: 244–249.

Sahota A.W., Gillani A.H., Ullah M.F. (1994). Haematological studies on heat stressed chickens supplemented with ascorbic acid. Pakistan Vet. J., 14: 30.

Shoukry H.M.S. (2001). Grow-out performance and carcass characteristics of heat-stressed broiler chicks as affected by high dietary tryptophan. Egypt. Poultry Sci. J., 21: 1059–1078.

Temim S.A., Chagneau M., Guillaumin S., Mitchel J., Peresson R., Tesseraud S. (2000). Does excess dietary protein improve growth performance and carcass characteristics in heat exposed chickens. Poultry Sci., 79: 312–317.

Tollba A.A.H., Sabry M.M., Medani G.G. (2004). Effect of microbial probiotics on performance of broiler chicks under normal or heat stress conditions. 1- lactobacillus or pediococcus. Egypt. Poultry Sci. J., 24: 351–367.

Traber M.G., Atkinson J. (2007). Vitamin E, antioxidant and nothing more. Free Rad. Biol. Med., 43: 4–15.

Trinder P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24–27.

Varasteh S., Braber S., Akbari P., Garssen J., Fink-Gremmels J. (2015). Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galactooligosaccharides. Plos One, 10: e0138975.

Vathana S., Kang K., Loan C.P., Thinggaard G., Kabasa J.D., Meulen U. (2002). Effect of vitamin C supplementation on performance of broiler chickens in Cambodia. Deutscher Tropentag 2002. Proc. Conference on International Agricultural Research for Development, 9–11.10.2002, Witzenhausen, Germany.

Volvoinskaia V.P., Kelman B.Y. (1962). Modification of water holding capacity method of meat. F. D. Industry. Musco., 11: 80.

Watson D. (1960). A simple method for determination of serum cholesterol. Clin. Chem. Acta, 5: 637–643.

Weichselbaum T.E. (1946). An accurate and rapid method for determination of proteins in small amount of blood, serum and plasma. Am. J. Clin. Pathol., 16: 40–48.

Zeferino C.P., Komiyama C.M., Pelicia V.C., Fascina V.B., Aoyagi M.M., Coutinho L.L., Moura A.S.A.M.T. (2016). Carcass and meat quality traits of chickens fed diets concurrently supplemented with vitamins C and E under constant heat stress. Animal, 10: 163–171.

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