

## ALLEVIATION OF CHRONIC HEAT STRESS IN BROILERS BY DIETARY SUPPLEMENTATION OF NOVEL FEED ADDITIVE COMBINATIONS

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**Abstract:** Heat stress causes economic losses via decreasing feed intake, nutrient digestion, weight gain, feed conversion, immunity, carcass quality and increasing mortality in broilers. The aim of the study was to assess the ability of dietary additive combinations to ameliorate the detrimental effect caused by chronic heat stress ( $32 \pm 2^\circ\text{C}$  for 24 h per d) on performance, carcass traits, metabolic status and economic efficiency of broiler chickens. A total of 420, one-day old Cobb-500 broiler chicks were assigned into seven treatment groups (n=60) of five replicates. Each replicate contained 12 unsexed chicks. The control groups did not receive any supplementation to the basal diet (thermoneutral control (TNC) and heat stress control (HSC) groups). The other groups received control diet supplemented with 1 % cumin plus 1 % turmeric powders (T1); 1.5 g/kg potassium chloride plus 2 g/kg sodium bicarbonate (T2); 1000 ppm propolis plus 15000 IU vitamin A (T3); 1200 ppb chromium plus 500 ppm vitamin C (T4) ; 1200 ppm betaine plus 500 ppm vitamin E (T5). The results indicated that at 42 d of age, all dietary additive combinations improved the growth performance indices, carcass traits, concentrations of serum antioxidant enzyme biomarkers, stress biomarkers and economic efficiency in comparison to HSC group. Based on the obtained results, it could be concluded that dietary supplementation with betaine and vitamin E followed by chromium and vitamin C combinations offers a good management practice for alleviating heat stress related depression in the performance of broiler chickens.

**Key words:** heat stress; broilers; vitamin C; betaine; chromium; propolis; turmeric

### Introduction

The most obvious constraint on poultry production in Saudi Arabia, especially during summer months is high temperature with high humidity, which causes severe stress on birds and reduces performance. The continuous challenge

for the poultry production in tropical and sub-tropical region all over the world remains heat stress. Hot environments making several alterations in blood hormones, glucose levels, leukocytes, electrolytes and organ functions (1).

Heat stress causes economic losses via decreasing feed intake, nutrient digestion, weight gain (WG), egg production, feed conversion (FC), immunity, carcass quality and increasing mortality (2).

Heat stress trigger the hypothalamo-hypophyseal adrenocortical axis to secrete corticosteroids (3). The catabolic effects of higher blood corticosteroids elevate the free radicals through changing oxidative metabolism, producing huge damage of cellular functions and cell membrane, which causing muscle degeneration and growth retardation (3). The greatest part of the energy production is directed to thermoregulation mechanisms, which develop stress-induced immunosuppression. The immune suppressed birds are predisposed to numerous dangerous diseases associated with high mortality rates (4)

Dietary modifications by the use of commercial feed additives are considered the most favored and practical ways to mitigate the effect of heat stress in poultry (5). Therefore, dietary inclusion of some selected feed additives could also be effective for the preservation of growth and immunity for broilers during periods of heat stress. Many previous studies illustrated the beneficial effect different dietary supplements on the performance of broilers under heat stress independently (6, 7, 8, 9, 10, 11, 12). These studies revealed that Vitamin A, E, C, play important roles in the performance and immune function of poultry under heat stress. Betaine is a methyl donor and it has methionine sparing effect, help in maintaining cellular water balance, protect the integrity of cell membrane and improve broiler performance and to overcome stress. In addition, the supplementation of dietary electrolytes increase tolerance to heat stress improve performance of broilers under heat stress. Electrolyte prevent acid base balance disturbances and decrease the occurrence of respiratory alkalosis during heat stress. Herbal additive like cumin, turmeric and propolis very rich in flavonoid and aromatic acids and they have antioxidant, anti-inflammatory, antibacterial, antifungal activity and have a positive effect on broiler performance under heat

stress. However, few studies have been conducted to detect the best combination at the recommended concentrations suggested by previous studies to mitigate the decline in performance caused by heat stress in broiler chickens. Therefore, the aim of the current study was to detect the best commercial additive combinations to ameliorate the detrimental effect caused by chronic heat stress on performance, carcass traits, metabolic status, antioxidant capabilities and economic efficiency of broiler chickens.

## Material and methods

### *Experimental animals and protocol*

The current experiment was accomplished according to King Faisal University Animal Care and Use Committee and the experimental protocol was approved by the Deanship for Scientific Research with reference number (Decision No: KFU-REC/2017-04-01).

The experiment was carried out on 420 one-day-old broiler chickens (Cobb - 500) purchased from a marketable hatchery (Al-Ahsa, KSA). Birds were housed in pens with wood shavings floor with 10 birds/m<sup>2</sup> at the Research and Agriculture Station, King Faisal University, Al-Ahsa, Saudi Arabia from 1 to 42 d old. Water and feed provided ad libitum during 24 hours. Constant observation for health status and behavior usually carried out for broiler chickens. The temperature was kept at  $32 \pm 2$  °C with 65 % relative humidity (RH) for 24 h for all treatment groups except thermoneutral control (TNC). For maintaining the desired temperature and relative air humidity air conditioners, heaters with thermostats, and air circulators were applied. The thermoneutral control group (TNC) were held at thermoneutral chamber with normal ambient temperature ( $22 \pm 4$  °C) and relative humidity (RH) of commercial management program ( $55 \pm 3$  %, RH) at the same experimental place.

### *The experimental design*

A total of 420 one-day-old unsexed broiler chickens (Cobb - 500) randomly allocated to 7 experimental groups (60 chick/each). Each group consisted of 5 replicate pens with 12

chicks each. The control groups did not receive any supplementation to the basal diet (thermoneutral control (TNC) and heat stress control (HSC) groups). The other groups received control diet supplemented with 1 % cumin plus 1 % turmeric powders (T1); 1.5 g/kg potassium chloride plus 2 g/kg sodium bicarbonate (T2); 1000 ppm propolis plus 15000 IU vitamin A (T3); 1200 ppb chromium (Cr) plus 500 ppm vitamin C (T4) ; 1200 ppm betaine (Bet) plus 500 ppm vitamin E (VE) (T5). According to NRC (13) guidelines, fourteen experimental diets, seven in each feeding phase (starting and growing) were formulated. The basal diets contained 23% and 20 % protein and 3200 kcal of ME/kg for starter and grower diets, respectively. Composition and chemical analysis of broiler basal diets are presented in (Table 1). Proximate composition of feed ingredients were performed according to AOAC (14) and the experimental diets were formulated based on these values. The selected feed additives were thoroughly mixed with small amount of feed on the expense of corn, then homogenized with the total amounts and added to the diet. Prophylactic vaccination against viral diseases was carried out. All chicks were immunized with eye drops by weak Newcastle virus vaccine at age of 7 days and with a live attenuated freeze-dried vaccine (Nobilis® Ma5 + Clone 30) at the age of 20 days.

#### *Broiler performance*

Feed consumption and body weight for each pen were recorded weekly during the whole experiment. Average daily gain, feed intake and feed conversion ratio were calculated during starter (1 to 21 d), grower (22 to 42 d), and overall (1 to 42 d) phases.

#### *Blood Sampling*

Ten birds from each group were used for collection of blood samples at days 21 and 42. The samples were collected into two tubes, one contained EDTA (as anticoagulant) and the other had no anticoagulant. Samples were centrifuged at 3500 g for 15 min for gathering plasma and serum, respectively.

#### *Determination of New castle serum antibody titre*

The log10 of serum antibody titer of new castle disease was determined by using commercial ELISA kits (IDEXX® Laboratories, B.V., Netherlands) according to the recommendation of the supplier.

#### *Determination of blood antioxidant enzymes and oxidative stress biomarkers*

The activities of antioxidant biomarkers (glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and malondialdehyde (MDA) were analyzed by spectro-photometrical method with commercial kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All procedures were achieved according to manufacturer's instructions. Plasma corticosterone (CS) levels was assayed by the use of ELISA kit (DetecteX® Corticosterone, EIA kit, Arbor Assays®, USA), according to procedures of Quintero-Filho et al. (3).

#### *Determination of blood biochemical profile*

Serum glucose, total protein, albumin, total cholesterol and globulin levels were analyzed spectrophotometrically by using commercial kits from Spinreact company ® (Spain) and according to manufacturer's instructions.

#### *Heterophil/lymphocyte (H/L) ratio*

At the end of the experiment d 42, 10 birds from each group were used for determination of H/L ratio. Smears were prepared using the standard two-slide wedge procedure for differential leukocyte counts. Subsequently, blood films were air dried and stained with Wright-Giemsa Quick stain. The H/L ratio was calculated according to method adopted from (15)

#### *Carcass characteristics and relative organ weight*

At 42 day of age, 10 bird from each treatment (2 from each pen) were randomly selected and humanely slaughtered according to Islamic method for carcass traits characteristics. The gizzard, bursa of Fabricius, liver, spleen, ab-

dominal fat, heart, thymus, pancreas, and stomach were removed and blot dry weighed. Relative organ weight was calculated as a percentage of body weight.

#### *Economic efficiency*

Economic efficiency was estimated according to Wealleans et al., (16). Economic efficiency (EE) = (Net revenue/ total costs)  $\times$  100. Relative economic efficiency (REE) = Economic efficiency/economic efficiency of the positive control (17)

#### *Statistical analysis*

Data analysis was accomplished by one-way ANOVA using the procedure of SPSS software version 19 (18). Data were tested for normality before analysis by the use of the Kolmogorov–Smirnov test. Duncan is multiple range test (19) was used to detect the differences among means and significance level was set at  $P < 0.05$ .

### **Results**

The effect of diet supplementation with novel feed additives on growth performance of 42-day broilers was presented in (Table 2). The results presented in (Table 2) indicated the presence of statistically significant differences ( $P < 0.05$ ) in the broiler growth performance indices such as body weight, feed intake, body weight gain and feed conversion ratio between the different treatment groups because of the dietary inclusion of all supplements. All dietary additive combinations induced a significant improvement in body weight gain, feed intake, feed conversion rate in birds of treatment groups during the entire period of the study (d 0–42). On the other side, chronic heat stress (HSC group) induced a significant reduction in body weight, weight gain, feed intake, feed conversion efficiency and mortality during the entire period of the study. It is interesting to note that, dietary supplementation with the combination of 1200 ppm betaine and 500 ppm vitamin E followed by 1200 ppb chromium and 500 ppm vitamin C showed the most favorable and the best results regarding all growth performance indices of broilers subjected to heat stress in comparison with other treatment groups.

All the dietary supplements significantly increased ( $P < 0.05$ ) the carcass weight, dressing weight, dressing percentage or carcass yield and relative liver, heart, gizzard weights in comparison with the HSC group (Table 3). The greater relative organ weights were recorded in T5, T4, T3 when compared with T1 and T2. The major impact of the heat stress on the carcass traits was showed in the increase of the relative abdominal fat in the HSC to about 1.98 %, which decreased to 1.03 and 1.12 % in birds receiving both the combination of betaine and vitamin E and the chromium and vitamin C, respectively. The highest dressing percentage value (70.27%) was observed in birds of T5 group in comparison with other treatments groups. The lowest dressing percentage was recorded in the heat stressed control group (68.42 %). The greater response of the dietary supplements on carcass traits of broilers under chronic heat stress was obtained by dietary supplementation of betaine and vitamin E combination.

Heat stressed broilers had higher ( $P < 0.05$ ) levels of blood glucose, total cholesterol and albumen and lower ( $P < 0.05$ ) level of total protein and globulin when compared with all treated groups and TNC (Table 4). The blood levels of triglycerides and total cholesterol were significantly reduced ( $p < 0.01$ ) in all treated groups in comparison with heat stressed control (HSC).

In comparison with the heat stressed control, the dietary inclusion of all supplement combinations in broiler diets significantly increased ( $P < 0.001$ ) the activities of antioxidant enzymes (SOD, GSH-Px,) and TAOC but reduced ( $P < 0.001$ ) the MDA content in the serum of chicks at the ages 42 d (Table 4). Heat stress reduce blood antioxidant status in broilers and increased the oxidative stress indicators. It was noted that dietary supplementation with selected additive combinations had decreased the concentrations of both H: L ratio and corticosterone significantly ( $P < 0.001$ ) of broilers under heat stress (Table 4) in comparison with HSC group. The concentrations of ND antibodies titer increased significantly ( $P < 0.001$ ) at 21 and 42 d of age by dietary additives in treatment groups in comparison with heat stressed

control and the highest values were noticed in birds of T5, T4 and T3 groups, respectively. This research extends our knowledge that dietary supplementation of betaine and vitamin E, chromium and vitamin C, propolis and vitamin A combinations are better option for enhancing serum antioxidant status and immune response (ND antibodies titer) for broilers exposed to chronic heat stress.

The economic efficiency of 6 weeks old broiler chicks fed diet fortified with different

supplement combinations under heat stress are shown on (Table 5). The dietary additive supplementation increased ( $P < 0.001$ ) the net return and economic efficiency in all treated groups in comparison with HSC group. Concerning the economic feed efficiency and the relative economic efficiency, the highest values were recorded for the treatment groups (T5, T4, T3 and T2) in comparison with T1 and HSC groups.

**Table 1:** Ingredients and analyzed chemical composition of basal diets

Item* (% unless noted)	Starter diet	Grower and finisher diet
Ingredient, %		
Corn	54.64	62.55
Corn gluten meal (60%)	4.11	3
Soybean meal, 44%	32.5	26.4
Sunflower oil	4.4	3.7
Limestone	1.5	1.5
Dicalcium phosphate	1.8	1.8
Salt	0.4	0.4
Vitamin - mineral premix	0.3	0.3
DL-methionine	0.2	0.2
L- lysine	0.1	0.1
Antioxidant	0.05	0.05
Total	100	100
Chemical analysis		
Dry matter	90.11	89.94
Crude protein	23.01	20.05
ME(Kcal/kg diet) <sup>b</sup>	3200	3200
Calcium	1.08	1.06
Non-phytae phosphorus	0.45	0.43
Na	0.20	0.17
Lysine	1.25	1.07
DL-Methionine	0.58	0.53
Methionine+cystine	1.05	0.87
Crude fiber	2.96	2.95
Crude fat	6.38	6.04

\*Ingredient and nutrient composition are reported on as-fed basis.

<sup>a</sup>The vitamin and mineral premix provided per kg of diet: vitamin A, 4000000 IU; vitamin D3, 667000 IU; vitamin E 3334 mg; vitamin K3, 1167 mg; vitamin B1, 334 mg; vitamin B2, 1667 mg; vitamin B3, 3334 mg; B6, 500 mg; vitamin B12 33.4 mg, Folic acid, 334 mg; Biotin, 17 mg; Iron, 10 ; Copper, 2.167; Zinc, 18.334; Manganese 20.0; Iodine, 0.167; Cobalt, 0.034 and Selenium, 0.034.

<sup>b</sup>ME Calculated based on NRC (1994) feed composition tables

**Table 2:** Effect of dietary treatments on body weight (BW), feed intake (FI) and feed conversion ratio (FCR) of boilers under thermoneutral (TN) and heat stress (Mean± Pooled SE)

Item	TNC	HSC	T1	T2	T3	T4	T5	SEM	P
Initial BW (day1)	40.56	41.23	40.86	41.25	42.07	41.33	40.07	0.23	0.332
<b>0 to 21 day</b>									
BW (day 21 W3)	904.19 <sup>a</sup>	417.82 <sup>d</sup>	621.73 <sup>b</sup>	608.06 <sup>b</sup>	599.33 <sup>b</sup>	572.40 <sup>c</sup>	625.31 <sup>b</sup>	13.39	<0.001
Feed intake (g/bird)	1191.04 <sup>a</sup>	866.2 <sup>e</sup>	1077.37 <sup>b</sup>	1013.98 <sup>cd</sup>	1027.17 <sup>c</sup>	1001.76 <sup>d</sup>	1019.69 <sup>cd</sup>	9.05	<0.001
BW gain (g/bird)	863.62 <sup>a</sup>	376.59 <sup>e</sup>	580.86 <sup>bc</sup>	566.81 <sup>bc</sup>	557.27 <sup>c</sup>	531.07 <sup>d</sup>	585.31 <sup>b</sup>	13.41	<0.001
FCR (g/g)	1.38 <sup>d</sup>	2.32 <sup>a</sup>	1.86 <sup>bc</sup>	1.83 <sup>bc</sup>	1.84 <sup>bc</sup>	1.89 <sup>b</sup>	1.74 <sup>c</sup>	0.02	<0.001
<b>22-42 day</b>									
Feed intake (g/bird)	3475.15 <sup>a</sup>	2431.59 <sup>c</sup>	2612.92 <sup>b</sup>	2478.13 <sup>c</sup>	2512.65 <sup>c</sup>	2486.72 <sup>c</sup>	2479.62 <sup>c</sup>	34.7	<0.001
BW gain (g/bird)	1740.81 <sup>a</sup>	1045.52 <sup>d</sup>	1142.93 <sup>c</sup>	1151.5 <sup>c</sup>	1169.67 <sup>c</sup>	1217.33 <sup>b</sup>	1177.81 <sup>c</sup>	21.07	<0.001
FCR (g/g)	1.99 <sup>d</sup>	2.33 <sup>a</sup>	2.29 <sup>a</sup>	2.16 <sup>b</sup>	2.15 <sup>b</sup>	2.05 <sup>cd</sup>	2.11 <sup>bc</sup>	0.01	<0.001
<b>0-42 day</b>									
Final BW (day 42)	2645.00 <sup>a</sup>	1463.35 <sup>d</sup>	1664.66 <sup>c</sup>	1759.56 <sup>c</sup>	1769.00 <sup>b</sup>	1789.73 <sup>bc</sup>	1803.13 <sup>bc</sup>	21.64	<0.001
Feed intake (g/bird)	4666.18 <sup>a</sup>	3297.79 <sup>d</sup>	3690.28 <sup>b</sup>	3492.11 <sup>c</sup>	3539.81 <sup>c</sup>	3488.49 <sup>c</sup>	3499.31 <sup>c</sup>	42.27	<0.001
BW gain (g/bird)	2604.43 <sup>a</sup>	1422.11 <sup>d</sup>	1723.8 <sup>c</sup>	1718.31 <sup>c</sup>	1726.93 <sup>c</sup>	1748.4 <sup>bc</sup>	1763.13 <sup>b</sup>	33.33	<0.001
FCR (g/g)	1.79 <sup>d</sup>	2.32 <sup>a</sup>	2.14 <sup>b</sup>	2.03 <sup>c</sup>	2.04 <sup>c</sup>	1.99 <sup>d</sup>	1.98 <sup>d</sup>	0.01	<0.001
<b>Mortality, %</b>	1.75	12.77	4.44	3.88	4.88	3.33	2.22	1.09	0.08

BW, body weight, FCR, feed conversation ratio = feed intake/ body weight gain

a, b, c, d, e means with different letters in the same row are significantly different at P<0.05.

TNC, thermoneutral control, HSC, heat stress control; basal diet supplemented with: 1 % Cumin and 1 % turmeric powder (T1) ; 1.5 g/kg potassium chloride and 2 g/kg sodium bicarbonate (T2) ; 15000 IU vitamin A and 1000 ppm propolis (T3); 500 ppm vitamin C and 1200 ppb chromium (T4); 1200 ppm betaine and 500 ppm Vitamin E (T5).

**Table 3:** Effect of dietary additives on carcass characteristics and relative weight (% of live weight) of internal organs of broilers under thermoneutral (TN) and heat stress (Mean± Pooled SE)

Item	TNC	HSC	T1	T2	T3	T4	T5	SEM	P
Preslaughter weight	2665.00 <sup>a</sup>	1621 <sup>e</sup>	1722 <sup>d</sup>	1730 <sup>d</sup>	1788.00 <sup>b</sup>	1798.00 <sup>bc</sup>	1831.2 <sup>b</sup>	56.21	<0.001
Carcass weight	1872.6 <sup>a</sup>	1109 <sup>e</sup>	1206 <sup>d</sup>	1210 <sup>d</sup>	1247 <sup>c</sup>	1258 <sup>c</sup>	1287 <sup>b</sup>	40.29	<0.001
Dressing, %	70.28 <sup>a</sup>	68.42 <sup>c</sup>	70.08 <sup>ab</sup>	69.94 <sup>ab</sup>	69.74 <sup>b</sup>	69.97 <sup>ab</sup>	70.27 <sup>a</sup>	0.33	<0.001
Liver, %	2.78 <sup>a</sup>	2.15 <sup>c</sup>	2.62 <sup>b</sup>	2.46 <sup>b</sup>	2.5 <sup>b</sup>	2.68 <sup>b</sup>	2.74 <sup>a</sup>	0.04	0.04
Heart %	0.76 <sup>a</sup>	0.45 <sup>d</sup>	0.71 <sup>b</sup>	0.65 <sup>c</sup>	0.62 <sup>c</sup>	0.72 <sup>b</sup>	0.75 <sup>b</sup>	0.11	0.001
Gizzard, %	1.73 <sup>a</sup>	1.35 <sup>c</sup>	1.54 <sup>b</sup>	1.52 <sup>b</sup>	1.55 <sup>b</sup>	1.58 <sup>b</sup>	1.61 <sup>b</sup>	0.02	0.01
Abdominal fat, %	1.65 <sup>b</sup>	1.98 <sup>a</sup>	1.23 <sup>d</sup>	1.48 <sup>bc</sup>	1.39 <sup>cd</sup>	1.12 <sup>d</sup>	1.03 <sup>e</sup>	0.05	<0.001

TNC, thermoneutral control, HSC, heat stress control; basal diet supplemented with: 1 % Cumin and 1 % turmeric powder (T1) ; 1.5 g/kg potassium chloride and 2 g/kg sodium bicarbonate (T2) ; 15000 IU vitamin A and 1000 ppm propolis (T3); 500 ppm vitamin C and 1200 ppb chromium (T4); 1200 ppm betaine and 500 ppm Vitamin E (T5)

**Table4:** Effect of dietary additives on blood biochemical profile, oxidative stress biomarkers, and ND antibody titre (Mean± Pooled SE)

Item	TNC	HSC	T1	T2	T3	T4	T5	SEM	P
Glucose, mg/dl	221.67 <sup>d</sup>	281.69 <sup>a</sup>	240.27 <sup>c</sup>	251.16 <sup>b</sup>	230.48 <sup>cd</sup>	224.61 <sup>d</sup>	226.81 <sup>d</sup>	3.533	<0.001
Total cholesterol, mg/dl	126.83 <sup>e</sup>	174.92 <sup>a</sup>	153.08 <sup>b</sup>	146.17 <sup>c</sup>	138.08 <sup>d</sup>	135.25 <sup>d</sup>	121.25 <sup>e</sup>	2.888	<0.001
Total protein, g/dl	3.79 <sup>a</sup>	2.79 <sup>e</sup>	3.24 <sup>cd</sup>	3.37 <sup>c</sup>	3.47 <sup>bc</sup>	3.62 <sup>b</sup>	3.94 <sup>a</sup>	0.062	<0.001
Albumen, g/dl	1.84 <sup>a</sup>	1.86 <sup>a</sup>	1.72 <sup>ab</sup>	1.63 <sup>b</sup>	1.62 <sup>b</sup>	1.62 <sup>b</sup>	1.53 <sup>b</sup>	0.028	0.004
Globulin, g/dl	1.95 <sup>b</sup>	0.93	1.52 <sup>d</sup>	1.73 <sup>c</sup>	1.85 <sup>bc</sup>	2.00 <sup>b</sup>	2.41 <sup>a</sup>	0.075	<0.001
Triglycerides, mg/dl	99.17 <sup>a</sup>	94.92 <sup>a</sup>	86.72 <sup>b</sup>	82.29 <sup>bc</sup>	77.02 <sup>d</sup>	85.96 <sup>b</sup>	80.86 <sup>bc</sup>	1.59	<0.001
SOD, U/ml	152.37 <sup>bc</sup>	127.80 <sup>d</sup>	152.60 <sup>bc</sup>	154.4 <sup>abc</sup>	148.4 <sup>c</sup>	160.60 <sup>a</sup>	155.60 <sup>ab</sup>	1.77	<0.001
GSH-PX, n mol/ml	11.02 <sup>a</sup>	5.98 <sup>e</sup>	7.48 <sup>d</sup>	8.08 <sup>cd</sup>	8.62 <sup>c</sup>	9.52 <sup>b</sup>	8.19 <sup>c</sup>	0.266	<0.001
MDA, nmol/ml	6.90 <sup>e</sup>	11.77 <sup>a</sup>	9.91 <sup>b</sup>	9.10 <sup>c</sup>	7.98 <sup>d</sup>	7.06 <sup>e</sup>	8.92 <sup>c</sup>	0.273	<0.001
TAOC, U/ml	8.02 <sup>e</sup>	8.42 <sup>e</sup>	9.64 <sup>d</sup>	10.32 <sup>c</sup>	10.69 <sup>bc</sup>	12.05 <sup>a</sup>	10.85 <sup>b</sup>	0.233	<0.001
Heterophils	21.51 <sup>e</sup>	28.9 <sup>a</sup>	23.88 <sup>ab</sup>	24.5 <sup>b</sup>	22.8 <sup>bcde</sup>	23.1 <sup>abcd</sup>	22.02 <sup>de</sup>	23.75	<0.001
lymphocytes	77.23 <sup>abc</sup>	71.34 <sup>d</sup>	76.30 <sup>cd</sup>	76.94 <sup>abc</sup>	77.56 <sup>ab</sup>	76.60 <sup>bc</sup>	77.80 <sup>a</sup>	0.365	<0.001
H/L ratio	0.28 <sup>d</sup>	0.41 <sup>a</sup>	0.31 <sup>b</sup>	0.32 <sup>a</sup>	0.29 <sup>cd</sup>	0.30 <sup>bc</sup>	0.28 <sup>d</sup>	0.006	<0.001
Corticosterone, ng/ml	10.92 <sup>a</sup>	10.46 <sup>b</sup>	9.57 <sup>c</sup>	9.51 <sup>c</sup>	9.52 <sup>c</sup>	9.13 <sup>d</sup>	9.07 <sup>d</sup>	0.11	<0.001
ND titer (log 10)									
21 days	1.95 <sup>g</sup>	2.27 <sup>f</sup>	2.47 <sup>e</sup>	2.58 <sup>d</sup>	2.76 <sup>c</sup>	2.87 <sup>b</sup>	3.11 <sup>a</sup>	0.06	<0.001
42 days	3.14 <sup>e</sup>	3.51 <sup>d</sup>	3.67 <sup>c</sup>	3.74 <sup>bc</sup>	3.86 <sup>b</sup>	4.44 <sup>a</sup>	4.48 <sup>a</sup>	0.08	<0.001

a, b, c, d, e means with different letters in the same row are significantly different at P<0.05. ND= Newcastle disease  
 GSH-Px, glutathione peroxidase; SOD, total superoxide dismutase and; MDA, malondialdehyde; TAOC, total antioxidant capacity, H/L heterophile/lymphocyte

TNC, thermoneutral control, HSC, heat stress control; basal diet supplemented with: 1 % Cumin and 1 % turmeric powder (T1) ; 1.5 g/kg potassium chloride and 2 g/kg sodium bicarbonate (T2) ; 15000 IU vitamin A and 1000 ppm propolis (T3); 500 ppm vitamin C and 1200 ppb chromium (T4); 1200 ppm betaine and 500 ppm Vitamin E (T5)

**Table5:** Economic analysis of 6-week broilers fed different dietary treatments (Mean± Pooled SE)

Item	TNC	HSC	T1	T2	T3	T4	T5	SEM	P
Cumulative feed intake (g/bird)	4666.18 <sup>a</sup>	3297.79 <sup>d</sup>	3690.28 <sup>b</sup>	3492.11 <sup>c</sup>	3539.81 <sup>c</sup>	3488.49 <sup>c</sup>	3499.31 <sup>c</sup>	42.27	<0.001
Feed cost / bird (SR)	5.6 <sup>a</sup>	3.96 <sup>d</sup>	4.42 <sup>b</sup>	4.19 <sup>c</sup>	4.25 <sup>c</sup>	4.18 <sup>c</sup>	4.19 <sup>c</sup>	0.02	<0.001
Total Cost per bird (SR)*	8.6 <sup>a</sup>	6.96 <sup>d</sup>	7.42 <sup>b</sup>	7.19 <sup>c</sup>	7.24 <sup>c</sup>	7.18 <sup>c</sup>	7.19 <sup>c</sup>	0.02	<0.001
Total return (SR/bird)	31.25 <sup>a</sup>	17.06 <sup>d</sup>	20.68 <sup>c</sup>	20.61 <sup>c</sup>	20.72 <sup>c</sup>	20.98 <sup>bc</sup>	21.16 <sup>b</sup>	0.4	<0.001
Net profit (SR/bird)	22.65 <sup>a</sup>	10.11	13.26	13.42	13.47	13.79	13.95	0.35	>0.05
Economic feed efficiency	263.47 <sup>a</sup>	145.32 <sup>e</sup>	178.45 <sup>d</sup>	186.78 <sup>c</sup>	185.94 <sup>c</sup>	191.94 <sup>b</sup>	193.91 <sup>b</sup>	3.23	<0.001
Relative economic efficiency	1 <sup>a</sup>	0.56 <sup>e</sup>	0.68 <sup>d</sup>	0.72 <sup>c</sup>	0.71 <sup>c</sup>	0.73 <sup>b</sup>	0.75 <sup>b</sup>	0.01	<0.001

SR, Saudi riyal

TNC, thermoneutral control, HSC, heat stress control; basal diet supplemented with: 1 % Cumin and 1 % turmeric powder (T1) ; 1.5 g/kg potassium chloride and 2 g/kg sodium bicarbonate (T2) ; 15000 IU vitamin A and 1000 ppm propolis (T3); 500 ppm vitamin C and 1200 ppb chromium (T4); 1200 ppm betaine and 500 ppm Vitamin E (T5).

\*Total cost include (chick price, light, electricity, workers, vaccinations and bedding)

## Discussion

The current study confirmed the detrimental impacts of heat stress on broilers performance, carcass traits, blood metabolites, antioxidant capabilities and economic efficiency. The harmful effects of heat stress on growth performance indices detected in the current experiment were consistent with previous studies (8, 10, 11). Heat stress reduces feed intake as an adaptation defense mechanism started by birds to reduce metabolic heat production (20). Heat stress increases free radical generation and lipid peroxidation, which impair growth performance. The improved broiler performance by the selected additive combination in the current studies are matching the findings of earlier studies (6, 7, 8, 9, 10, 11, 12).

Antioxidant vitamins (C and E) had a beneficial effect in relieving heat stress in broilers through their antioxidant effects (21). Betaine is crucial to be supplied during heat stress, as poultry cannot synthesize sufficient amount and poultry feed considers as a poor source (22). This mixture vitamin E and betaine (Bet with VE) resulted in complete reduction of the negative effect of chronic heat stress (CHS) on broiler performance. The synergetic effect of Bet and vitamin E may reveal the different mode of action of both. The supplementation of betaine and vitamin E elicit the best significant effect on growth performance and this finding could have important implications in poultry production in subtropical and tropical regions. The potential effect of betaine represented by improving the digestibility of specific nutrients as energy and protein through enhancing the structural and functional characteristics of intestinal epithelia (23). Moreover, dietary supplementation of betaine and vitamin E combination provides a more potent antioxidant effect, and spares essential amino acids like choline and methionine (12, 23). A positive effect was detected between the dietary combination vitamin C and Cr as evidenced by their potent antioxidant property against oxidative stress in the current study and confirm the finding of previous studies (5, 24, 25). There are several explanation for this result, vitamin C and chromium

supplementation increased serum Vit C, Cr, Vit E, Fe, Zn, Mn and improved nutrient digestibility in laying hens under cold stress (24). The rate of ascorbic acid uptake into the cells was decreased during heat stress because of the decrease in insulin level and hyperglycemia (25). Chromium indirectly increases the intracellular availability of Vit C by intensifying the action of insulin (24). The positive impact of the combination of vitamin A and propolis could be due to the role played by vitamin A as an effective radical-trapping antioxidant (26). Heat stress reduce the conversion of carotene to vitamin A and the concentration of vitamin A decreases during stress conditions so much vitamin A needed during heat stress (26).

The observed beneficial effects of cinnamon and turmeric in the current study could be attributed to their stimulation of appetite, feed intake, digestive enzyme secretions, immune response and the antioxidant actions (27). Increased growth performance and carcass traits by KCl supplementation could be ascribed to decreased body temperature by more water ingestion after electrolyte supplementation that caused divert more energy toward BW gain. In accordance with the present results, previous studies have demonstrated that, the addition of KCl and  $\text{NaHCO}_3$  during heat stress, at levels of 1.5% and 0.5% respectively, improved final weight, feed efficiency and improved ( $P < 0.05$ ) serum potassium and bicarbonate level through providing of potassium and bicarbonate ions (2).

It has been established that heat stress alter the carcass composition of birds through increasing fat deposition and reducing body protein content (6). The improved carcass characteristics due to the dietary additive in the current study are in line with the finding of previous studies (6, 28, 29) who reported that supplementation of propolis, ascorbic acid, chromium, and turmeric significantly increased the dressing percentage and improved carcass characteristics in chicken reared under heat stress. The increase in dressing percentage in bird receiving vitamin E and Bet may be due to the osmotic effects of Bet, which increases water re-



tention and improving the digestibility of energy and protein through enhancing the structure and function of intestinal epithelia and sparing essential amino acids (23). The present findings seem to be consistent with other researches which found that dietary inclusion of vitamin C (5, 24), or Cr (24) improved all carcass characteristics in broilers. Another study illustrated that Cr supplementation increased carcass yield and decreased abdominal fat in heat stressed chickens (28).

The increase in glucose concentration in the current study could be ascribed to the increase in glucocorticoids that can result from heat stress as previously explained by (3). Glucocorticoids stimulate gluconeogenesis from muscle tissue proteins. Heat stress increased the plasma level of glucose and cholesterol and decreased total protein levels (30). Both Ascorbic acid and chromium supplementation enhance the utilization of corticosteroids released during stress (24), thus playing a crucial role in response to stress. Vitamin C mitigates the negative effects of stress related depression in poultry performance through reducing synthesis and secretion of corticosteroids (26). The increase in blood lipids under heat stress caused by compensation of energy needed due to the reduction in feed intake through lipolysis of body lipid which resulting in increasing the blood cholesterol and triglyceride levels (30).

The improved in blood oxidant and antioxidant biomarkers caused by dietary additives was in line with the results of previous studies (4, 7, 10, 21). These studies reported that dietary supplementation of chromium, betaine, vitamin E, C, betaine and propolis significantly improved primary and secondary immune response ( $P<0.01$ ), and improved H/L ratio ( $P<0.05$ ). This finding supports previous research of Niu et al. (4) dietary supplementation of Vitamin improved performance and immune competence of broilers under HS.

The improvement in economic efficiency in all treated groups after dietary supplementation of different additive combinations may be attributed to the ability of these additives to increase broiler performance, feed efficiency and their ability to stimulate bird immunity and to

reduce mortality rate. This result match those observed in earlier studies (7, 31).

## Conclusion

Based on the obtained results, it could be concluded that dietary supplementation of broiler under chronic heat stress with selected additive combinations improved the growth performance, carcass traits, concentrations of serum antioxidant enzyme biomarkers and economic efficiency. From the tested dietary additive combinations, the use of betaine and vitamin E or chromium and vitamin C together gave the best results in terms of improved growth performance, feed efficiency, immune response, antioxidant status and profitability of broilers under chronic heat stress. From the findings of the current study, it could be recommend the use of dietary either betaine and vitamin E or chromium or vitamin C combinations as a good management practice for mitigating heat stress related depression in the performance of broiler chickens.

## Conflict of interest

The authors declare that they have no conflict of interest

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