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Research Article
Performance of Heat-Stressed Broilers Supplemented with Dietary Choline and Betaine

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Abstract
Objective: A study was conducted to determine the effects of supplemental choline (CHO) and betaine (BET) on broiler performance and carcass characteristics under different temperature conditions, thermoneutral (TN: 23.9°C) and heat stress (HS: 28-36°C). **Materials and Methods:** The corn-soy bean basal (Control) diet was formulated to meet NRC requirements for broilers and supplemented with CHO and BET at 500 or 1000ppm (CHO500, CHO1000, BET500 and BET1000). Feed intake and body weight were recorded weekly by pen. On day 42, foot pads were assessed for health and litter samples collected from each pen. At slaughter on day 52, breast meat was collected for drip loss evaluation at 4 and 7 days post slaughter. Breast meat lightness (L*), redness (a*) and yellowness (b*) were measured. **Results:** Results showed that HS birds consumed 20.59% less feed and gained 23.34% less weight than TN birds ($p<0.05$) during 22-49 days of age. The overall feed intake and weight gain during days 1-49 were similarly reduced in HS birds. Heat stress decreased feed efficiency during days 22-49 ($p = 0.02$). HS birds had lower feed efficiency (0.54) when compared to TN birds (0.56). Drip loss 4 days post slaughter was affected by diet×temperature interaction ($p = 0.04$) The lowest drip loss occurred with CHO500 (0.60%) in HS and BET1000 (0.83%) in TN birds. Breast meat color of HS birds was lighter ($p = 0.02$) while that of TN birds was more yellow ($p = 0.004$). Temperature did not affect pododermatitis ($p = 0.22$) however, there was an effect of diet ($p = 0.003$) with CHO500 and BET1000 showing the lowest occurrence of foot pad dermatitis. **Conclusion:** In this study, breast meat drip loss was influenced by dietary CHO and BET while meat color was affected by rearing temperature. Dietary CHO and BET supplementation did not improve weight loss induced by HS conditions.

Key words: Broilers, heat-stress, choline, betaine, foot pad dermatitis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heat stress (HS) is one of the major problems facing the poultry industry[1]. Under high environmental temperature and high relative humidity conditions, chickens adapt by reducing feed intake to decrease endogenous heat production and hyperventilating or panting to eliminate extra heat. However, the results are detrimental to the birds' physiology because it causes slow growth rate, low body weight gain[2-4] and respiratory alkalosis[5,6]. Several management practices have been developed to improve birds' comfort and survival and to mitigate the negative impact of heat stress on broilers. These include housing insulation, ventilation, evaporative cooling systems, feed withdrawal[7,8], fasting before the onset of heat stress and acclimation of high temperature in younger age[9,10] among others. These practices do not directly address the acid-base imbalance (respiratory alkalosis) even though they improve broilers performances.

Respiratory alkalosis caused by excessive loss of carbon dioxide due to hyperventilation[11,12] subsequently results in electrolytes imbalance in chickens primarily due to potassium excretion. Smith and Teeter[13] reported a 633% increase in potassium (K^+) excretion via fecal excreta during heat stress (35°C) compared to a thermoneutral (24°C) environment. Supplementing heat-stressed broilers with essential electrolytes could be beneficial in correcting acid-base imbalance thereby improving broilers comfort and performance. Supplementation of water with potassium chloride (KCl) was shown to increase body weight gain[13,14] of heat-stressed broilers. According to Borges *et al.*[15], dietary electrolytes balance for maximum production was estimated to be 240 mEq kg^{-1}. However, close attention must be paid to the use of dietary electrolytes to alleviate the negative impacts of heat stress on broilers. Supplementation of the electrolytes sodium (Na^+), chloride (Cl^-) and K^+ in drinking water was shown to cause an increase in water consumption that resulted in increased litter wetness, the primary cause of foot pad dermatitis[16]. Additionally, excess accumulation of Na^+ and Cl^- may inhibit cells proliferation and protein synthesis as opposed to organic osmolytes[17].

Organic osmolytes such as choline and betaine could be alternatives to electrolytes. Currently, betaine is not required in a typical broiler diet, however choline is usually added at different rates depending on age: 1300 mg kg^{-1} for 0-3 weeks of age, 1000 mg kg^{-1} for 3-6 weeks of age and 750 mg kg^{-1} for 6-9 weeks of age[18]. Betaine can be synthesized from choline and has been shown to affect the movement of water across the small intestine of broiler chicks[19]. The supplementation of choline and betaine in broiler diets may be beneficial in improving osmotic pressure and decreasing excreta moisture in heat-stressed chickens thereby possibly improving their performance and foot health. Even though the effect of supplemental choline and betaine on broiler performance has been the objective of several studies, very little has been done concerning their osmoprotectant benefit if any, under heat stress conditions. Therefore, this study was designed to evaluate the impact of dietary supplementation of choline and betaine on the performance and carcass characteristics of broilers under HS conditions.

MATERIALS AND METHODS

Birds and housing: The procedures were reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee (IACUC) and the research was conducted at the Johnson Animal Research and Teaching Unit (JARTU). Two adjacent rooms, thermoneutral (TN) and heat stress (HS) were used. Each room was divided into 40 pens equipped with individual gravity feeders, nipple drinkers and contained bedding made of shredded paper. The rooms were equipped with data loggers (HOBO; data logger temperature/relative humidity; Onset; Bourne, MA)to record temperature and humidity daily. In addition, a humidifier was placed in the heat stress room to ensure that humidity remained above 50%. Eight hundred day-old mixed sex Cobb500 (female) × Hubbard (M99 male) chicks were obtained from Pilgrim's Pride hatchery in Cohutta, Georgia. They were vaccinated at the hatchery against Marek's and Gumboro diseases (VAXXITEK HVT-IBD); *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* (GARASOL); Coccidiosis (COCCIVAC-B), Newcastle (NEWHATCHC2-M) and Infectious bronchitis (MILDVAC ARK and GA 2008). The birds were weighed in groups of 10 and assigned to 5 dietary treatments in 8 replicate pens per room.

Temperature treatments: During the first 21 days, birds were brooded under similar conditions in the two rooms. The temperature was set at 32.22°C in the first week and decreased by 2.78°C until it reached 23.9°C by the end of the third week. From day 22 to the end of the study (day 52), the temperature was maintained at 23.9°C in the TN room and cycled in the HS room. To achieve the cycling temperature, the ambient temperature was set at 28°C from 0100h to 0900h, then it was gradually increased to 36°C by 0400h and gradually decreased until 28°C was reached by 0100h (Fig. 1). The photoperiod regimen was 23 h of light followed by 1 h of dark in both environments. The humidity was maintained at approximately 50% in the HS room.

Table 1: Nutritional composition of starter diet (%)

Ingredients	CON	CHO500	CHO1000	BET500	BET1000
Corn, grain	62.8600	62.8600	62.8600	62.8600	62.8600
Soybean meal	31.0000	31.0000	31.0000	31.0000	31.0000
Vitamin premix[1]	0.0625	0.0625	0.0625	0.0625	0.0625
Filler, sand	0.2000	0.1050	0.0100	0.1430	0.0853
Choline (mg kg^{-1})[2]	0.0000	0.0950	0.1900	0.0000	0.0000
Betaine (mg kg^{-1})[2]	0.0000	0.0000	0.0000	0.0570	0.1147
DL methionine	0.1500	0.1500	0.1500	0.1500	0.1500
Salt	0.3500	0.3500	0.3500	0.3500	0.3500
Limestone	1.1000	1.1000	1.1000	1.1000	1.1000
Dical phosphate	2.1000	2.1000	2.1000	2.1000	2.1000
Trace min. premix[3]	0.1250	0.1250	0.1250	0.1250	0.1250
Fat, animal	1.9000	1.9000	1.9000	1.9000	1.9000
Coban	0.0495	0.0495	0.0495	0.0495	0.0495
Lysine	0.1000	0.1000	0.1000	0.1000	0.1000
Total	100.0000	100.0000	100.0000	100.0000	100.0000
Nutrient composition					
ME (kcal kg^{-1})	3024.0000	3024.0000	3024.0000	3024.0000	3024.0000
Crude protein (%)	20.5900	20.5900	20.5900	20.5900	20.5900
Calcium (%)	0.9800	0.9800	0.9800	0.9800	0.9800
Available phosphorus (%)	0.5100	0.5100	0.5100	0.5100	0.5100
Potassium (%)	0.8100	0.8100	0.8100	0.8100	0.8100
Chloride (%)	0.2500	0.2500	0.2500	0.2500	0.2500
Sodium (%)	0.1600	0.1600	0.1600	0.1600	0.1600
Zinc (mg kg^{-1})	92.9900	92.9900	92.9900	92.9900	92.9900
Choline (mg kg^{-1})	1236.3800	2186.4400	3136.4400	1236.3800	1236.3800
Betaine (mg kg^{-1})	0.0000	0.0000	0.0000	585.0100	1147.0300
Lysine (%)	1.1800	1.1800	1.1800	1.1800	1.1800
Methionine (%)	0.4700	0.4700	0.4700	0.4700	0.4700
Methionine+Cysteine (%)	0.8100	0.8100	0.8100	0.8100	0.8100
Threonine (%)	0.7600	0.7600	0.7600	0.7600	0.7600
Fat (%)	4.6000	4.6000	4.6000	4.6000	4.6900
Salt (%)	0.5300	0.5300	0.5300	0.5300	0.5300

[1]Vitamin premix supplied per kg of feed; Vitamin A: 17636684 IU, Vitamin D3: 5952381 ICU, Vitamin E: 35000 IU, Vitamin B12: 16 mg, Biotin: 176 mg, Menadione: 3858 mg, Thiamin: 3307 mg, Riboflavin: 15432 mg, Pantothenic acid: 24250 mg, Vitamin B6: 4409 mg, Niacin: 88180 mg, Folic acid: 1658 mg. [2]Choline chloride (60%), Betaine anhydrous, provided by Balchem Corporation and added at the expense of the filler, sand. [3]Trace minerals premix supplied at 1.14 kg per ton of feed; Calcium: 3% min and 4% max, Manganese: 9.70%, Zinc: 7.50%, Copper: 3.53%, Iron: 4400 ppm, Iodine: 1586 ppm, Selenium: 240 ppm

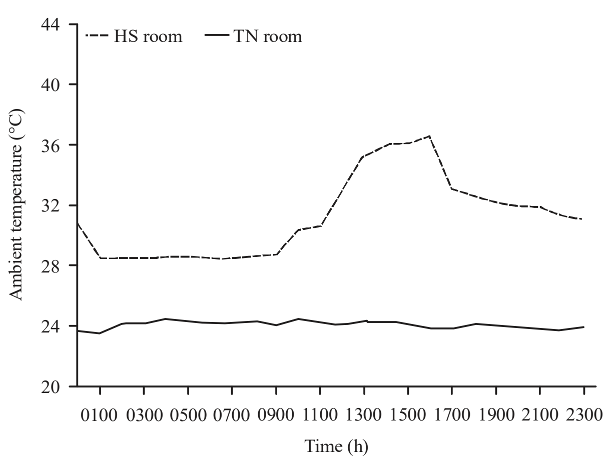

Fig. 1: Ambient temperature by time during 24 h in the heat stress (HS) and thermoneutral (TN) rooms

Dietary treatments: Five dietary treatments were tested. The basal formulation with choline or betaine supplementation was used to make the following diets:

- Control (CON), basal formulation with no added choline or betaine
- Low choline (CHO500), basal diet plus 500 ppm methyl equivalents added choline
- High choline (CHO1000), basal diet plus 1000 ppm methyl equivalents added choline
- Low betaine (BET500), basal diet plus 500 ppm methyl equivalents added betaine
- High betaine (BET1000), basal diet plus 1000 ppm methyl equivalents added betaine

The basal diet was formulated to meet broilers energy, protein, minerals and vitamins requirements[18] except for methionine which was 20% lower. The birds were fed starter diet (Table 1) from days 1-21, grower diet (Table 2) from days 22-35 and finisher diet (Table 3) from days 36-52.

Growth performance: Feed was weighed and added every three days to ensure continuous access. Once per week (from

Table 2: Nutritional composition of grower diet (%)

Ingredients	CON	CHO500	CHO1000	BET500	BET1000
Corn, grain	62.3200	62.3200	62.3200	62.3200	62.3200
Soybean meal	31.0000	31.0000	31.0000	31.0000	31.0000
Vitamin premix[1]	0.0625	0.0625	0.0625	0.0625	0.0625
Filler, sand	0.3000	0.1884	0.0768	0.2430	0.1853
Choline (mg kg^{-1})[2]	0.0000	0.1116	0.2232	0.0000	0.0000
Betaine (mg kg^{-1})[2]	0.0000	0.0000	0.0000	0.0570	0.1147
DL methionine	0.2000	0.2000	0.2000	0.2000	0.2000
Salt	0.3500	0.3500	0.3500	0.3500	0.3500
Limestone	1.1000	1.1000	1.1000	1.1000	1.1000
Dical phosphate	1.6900	1.6900	1.6900	1.6900	1.6900
Trace min. premix[3]	0.1250	0.1250	0.1250	0.1250	0.1250
Fat, animal	2.7000	2.7000	2.7000	2.7000	2.7000
Coban	0.0495	0.0495	0.0495	0.0495	0.0495
Lysine	0.1000	0.1000	0.1000	0.1000	0.1000
Total	100.0000	100.0000	100.0000	100.0000	100.0000
Nutrient composition					
ME (kcal kg^{-1})	3072.0000	3074.0000	3072.0000	3072.0000	3072.0000
Crude protein (%)	19.9500	19.9500	19.9500	19.9500	19.9500
Calcium (%)	0.8900	0.8900	0.8900	0.8900	0.8900
Available phosphorus (%)	0.4300	0.4300	0.4300	0.4300	0.4300
Potassium (%)	0.8000	0.8000	0.8000	0.8000	0.8000
Chloride (%)	0.2500	0.2500	0.2500	0.2500	0.2500
Sodium (%)	0.1600	0.1600	0.1600	0.1600	0.1600
Zinc (mg kg^{-1})	92.4800	92.4900	92.4800	92.4800	92.4800
Choline (mg kg^{-1})	1233.0300	2349.3700	3465.1000	1233.0200	1233.0300
Betaine (mg kg^{-1})	0.0000	0.0000	0.0000	585.0100	1147.0300
Lysine (%)	1.1800	1.1800	1.1800	1.1800	1.1800
Methionine (%)	0.5200	0.5200	0.5200	0.5200	0.5200
Methionine+cysteine (%)	0.8500	0.8500	0.8500	0.8500	0.8500
Threonine (%)	0.7600	0.7600	0.7600	0.7600	0.7600
Fat (%)	5.3800	5.3800	5.3800	5.3800	5.3800
Salt (%)	0.5300	0.5300	0.5300	0.5300	0.5300

[1]Vitamin premix supplied per kg of feed; Vitamin A: 17636684 IU, Vitamin D3: 5952381 ICU, Vitamin E: 35000 IU, Vitamin B12: 16 mg, Biotin: 176 mg, Menadione: 3858 mg, Thiamin: 3307 mg, Riboflavin: 15432 mg, Pantothenic acid: 24250 mg, Vitamin B6: 4409 mg, Niacin: 88180 mg, Folic acid: 1658 mg. [2]Choline chloride (60%), Betaine anhydrous, provided by Balchem Corporation and added at the expense of the filler, sand. [3]Trace minerals premix supplied at 1.14 kg per ton of feed; Calcium: 3% min and 4% max; Manganese: 9.70%, Zinc: 7.50%, Copper: 3.53%, Iron: 4400 ppm, Iodine: 1586 ppm, Selenium: 240 ppm

days 1-49), the remaining feed was weighed to determine feed intake. Similarly, the birds were weighed per pen to determine body weight gain. When a bird died, the weight was recorded and accounted for in the calculation of weekly average weight gain and feed intake.

Litter samples and foot pad dermatitis: The litter made of shredded paper was not changed during the entire experiment. On day 42, litter samples were collected from each pen at approximately the same area between the nipple lines and the feeders and stored at -20°C for later determination of moisture content. On day 42, all the birds were scored for foot pad dermatitis. The pads of both feet were observed and necrotic patterns ranked using the following scores: 0-no lesions, 1-small lesion, 2-moderate lesion on one foot or on both feet and 3-extensive lesion on one foot or on both feet as outlined by Mello *et al.*[20]. For consistency, all scoring was done by the same individual. To determine litter moisture, samples were thawed at room temperature, weighed and dried in the oven for 48 h at 16°C. After 48 h, the samples were weighed and moisture content determined as difference between wet and dried litter weight and expressed as a percent of wet litter.

Processing of birds: Prior to harvesting on day 52, birds were deprived of feed for 12 h to allow for the emptying of the digestive track. Eighty birds randomly selected from each treatment per room were weighed, hung on shackles, stunned with an electric knife and then killed by severing the jugular vein. They were allowed to bleed for 3 min then scalded in hot water (59.44°C) for 40 sec and their feathers removed using a de-feathering machine. Birds were then eviscerated and carefully dissected. The carcasses were weighed and the breast meat separated. The breasts were cut into half, weighed, individually bagged and chilled on ice for later drip loss determination.

Table 3: Nutritional composition of finisher diet (%)

Ingredients	CON	CHO500	CHO1000	BET500	BET1000
Corn, grain	66.0000	66.0000	66.0000	66.0000	66.0000
Soybean meal	27.3700	27.3700	27.3700	27.3700	27.3700
Vitamin premix[1]	0.0625	0.0625	0.0625	0.0625	0.0625
Filler, sand	0.3000	0.1884	0.0768	0.2430	0.1853
Choline (mg kg^{-1})[2]	0.0000	0.1116	0.2232	0.0000	0.0000
Betaine (mg kg^{-1})[2]	0.0000	0.0000	0.0000	0.0570	0.1147
DL methionine	0.1500	0.1500	0.1500	0.1500	0.1500
Salt	0.3500	0.3500	0.3500	0.3500	0.3500
Limestone	1.1000	1.1000	1.1000	1.1000	1.1000
Dical phosphate	1.6900	1.6900	1.6900	1.6900	1.6900
Trace min. premix[3]	0.1250	0.1250	0.1250	0.1250	0.1250
Fat, animal	2.7000	2.7000	2.7000	2.7000	2.7000
Coban	0.0495	0.0495	0.0495	0.0495	0.0495
Lysine	0.1000	0.1000	0.1000	0.1000	0.1000
Total	100.0000	100.0000	100.0000	100.0000	100.0000
Nutrient composition					
ME (kcal kg^{-1})	3105.0000	3107.0000	3105.0000	3108.0000	3105.0000
Crude protein (%)	18.5500	18.5500	18.5500	18.5500	18.5500
Calcium (%)	0.8800	0.8800	0.8800	0.8800	0.8800
Available phosphorus (%)	0.4300	0.4300	0.4300	0.4300	0.4300
Potassium (%)	0.7400	0.7400	0.7400	0.7400	0.7400
Chloride (%)	0.2500	0.2500	0.2500	0.2500	0.2500
Sodium (%)	0.1600	0.1600	0.1600	0.1600	0.1600
Zinc (mg kg^{-1})	91.1500	91.1600	91.1500	91.1600	91.1500
Choline (mg kg^{-1})	1156.7100	2273.0500	3388.7800	1157.2500	1156.7100
Betaine (mg kg^{-1})	0.0000	0.0000	0.0000	569.9800	1147.0300
Lysine (%)	1.0800	1.0800	1.0800	1.0800	1.0800
Methionine (%)	0.4500	0.4500	0.4500	0.4500	0.4500
Methionine+cysteine (%)	0.7700	0.7700	0.7700	0.7700	0.7700
Threonine (%)	0.7000	0.7000	0.7000	0.7000	0.7000
Fat (%)	5.4800	5.4800	5.4800	5.4900	5.4800
Salt (%)	0.5300	0.5300	0.5300	0.5300	0.5300

[1]Vitamin premix supplied per kg of feed; Vitamin A: 17636684 IU, Vitamin D3: 5952381 ICU, Vitamin E: 35000 IU, Vitamin B12: 16 mg, Biotin: 176 mg, Menadione: 3858 mg, Thiamin: 3307 mg, Riboflavin: 15432 mg, Pantothenic acid: 24250 mg, Vitamin B6: 4409 mg, Niacin: 88180 mg, Folic acid: 1658 mg. [2]Choline chloride (60%), Betaine anhydrous, provided by Balchem Corporation and added at the expense of the filler, sand. [3]Trace minerals premix supplied at 1.14 kg per ton of feed; Calcium: 3% min and 4% max; Manganese: 9.70%, Zinc: 7.50%, Copper: 3.53%, Iron: 4400 ppm, Iodine: 1586 ppm, Selenium: 240 ppm

Drip loss: The left half of each breast was hung in the refrigerator at 4°C for drip loss determination. The breasts were weighed on day four and again on day seven post slaughter. Drip loss constitutes water loss during storage and was determined by the difference in weights between the initial Day one weight and each period of storage time. For four days post slaughter, drip loss was determined by taking the difference in breast weights between days four and one and is expressed as a percent of day one breast weight. Drip loss seven days post slaughter was determined using the same principle and expressed as a percent of day one breast weight.

Breast meat color: Color of breast meat was determined using photo colorimeter (Chroma Meter CR-400, Konica Minolta Inc., Tokyo, Japan) and reported using L* a* b* color space (CIELAB) from CIE (International Commission on Illumination). L* represents lightness and the breast meat is considered light when L* >50.0 and dark when L* <45.0. Redness (a*) and yellowness (b*) represent the chromaticity coordinates. Terms a* and b* indicate color direction: the more positive a* the higher the redness and the more negative a* the greener the color, the more positive b* the more yellow and the more negative b* the more pronounced the blue color[21]. Each color value is given by the average of three measurements on an individual breast sample.

Statistical analysis: The experimental design was a completely randomized split plot design with temperature in the whole plots and diet in the subplots. Data were analyzed using mixed model procedure in SAS 9.3 (SAS Institute, Cary, NC, USA) and least squares means were compared using protected LSD at 5% level of significance. Data on foot pad dermatitis were analyzed using contingency table.

RESULTS AND DISCUSSION

Growth performance: Average feed consumption for days 1-21, 22-49 and 1-49 are presented in Table 4. There was no diet or temperature effect nor was there an interaction with feed intake during days 1-21 ($p>0.20$). During days 22-49 there was no effect of diet×temperature interaction on feed intake ($p = 0.17$). However, temperature significantly affected feed intake ($p = 0.001$) with TN birds consumption 20.59% more than HS bird (4.08 kg vs. 3.24 kg). The feed consumption during days 1-49 was similarly affected only by temperature ($p = 0.0001$). The TN birds' average consumption was 17.67% higher than that of the heat-stressed birds (4.98 vs. 4.10 kg).

There was no diet or temperature effect nor was there any interaction on body weight gain during the starter period ($p>0.50$; Table 5). During days 22-49, only temperature significantly affected body weight gain ($p = 0.001$). The average body weight gain of HS birds (1.74 kg) was 23.35% lower when compared to TN birds (2.27 kg). During days 1-49, body weight gain was similarly affected by heat stress ($p = 0.001$) with heat-stressed birds (2.86 kg) gaining 19.23% less weight compared to TN birds (2.31 kg).

There was no effect of diet×temperature interaction (Table 6) on feed efficiency (gain:feed). However, during days 1-21, BET500 and BET1000 increased ($p = 0.04$) feed efficiency by 8.87% compared to the CON. During days 22-49 only temperature affected feed efficiency ($p = 0.02$) with HS birds having 3.57% reduction in feed efficiency compared to TN birds.

Diet and interaction of diet and temperature had no effect on mortality (Table 7). However, mortality in the heat stress treatment (11.25%) was significantly higher than that of the thermoneutral environment (3%) ($p = 0.0006$).

This study was conducted to evaluate the impact of dietary supplementation with choline or betaine on heat-stressed broilers. The performance parameters evaluated were weight gain, feed consumption, feed efficiency and mortality. Numerous studies have reported the effect of high environmental temperature on the performance of broilers. Heat stress has been shown to negatively impact broiler chicken performance[13,22]. In the current study, HS decreased feed intake and weight gain during days 22-49. The reduction in feed consumption could be due to an effort by the bird to reduce heat generated from feed consumption, digestion and

Table 4: Effect of choline and betaine supplementation on average feed intake (kg) [1] of heat-stressed broilers during 1-21, 22-49 and 1-49 days of age

Diet	Temperature[2]	Day 1-21	Day 22-49	Day 1-49
CON	TN	0.900	3.98	4.86
	HS	0.820	3.33	4.15
CHO500	TN	0.900	4.13	5.04
	HS	0.840	3.27	4.11
CHO1000	TN	0.920	4.03	4.96
	HS	0.890	3.30	4.19
BET500	TN	0.850	3.99	4.83
	HS	0.890	3.31	4.19
BET1000	TN	0.920	4.27	5.18
	HS	0.860	3.00	3.86
SEM		0.047	0.16	0.18
Effect		----------------	p-values	----------------
Diet		0.250	0.990	0.9900
Temperature		0.860	0.001	0.0001
Diet×temperature		0.730	0.170	0.2600
Main effect means				
Diet				
CON		0.860	3.650	4.5100
CHO500		0.870	3.700	4.5700
CHO1000		0.910	3.670	4.5700
BET500		0.870	3.650	4.5100
BET1000		0.890	3.640	4.5200
SEM		0.033	0.110	0.1300
Temperature				
TN		0.900	4.080[a]	4.9800[a]
HS		0.860	3.240[b]	4.1000[b]
SEM		0.023	0.098	0.1100

[1]Values are least squares means. [2]TN: Thermoneutral environment, HS: Heat stress environment. [a,b,c]Means within same column followed by different letters differ significantly ($p<0.05$)

Table 5: Effect of choline and betaine supplementation on average body weight gain (kg)[1] of heat-stressed broilers during 1-21, 22-49 and 1-49 days of age

Diet	Temperature[2]	Day 1-21	Day 22-49	Day 1-49
CON	TN	0.57	2.19	2.75
	HS	0.52	1.80	2.32
CHO500	TN	0.58	2.33	2.91
	HS	0.55	1.76	2.31
CHO1000	TN	0.61	2.24	2.85
	HS	0.60	1.78	2.38
BET500	TN	0.57	2.27	2.84
	HS	0.61	1.77	2.38
BET1000	TN	0.64	2.30	2.94
	HS	0.57	1.59	2.15
SEM		0.042	0.091	0.11
Effect		----------------	p-values	----------------
Diet		0.46	0.82	0.87
Temperature		0.53	0.001	0.001
Diet×temperature		0.65	0.35	0.36
Main effect means				
Diet				
CON		0.54	1.99	2.54
CHO500		0.57	2.04	2.61
CHO1000		0.60	2.01	2.61
BET500		0.59	2.02	2.61
BET1000		0.60	1.94	2.54
SEM		0.029	0.065	0.077
Temperature				
TN		0.59	2.27[a]	2.86[a]
HS		0.57	1.74[b]	2.31[b]
SEM		0.024	0.052	0.065

[1]Values are least squares means. [2]TN: Thermoneutral environment, HS: Heat stress environment. [a,b]Means within same column followed by different letters differ significantly (*$p<0.05$)

Table 6: Effect of choline and betaine supplementation on feed efficiency (Gain:feed)[1] of heat-stressed broilers during 1-21, 22-49 and 1-49 days of age

Diet	Temperature[2]	Day 1-21	Day 22-49	Day 1-49
CON	TN	0.63	0.550	0.560
	HS	0.62	0.540	0.560
CHO500	TN	0.64	0.560	0.580
	HS	0.66	0.540	0.560
CHO1000	TN	0.66	0.550	0.570
	HS	0.66	0.540	0.570
BET500	TN	0.68	0.570	0.590
	HS	0.68	0.530	0.570
BET1000	TN	0.69	0.540	0.570
	HS	0.65	0.530	0.560
SEM		0.022	0.013	0.010
Effect		----------------	p-values	----------------
Diet		0.040	0.700	0.420
Temperature		0.820	0.020	0.050
Diet×temperature		0.580	0.780	0.870
Main effect means				
Diet				
CON		0.620[b]	0.550	0.560
CHO500		0.650[ab]	0.550	0.570
CHO1000		0.660[ab]	0.550	0.570
BET500		0.680[a]	0.550	0.580
BET1000		0.670[a]	0.530	0.560
SEM		0.015	0.009	0.006
Temperature				
TN		0.660	0.560[a]	0.570
HS		0.660	0.540[b]	0.560
SEM		0.014	0.006	0.004

[1]Values are least squares means. [2]TN: Thermoneutral environment, HS: Heat stress environment. [a,b]Means within same column followed by different letters differ significantly ($p<0.05$)

Table 7: Effect of choline and betaine supplementation on mortality (%)[1] of heat-stressed broilers during 1-49 days of age

Diet	Temperautre[2]	Mortality
CON	TN	7.50
	HS	12.50
CHO500	TN	2.50
	HS	13.75
CHO1000	TN	1.25
	HS	3.75
BET500	TN	2.50
	HS	11.25
BET1000	TN	1.25
	HS	15.00
SEM		3.64
Effect		p-values
Diet		0.3200
Temperature		0.0006
Diet×temperature		0.5400
Main effect means		
Diet		
CON		10.0000
CHO500		8.1300
CHO1000		2.5000
BET500		6.8800
BET1000		8.1300
SEM		2.5800
Temperature		
TN		3.0000[b]
HS		11.2500[a]
SEM		1.6300

[1]Values are least squares means. [2]TN: Thermoneutral environment, HS: Heat stress environment. [a,b]Means within same column followed by different letters differ significantly ($p<0.05$)

Table 8: Effect of choline and betaine supplementation to broilers on litter moisture content[1] under thermoneutral and heat stress conditions on day 42

Diet	Temperature[2]	Day 42
CON	TN	41.43
	HS	26.32
CHO500	TN	41.30
	HS	18.16
CHO1000	TN	45.39
	HS	25.40
BET500	TN	42.67
	HS	18.16
BET1000	TN	42.87
	HS	15.72
SEM		2.86
Effect		p-values
Diet		0.0700
Temp		0.0001
Diet×temperature		0.1700
Main effect means		
Diet		
CON		33.8800
CHO500		29.7300
CHO1000		35.4000
BET500		31.8200
BET1000		29.3000
SEM		2.0200
Temperature		
TN		42.7300[a]
HS		21.3100[b]
SEM		1.8600

[1]Values are least squares means. [2]TN: Thermoneutral environment, HS: Heat stress environment. [a,b]Means within same column followed by different letters differ significantly ($p<0.05$)

metabolism. Feed efficiency was affected by diet during the starter phase with BET500 and BET1000 showed higher feed efficiency compared to the CON. During 22-49 days of age, feed efficiency was decreased only by HS. These results are consistent with those reported by Bartlett and Smith[23] who found that broilers under cyclic and chronic heat stress (23.9-37°C) had lower feed intake compared to thermoneutral birds (23.9°C).

In this study, dietary supplementation of choline and betaine to provide 500 ppm and 1000 ppm methyl equivalents to a diet low in methionine did not improve weight gain, feed intake or feed efficiency during the grower and finisher periods. These results are in accordance with previous reports. Esteve-Garcia and Mack[24] fed methionine diet supplemented with 0.5 g kg^{-1} betaine and found no positive effect on broiler performance. However, contrary to the results of the current study, Waldroup *et al.*[25] evaluated choline and betaine sparing effect of methionine by adding 500 mg or 1000 mg of choline or betaine to the diet and reported an improvement in feed intake in birds reared in thermoneutral environment.

Reasons for these differences are unclear but they could be due to the fact that in the current study, betaine and choline were added to a diet deficient in methionine. According to Baker *et al.*[26], the beneficial effect of choline regarding its methionine sparing effect is more likely in a diet already containing adequate levels of choline and deficient in methionine. Although, methionine level was deliberately decreased compared to NRC requirements, choline sparing effect of methionine and its translation into weight gain was not apparent in this study.

In the current study, HSresulted in 11.25% mortality compared to 3.00% in TNenvironment. De Basilio *et al.*[10] obtained a higher mortality rate of 38% with thermally challenged chickens but that study employed a high temperature of 38°C while in the current study the maximum temperature in the HS room was 36°C. Supplementing diets with choline and betaine did not reduce mortality rate. This is consistent with the results reported by Waldroup and Fritts[27] who found no effect of choline and betaine on broilers mortality.

Litter moisture: Litter moisture content expressed as a percent of wet litter weight is reported in Table 8. Litter

Table 9: Effect of choline and betaine supplementation on foot pad score (%)[1] of heat-stressed broilers on day 42

	Foot pad lesion score			
Temperature[2]	0	1	2	3
HS[a]	58.81	15.80	16.06	9.33
TN[a]	58.16	20.92	13.27	7.65
Diet[2]				
CON[abc]	56.29	19.87	14.57	9.27
CHO500[ab]	67.27	15.76	9.70	7.27
CHO1000[c]	47.30	19.59	23.65	9.46
BET500[bc]	51.61	21.94	18.06	8.39
BET1000[b]	68.55	15.09	8.18	8.18

[1] Values are least squares means. [2] Pairwise comparisons among temperature and dietary treatments respectively, TN: Thermoneutral environment, HS: Heat stress environment. [a] Temp followed by different letters differ significantly in foot pad lesion score patterns (p<0.05). [a,b,c] Diet followed by different letters differ significantly in foot pad lesion score patterns p<0.05)

moisture (p = 0.17) was not affected by diet×temperature interaction. However, litter moisture was reduced (p = 0.0001) in the HS (21.31%) room compared to TNroom (42.73%). CHO500 (29.73%) and BET1000 (29.73%) tended to lower litter moisture (p = 0.07).

Foot pad dermatitis: Interaction of diet and temperature had no effect on foot pad lesion score (p>0.05, Table 9). Scores did not differ among temperature treatments (p = 0.22) but were affected by diets (p = 0.003). The pairwise comparisons showed that the pattern of foot pad lesion scores for the CON did not differ from that of the rest of the diets. However, BET1000 and CHO500 showed similar pattern with the highest number of unaffected (score 0) birds being 68.55 and 67.27% respectively.

Litter moisture was reduced by 50% in the heat stress room. This is not surprising due to the fact that in the HSenvironment, the temperature cycled between 28 and 36°C. This allowed the moisture in the litter to evaporate and the bedding to remain drier. Litter wetness has been positively linked to foot pad dermatitis[28,29]. Hence, it is very important to maintain litter dryness. Various factors can increase litter wetness and contribute to the development of foot pad dermatitis[30]. These include infectious diseases, dietary composition and increased water consumption due to electrolytes supplementation[31]. Water consumption was not measured in this study. In evaluating the impact of choline and betaine supplementation on litter wetness, results showed that CHO500 and BET1000 tended to decrease litter moisture compared to the CON. The trend was similar in foot pad scores. Even though CHO500 and BET1000 did not statistically differ from the CON, they showed less incidence of foot pad dermatitis (highest score 0). Chicken feet health is a welfare issue and it has been shown that turkey poults with severe necrosis develop pain induced inappetence resulting in decreased feed intake and weight gain[32]. Additionally, there is an increasing export market for chickens' paws in certain countries thus ensuring a high 0 score is economically important for poultry producers.

Breast meat color: The mean L*, a* and b* values for breast meat are presented Table 10. Diet or diet×temperature interaction had no effect on lightness and yellowness. Lightness (54.51) was significantly higher (p = 0.02) for HS birds compared to TN (51.73) birds. Conversely, yellowness was significantly higher (p = 0.004) for TN (3.75) compared to HS (2.60) birds.

Meat color and drip loss are important quality parameters that could influence consumers' selection. The results of this study showed that choline and betaine supplementation did not affect breast meat color nor was there a diet×temperature interaction. However, heat stress increased breast meat lightness and decreased yellowness. Similarly, in an experiment conducted by Akşit, *et al.*[33] in which broilers were exposed to cyclic temperature, breast meat lightness was increased by heat stress, however, meat yellowness was unaffected.

Breast meat drip loss: Drip loss assessed on days four and seven post slaughter is presented in Table 11. Drip loss 4 days post slaughter was affected by diet×temperature interaction (p = 0.04) . The lowest drip loss occurred for CHO500 (0.60%) and BET1000 (0.80%) in HS and TNenvironments respectively. For seven days post slaughter, drip loss was not significantly affected by diet, temperature or by their interaction (p>0.30). Breast meat drip loss was affected by diet×temperature interaction. This was caused by BET1000 increasing drip loss by 0.75% in HS compared to TN birds, while CHO500 and BET500 decreased it by 0.4 and 0.79% respectively. This suggests that the supplementation with CHO500 under HS and with BET1000 under TN conditions could reduce water loss during short term storage (up to four days). The

Table 10: Effect of choline and betaine supplementation on breast meat color (L*, a*, b*) [1] from heat-stressed broilers

Diets	Temperature	L*	a*	b*
CON	TN	53.09	2.84	4.33
	HS	55.41	2.16	2.58
CHO500	TN	51.56	2.66	3.27
	HS	53.19	2.73	2.01
CHO1000	TN	50.36	2.97	4.04
	HS	54.57	3.06	3.43
BET500	TN	52.60	2.96	4.47
	HS	54.05	2.48	3.08
BET1000	TN	51.04	2.88	2.64
	HS	53.84	2.86	1.92
SEM		1.18	0.36	0.62
Effect		--------------------	p-values	--------------------
Diet		0.370	0.46	0.060
Temperature		0.020	0.64	0.004
Diet×temperature		0.730	0.72	0.880
Main effect means				
Diet				
CON		54.25	2.50	3.450
CHO500		52.38	2.69	2.640
CHO1000		52.46	3.02	3.730
BET500		53.32	2.72	3.780
BET1000		52.46	2.87	2.280
SEM		0.840	0.26	0.430
Temperature				
TN		51.73^{b}	2.86^{a}	3.750^{a}
HS		54.21^{a}	2.66^{a}	2.600^{b}
SEM		0.670	0.19	0.280

[1]Values are least squares means. L*: Lightness, a*: Redness, b*: Yellowness [2]TN: Thermoneutral environment, HS: Heat stress environment. [a,b]Means within same column followed by different letters differ significantly (p<0.05)

Table 11: Effect of choline and betaine supplementation on drip loss from breast meat (%) [1] of heat-stressed broilers 4 and 7 days post slaughter

Diet	Temperature[2]	Day 4 post slaughter	Day 7 post slaughter
CON	TN	0.98^{ab}	1.80
	HS	0.92^{ab}	1.80
CHO500	TN	1.00^{ab}	1.74
	HS	0.60^{b}	1.13
CHO1000	TN	1.25^{ab}	2.06
	HS	0.90^{ab}	1.89
BET500	TN	1.62^{a}	2.10
	HS	0.83^{b}	1.18
BET1000	TN	0.80^{b}	1.61
	HS	1.55^{a}	2.13
SEM		0.28	0.37
Effect		---------------p-values	---------------
Diet		0.39	0.62
Temp		0.35	0.32
Diet×temperature		0.04	0.35
Main effect means			
Diet			
CON		0.94	1.80
CHO500		0.80	1.43
CHO1000		1.08	1.97
BET500		1.23	1.64
BET1000		1.18	1.87
SEM		0.17	0.26
Temperature			
TN		1.13	1.86
HS		0.96	1.63
SEM		0.13	0.16

[1]Values are least squares means. [2]TN: Thermoneutral environment, HS: Heat stress environment. [a,b]Means within same column followed by different letters differ significantly (p<0.05)

supplementation of BET1000 under HS conditions may have caused betaine to effectively accumulate in cells and help retain water in HS birds. This could explain the higher drip loss of breast meat from HS birds. In order to fully understand the negative impact of HS and dietary supplementation of choline and betaineon the meat quality, further research need to be done including the determination of the meat pH, cooking loss and shear force.

CONCLUSION

In this study, diets supplemented with CHO500 or BET1000 tended to result in lower litter moisture and decreased the incidence of pododermatitis. Breast meat drip loss was lower in HS chickens fed CHO500 and in TN chickens fed BET 1000. Heat stress increased breast meat lightness regardless of betaine and choline supplementation. Heat stress decreased feed efficiency and weight gain of broilers during days 22-49 and the supplementation of betaine and choline did not prevent or reduce these negative impacts.

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