Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part I: Growth performance, body composition and intestinal nutrient transporters

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ABSTRACT High ambient temperature is one of the most common stressors in modern poultry production, resulting in reduced feed intake, weight gain, and increased mortality. This study evaluated the effects of vitamin E (**Vit E**) and organic selenium (**Se**) supplementation on performance, body composition, core body temperatures, and mRNA abundance of nutrient transporters in the jejunum of broilers exposed to daily 4-h elevated temperature during d 28 to 35. A total of 640 Cobb male birds were randomly allocated to 32 floor pens in a 2 × 2 factorial arrangement that included ambient temperature (thermoneutral, [**TN**]; or heat stress, [**HS**]) and dietary treatments (basal diet or Vit E + Se). Four rooms were used (2 TN and 2 HS) each housing half of the 8 replicate pens per group. Vit E and organic Se were added to the basal diet at the rate of 250 mg/kg and 1 mg/kg diet, respectively. Data were subjected to a 2-way ANOVA using the GLM procedure of JMP (SAS). During the HS period, birds fed the Vit E/Se diet had significantly lower mortality compared to nonsupplemented group (1.92% vs. 7.01%). Moreover, dietary Vit E/Se supplementation had a significant effect on performance by increasing BWG, FI, and European production efficiency factor (**EPEF**) during the entire experimental period (d 0−35). Dietary Vit E and Se supplementation significantly increased carcass, tissue, lean, and fat weights as well as bone mineral content (**BMC**) and bone mineral density (**BMD**) on d 35. Birds fed Vit E/Se supplemented diet had significantly lower ($P = 0.010$) core body temperature compared to birds fed the basal diet on d 30. Dietary treatment did not influence mRNA abundance of PepT1, SGLT1, or NaPi-IIb on d 28 or d 35. However, HS significantly upregulated levels of PepT1 and NaPi-IIb ($P < 0.001$) and downregulated that of SGLT1 ($P = 0.017$) on d 28. In conclusion, dietary Vit E and Se supplementation significantly improved broiler growth performance and carcass composition, and reduced heat-related mortality and core body temperature (on d 30) without influencing the mRNA abundance of intestinal nutrient transporters.

Key words: broiler, heat stress, nutrient transporter, selenium, vitamin E

INTRODUCTION

Modern poultry production is associated with a range of stressors including environmental, nutritional, and biological, and such stress factors inevitably influence the animal's physiology and performance with varying degrees (Surai et al., 2019). High ambient temperature is one of the most common stressors in commercial poultry production, resulting in reduced feed intake and body weight gain, and increased mortality (Lara and Rostagno, 2013; Emami et al., 2021). Because of their physiological state and greater metabolic activity, broilers are more susceptible to temperature-associated environmental challenges (Sahin et al., 2009; Lara and Rostagno, 2013; Emami et al., 2020). In addition to its effect on bird performance, it also causes alterations in intestinal nutrient transporters (Habashy et al., 2017), gut permeability and function (Slawinska et al., 2016), immune response (Habibian et al., 2014; Emami et al., 2021) and the endocrine system including cortisol and

thyroid hormones (Sohail et al., 2010). Moreover, elevated ambient temperatures have been reported to cause undesirable changes in carcass characteristics (De Antonio et al., 2017; Emami et al., 2021), bone mineralization (Yan et al., 2019), and meat quality (Cheng et al., 2018).

Exposure to high ambient temperature elevates the level of reactive oxygen species (**ROS**) and causes biological and physiological disturbances in cellular functions (Emami et al., 2020). An uncontrolled increase in ROS level leads to free radical mediated chain reactions, which further causes lipid peroxidation and oxidative damage to proteins, DNA, and RNA (Hai et al., 2006; Harsini et al., 2012; He et al., 2018). Birds respond to such conditions by reprogramming several defense mechanisms including antioxidant enzymes, heat-shock proteins, and cytokines to alleviate or reduce the negative effects of HS (Tan et al., 2010; Slimen et al., 2016). Moreover, environmental and nutritional strategies are available to improve the efficiency of these defense mechanisms (Flees et al., 2021; Greene et al., 2021). Among the strategies for coping with the impact of HS in animals, dietary interventions through supplementation of several feed additives including vitamins (e.g., A, E, and C) and minerals (e.g., zinc, selenium) can be utilized to improve the host's antioxidant defense mechanism (Sahin and Kucuk, 2003; Habibian et al., 2016; He et al., 2018).

Vitamin E (**Vit E**) is a biological antioxidant and a free radical scavenger that protects the cells and lipid rich-membranes from oxidative damage (Traber and Atkinson, 2007; Gao et al., 2010) and is considered as the core of the antioxidant system (Surai et al., 2019). Among the four tocopherols and four tocotrienols (designated as α-, β-, γ-, and δ-), only the α-tocopherol form has the biological activity to meet the animal's Vit E requirements. Dietary supplementation of various levels of Vit E can improve broiler performance (Sahin et al., 2002; Attia et al., 2017) and reduce the oxidative stress experienced under high environmental temperatures (Sahin et al., 2001).

Selenium is an essential trace mineral that plays a pivotal role within several metabolic pathways. It is a component of selenium-dependent antioxidant enzymes such as glutathione peroxidases (**GSH-Px**) (Habibian et al., 2015). Along with superoxide dismutase (**SOD**) and catalase (**CAT**), this enzyme protects the cells against free radicals and lipid peroxides especially under stress conditions (Sahin and Kucuk, 2001). Since the plant-based feed ingredients used in the diet are deficient in Se, inorganic (sodium selenite) and organic (selenomethionine and selenoyeast) forms are used to supply broiler needs (Habibian et al., 2015). Thus, these Se sources are usually added at 0.2 to 0.3 mg/kg diet to meet the requirements without relying on Se content of the feed ingredients for optimum broiler growth. The maximum allowable level of organic Se sources in complete feed is 0.3 mg/kg in US (FDA, 2017) and 0.2 mg/kg in Europe (EFSA, 2011). However, dietary addition of Se above the recommended level might have beneficial effects on broiler performance, oxidative status, and immune response during a stress challenge (Surai, 2018).

Elevated ambient temperature can impair the absorption of vitamins A, C, and E and reduce tissue mineral concentrations including iron, zinc, and selenium (Sahin and Kucuk, 2003; Harsini et al., 2012; Habibian et al., 2016). In line with published data suggesting the beneficial and protective effects of dietary Vit E and Se during HS conditions, the present study aimed to investigate the effects of dietary supplementation of Vit E and Se on broiler growth performance, mortality rate, body composition and mRNA abundance of several nutrient transporters in the jejunum.

MATERIALS AND METHODS

Experimental Diets, Birds, and Management

This project was approved and conducted under the guidelines of the Virginia Tech Institutional Animal Care and Use Committee. A total of 640 one-day-old Cobb male chicks were randomly allocated to 32 floor pens in a 2×2 factorial arrangement that included ambient temperature (thermoneutral, [**TN**]; or heat stress, [**HS**]) and dietary treatments (basal diet or Vit E + Se) as main factors. Four rooms were used (2 TN and 2 HS) each housing half of the 8 replicate pens per group. For the Vit E and Se diet, the basal diet was supplemented with 250 mg/kg Vit E (Rovimix E50, DSM Nutritional Products, Basel, Switzerland) and 1 mg/kg organic Se (Sel-Plex, Alltech Inc., Nicholasville, KY).

The birds were raised in a controlled environment for 35 d. The starter and grower diets were based on corn-soybean meal formulations and provided to the birds from d 0 to 21 and d 21 to 35 of age, respectively (Table 1). All diets were formulated to meet or exceed the NRC (1994) nutrient recommendations. Each pen was equipped with a plastic bucket feeder and an automatic nipple drinker. Water and experimental diets (in mash form) were provided ad libitum throughout the study period. All chicks were weighed on a per pen basis and feed intake (**FI**) was recorded at weekly intervals. Any mortality was removed and recorded (including bird weight) twice daily. Body weight gain (**BWG**), FI, and feed conversion ratio (**FCR**) were subsequently calculated based on performance values. European production efficiency factor (**EPEF**) was calculated using the following formula: [(liveability (%) $\times$ live weight (kg) / age (d) $\times$ FCR) $\times$ 100].

Heat Stress Protocol

The HS protocol was performed during the last week of the experiment (d 28–d 35). For the HS groups, temperature was raised and maintained at 35 $\pm$ 1°C from 10 AM to 2 PM (4 h), then reduced to 25 $\pm$ 1°C for the remainder of the day. Relative humidity was monitored and maintained at <50%. Birds in the TN groups were kept under recommended temperature (25 $\pm$ 1°C). The

Table 1. Basal diet formulation for starter (d 0−21) and grower (d 22−35) phases.

Ingredient, g/kg	0-21 d	22-35 d
Corn	59.73	64.44
Soybean meal, CP 48%	33.20	28.00
Vegetable oil	3.00	4.20
Limestone	0.68	0.60
Dicalcium phosphate	2.20	1.80
DL-Methionine (98%)	0.20	0.13
L-Lysine HCl (78%)	0.24	0.12
L-Threonine	0.09	0.05
Salt	0.30	0.30
Vit-Min (NB3000)[1,2]	0.36	0.36
Total	100.00	100.00
Chemical composition (Calculated)		
Dry Matter, %	87.75	87.75
Crude Protein, %	21.00	18.75
AME_n, kcal/kg	3060	3185
Lysine, %	1.32	1.08
Dig. Lysine, %	1.18	0.95
Methionine + cysteine, %	0.88	0.76
Dig. Methionine + cysteine, %	0.78	0.66
Threonine, %	0.90	0.77
Dig. Threonine, %	0.77	0.66
Calcium, %	0.90	0.76
Available phosphorus, %	0.45	0.38

[1]Vitamins supplied per kg diet: Retinol, 3.33 mg; Cholecalciferol, 0.1 mg; α-tocopherol acetate, 23.4 mg, Vitamin K_3, 1.2 mg; Thiamine, 1.6 mg; Riboflavin, 9.5 mg; Niacin, 40 mg; Pantothenic acid, 9.5 mg; Pyridoxine, 2 mg; Folic acid, 1 mg; Vitamin B_{12}, 0.016 mg; Biotin, 0.05 mg; Choline, 556 mg.

[2]Minerals supplied per kg diet: Mn, 144 mg; Fe, 72 mg; Zn, 144 mg; Cu, 16.2 mg; I, 2.15 mg; Se 0.22 mg.

birds' core body temperature (2 birds/pen) was monitored daily with a rectal probe during the HS period (d 28−35).

Carcass Composition Analysis (DEXA)

Dual Energy X-Ray Absorptiometry (**DEXA**) analysis was performed on 3 birds per pen on d 35 to assess various measurements of carcass/body composition. Birds were individually wing-banded, sacrificed by cervical dislocation and subsequently defeathered. Carcasses were stored at −20°C until DEXA analysis. Birds were thawed and 10 birds were scanned at a time using a GE Healthcare Lunar Prodigy Advance System (General Electric, Madison, WI). Individual bird scans were used to calculate measurements.

Total RNA Extraction and Reverse Transcription

On d 28 and d 35, 2 birds/pen were selected, euthanized, and jejunal sections (1 cm) were excised and snap-frozen in liquid nitrogen to later assess the mRNA abundance of several nutrient transporters. A 20 to 30 mg aliquot of jejunal tissues was weighed into a 2 mL microcentrifuge tube and homogenized in 600 μL TRI Reagent (Zymo Research, Irvine, CA) by a TissueLyser II (Qiagen, Valencia, CA). Total RNA was extracted from the homogenate using the Direct-zol RNA Miniprep Plus Kit (Zymo Research, Irvine, CA) according to the manufacturer's recommendations. Total RNA concentration was determined at optical density (**OD**) of 260 using a NanoDrop-1000 (Thermo Fisher Scientific, Waltham, MA), and RNA purity was verified by evaluating the 260/280 OD ratios. RNA integrity was evaluated by gel electrophoresis on 1.5% agarose gel in 0.5 × TAE buffer. After extraction, 2 μg of total RNA were used to synthesize first-strand cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendation and the cDNA was stored at −20°C until further analysis.

Quantitative Real-Time PCR

The mRNA abundance of peptide transporter 1 (**PePT1**), sodium-glucose cotransporter 1 (**SGLT1**), and type IIb sodium-phosphate cotransporter (**NaPi-IIb**) were determined by quantitative real-time PCR (ABI 7500 Fast Real-Time PCR System, Applied Biosystems, Foster City, CA) using Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA). Details of primer sets are provided in Table 2. Product specificity was confirmed by analysis of the melting curves produced by the ABI 7500 software (version 2.0.3). mRNA abundance was analyzed using glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) as an endogenous control. Average mRNA abundance relative to GAPDH for each sample was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The calibrator for each gene was the average ΔCt value from the negative control group for each sampling day.

Table 2. Sequences of primer pairs used for amplification of target and reference genes.

Gene[1]	Primer Sequence	Size	Acc (Reference)
PepT1	CCCCTGAGGAGGATCACTGTT CAAAAGAGCAGCAGCAACGA	66	NM_204365 (Kaminski and Wong, 2018)
SGLT1	GCCATGGCCAGGGCTTA CAATAACCTGATCTGTGCACCAGTA	71	NM_001293240 (Kaminski and Wong, 2018)
NaPi-IIb	GGCTGGCATGACTTTCGTTG GGGTGAGGGGATAAGAACGC	107	NM_204474

[1]PepT1: peptide transporter-1 (SLC15A1), SGLT1: sodium-glucose cotransporter-1 (SLC5A1), NaPi-IIb: type IIb sodium-phosphate cotransporter (SLC34A2).For each gene, the primer sequence for forward (F) and reverse (R) (5'-3') primers, the amplicon size (bp) and the NCBI Accession number (Acc) used for the primer design are listed.

Statistical Analysis

Data were subjected to a 2-way ANOVA using the GLM procedure of JMP (Pro 13). The model included ambient temperature (TN or HS) and dietary treatments (basal diet or Vit E/Se) as the main factors, and the 2-way interactions. Tukey's multiple range test was only carried out for significant interactions and was performed using simple effect analysis. Mortality rates were compared using a chi-square test. The probability $P < 0.05$ was considered significant unless otherwise noted.

RESULTS

Growth Performance

The effects of dietary Vit E and Se administration on the growth performance of broilers under TN and HS conditions are shown in Tables 3 and 4, respectively. There was no significant interaction effect on the growth performance of the birds in terms of BWG, FCR, and FI between dietary and ambient temperature treatments during different experimental periods, or the entire course of the experiment. Under TN condition, dietary supplementation of Vit E and Se significantly improved BWG between 0 to 7, 0 to 14, 0 to 21, and 0 to 27 days of the study ($P < 0.05$). Moreover, dietary intervention significantly influenced FCR ($P = 0.040$) during the first week of the study. Except for week one (d 0–7), birds in the Vit E/Se group consumed more feed compared to the control group ($P < 0.05$).

The environmental heat challenge caused a significant reduction in BWG and FI, and increased FCR from d 28 to 35 as well as during the entire experimental period (d 0–35). During the HS period (d 28–35), mortality rate was significantly higher ($P < 0.001$) in the heat-challenged groups in comparison to control birds (4.47% vs. 0%, respectively). However, the mortality rate was found to be lower ($P = 0.030$) in birds fed the Vit E/Se

Table 3. Dietary Vit E/Se supplementation on broiler performance between d 0 to d 27.[1]

Item[3]	Treatment Groups[2]		Statistics	
	Control	Vit E/Se	SEM	P value
0 to 7 d				
BWG (g)	105.3	110.8	1.40	0.049
FI (g)	146.2	147.7	1.85	0.695
FCR	1.389	1.334	0.01	0.040
0 to 14 d				
BWG (g)	390.1	409.7	3.87	0.009
FI (g)	542.9	572.3	5.60	0.006
FCR	1.392	1.399	0.01	0.738
0 to 21 d				
BWG (g)	888.2	913.3	6.41	0.049
FI (g)	1230.7	1279.5	9.20	0.006
FCR	1.386	1.401	0.01	0.281
0 to 27 d				
BWG (g)	1357.4	1392.9	8.36	0.031
FI (g)	1993.4	2050.4	11.57	0.011
FCR	1.469	1.472	0.01	0.763

[1]Data represent mean values of 16 replicates per treatment.
[2]Control: birds fed a basal diet; Vit E/Se: birds fed a basal diet supplemented with 250 mg/kg vitamin E and 1 mg/kg selenium.
[3]BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

supplemented diet during d 28 to 35. Dietary Vit E/Se supplementation had a pronounced effect on broiler performance by increasing BWG, FI, and EPEF during the overall experimental period (d 0 to 35). Moreover, the cumulative mortality rate tended to be lower ($P = 0.086$) in the Vit E/Se birds compared to control birds during the overall study period (d 0 to 35).

Carcass and Body Composition

The effects of dietary supplementation of Vit E/Se on broiler body/carcass composition (carcass weight, tissue weight, lean weight, fat weight, tissue percentage, bone mineral content [**BMC**], bone mineral density [**BMD**] and surface area of defeathered whole carcass) under HS condition are shown in Table 5. There was no significant interaction effect on broiler body/carcass composition between the dietary and ambient temperature

Table 4. Effects of dietary Vit E/Se supplementation on body weight gain, feed intake, and feed conversion ratio of broilers under heat stress.[1]

Item[4]	Treatments[2]									Statistics[3]		
	Control		Vit E/Se		Diet		Ambient Temperature			P value		
	TN	HS	TN	HS	Control	Vit E/Se	TN	HS	RMSE	Diet	AT	D × AT
28 to 35 d												
BWG (g)	651.2	606.4	693.5	614.7	628.8	654.1	672.3	610.5	44.43	0.118	0.001	0.288
FI (g)	1118	1073	1145	1087	1096	1116	1127	1080	47.83	0.155	0.010	0.519
FCR	1.709	1.771	1.651	1.773	1.740	1.712	1.680	1.772	0.06	0.235	<0.001	0.215
Mortality (%)	0.00	7.01	0.00	1.92	3.57	0.97	0.00	4.47	-	0.030	<0.001	-
0 to 35 d												
BW (g)	2061	2004	2145	2040	2032	2093	2103	2022	66.21	0.015	0.002	0.320
BWG (g)	2018	1961	2103	1998	1989	2050	2060	1979	66.45	0.015	0.002	0.319
FI (g)	3104	3075	3218	3115	3089	3166	3156	3095	81.18	0.008	0.042	0.160
FCR	1.535	1.570	1.530	1.560	1.553	1.545	1.533	1.565	0.003	0.467	0.003	0.808
EPEF	362.1	333.3	385.8	357.9	347.7	371.9	373.9	345.6	29.14	0.026	0.010	0.967
Mortality (%)	5.63	8.75	3.75	4.38	7.19	4.06	4.69	6.56	-	0.086	0.303	-

[1]Data represent mean values of 8 replicates per treatment.
[2]Control: birds fed a basal diet; Vit E/Se: birds fed a basal diet supplemented with 250 mg/kg vitamin E and 1 mg/kg selenium; TN: thermoneutral; HS: heat stress.
[3]AT: Ambient Temperature; D: Diet
[4]BW: body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; EPEF: European production efficiency factor.

Table 5. Effects of dietary Vit E/Se supplementation on body composition of broilers under heat stress.[1]

	Treatments[2]				Diet		Ambient Temperature			Statistics[3]		
	Control		Vit E/Se							P-value		
Item[4]	TN	HS	TN	HS	Control	Vit E/Se	TN	HS	RMSE	Diet	AT	D × AT
Carcass, (g)	2149	2110	2318	2206	2130	2262	2234	2158	167.1	<0.001	0.029	0.289
Tissue, (g)	1973	1929	2141	2050	1951	2096	2057	1990	160.9	<0.001	0.042	0.471
Lean, (g)	1572	1546	1729	1633	1559	1681	1651	1590	137.8	<0.001	0.033	0.218
Fat, (g)	400.9	382.9	412.2	416.9	391.9	414.5	406.5	399.8	50.88	0.032	0.524	0.277
Tissue (%Fat)	20.33	19.85	19.31	20.30	20.10	19.80	19.82	20.08	2.04	0.505	0.537	0.080
BMC, (g)	25.38	25.42	26.87	26.45	25.40	26.60	26.13	25.94	2.61	0.020	0.723	0.665
BMD, (g/cm^2)	0.193	0.199	0.202	0.205	0.196	0.203	0.197	0.202	0.01	0.003	0.062	0.601
Area, (cm^2)	161.1	156.5	163.5	157.8	158.8	160.6	162.3	157.1	14.86	0.538	0.089	0.848

[1]Data represent mean values of 24 replicates per treatment.
[2]Control: birds fed a basal diet; Vit E/Se: birds fed a basal diet supplemented with 250 mg/kg vitamin E and 1 mg/kg selenium; TN: thermoneutral; HS: heat stress.
[3]AT: Ambient Temperature; D: Diet.
[4]BMC: bone mineral content; BMD: bone mineral density.

treatments at the end of the study. As expected, HS significantly reduced broiler carcass, tissue, and lean weights on d 35. In accordance with the performance data, dietary Vit E/Se supplementation significantly increased carcass, tissue, lean, and fat weights, as well as BMC and BMD on d 35.

Body Temperature

As expected, elevated environmental temperature significantly increased broiler core body temperature between d 28 and d 35 (Table 6). There was a significant

interaction ($P = 0.033$) between dietary and ambient temperature treatments in terms of core body temperature on d 30. Broiler core body temperature was not affected by dietary treatment between d 28 and 35 except for d 30. Birds fed a diet containing Vit E/Se had significantly lower ($P = 0.010$) core body temperature compared to birds fed a control diet on d 30.

mRNA Abundance of Nutrient Transporters

The effects of dietary Vit E/Se administration on the mRNA abundance of PepT1, SGLT1, and NaPi-IIb in

Table 6. Effects of dietary Vit E/Se supplementation on rectal temperature of broilers under heat stress.[1]

	Treatments[2]				Diet		Ambient Temperature			Statistics[3]		
	Control		Vit E/Se							P value		
Item	TN	HS	TN	HS	Control	Vit E/Se	TN	HS	RMSE	Diet	AT	D × AT
D28	41.74	44.18	41.82	44.04	42.96	42.93	41.78	44.11	0.36	0.729	<0.001	0.215
D29	41.78	43.92	41.76	43.88	42.85	42.82	41.77	43.90	0.23	0.622	<0.001	0.869
D30	41.76^c	44.23^a	41.69^c	43.60^b	42.99	42.65	41.72	43.92	0.52	0.010	<0.001	0.033
D31	41.49	43.77	41.59	43.70	42.63	42.65	41.54	43.73	0.40	0.877	<0.001	0.406
D32	41.63	43.78	41.53	43.73	42.70	42.63	41.58	43.75	0.30	0.344	<0.001	0.710
D33	41.63	43.69	41.49	43.67	42.66	42.58	41.56	43.68	0.46	0.503	<0.001	0.610
D34	41.69	44.13	41.83	43.96	42.91	42.89	41.76	44.04	0.40	0.900	<0.001	0.137
D35	41.68	43.69	41.74	43.48	42.68	42.61	41.71	43.58	0.41	0.506	<0.001	0.186

$^{a-c}$Means with different superscripts in the same row are significantly different ($P < 0.05$).
[1]Data represent mean values of 16 replicates per treatment.
[2]Control: birds fed a basal diet; Vit E/Se: birds fed a basal diet supplemented with 250 mg/kg vitamin E and 1 mg/kg selenium; TN: thermoneutral; HS: heat stress.
[3]AT: Ambient Temperature; D: Diet.

Table 7. Relative mRNA abundance of PepT1, SGLT1, and NaPi-IIb in the jejunum of broiler chickens on d 28 and d 35.[1]

	Treatments[2]				Diet		Ambient Temperature			Statistics[3]		
	Control		Vit E/Se							P-value		
Item[4]	TN	HS	TN	HS	Control	Vit E/Se	TN	HS	RMSE	Diet	AT	D × AT
d 28												
PepT1	1.18	2.02	1.08	2.24	1.60	1.66	1.13	2.13	0.91	0.814	0.001	0.483
SGLT1	1.04	0.75	0.91	0.70	0.89	0.80	0.97	0.72	0.40	0.383	0.017	0.665
NaPi-IIb	1.24	2.09	1.26	1.93	1.67	1.60	1.25	2.01	0.85	0.751	0.001	0.675
d 35												
PepT1	1.12	1.42	1.37	1.60	1.27	1.48	1.24	1.51	0.64	0.184	0.096	0.833
SGLT1	1.07	0.92	1.22	0.95	1.00	1.08	1.15	0.93	0.45	0.433	0.062	0.624
NaPi-IIb	1.03	1.17	1.16	0.97	1.10	1.06	1.10	1.07	0.42	0.735	0.779	0.129

[1]Data represent mean values of 16 replicates per treatment.
[2]Control: birds fed a basal diet; Vit E/Se: birds fed a basal diet supplemented with 250 mg/kg vitamin E and 1 mg/kg selenium; TN: thermoneutral; HS: heat stress.
[3]AT: Ambient Temperature; D: Diet.
[4]PepT1: peptide transporter-1 (SLC15A1), SGLT1: sodium-glucose cotransporter-1 (SLC5A1), NaPi-IIb: type IIb sodium-phosphate cotransporter (SLC34A2).

the jejunum of broilers under HS conditions are shown in Table 7. There was no significant interaction on jejunal mRNA abundance of these nutrient transporters between the dietary treatment and ambient temperature groups on d 28 or d 35. HS significantly upregulated PepT1 and NaPi-IIb mRNA levels ($P < 0.001$) and downregulated that of SGLT1 ($P = 0.017$) on d 28. The dietary treatment did not influence mRNA abundance of PepT1, SGLT1, and NaPi-IIb on d 28 and d 35.

DISCUSSION

Heat stress causes an inevitable reduction in feed consumption and an increase in mortality that leads to reduced growth performance and significant economic losses in intensive poultry production systems (Lara and Rostagno, 2013). Several higher eukaryotic organisms, including poultry, have developed an integrated antioxidant defense system to reduce or eliminate the effects of such environmental challenges and maintain homeostasis and survival (Surai and Kochish, 2019). However, quite a long history of research revealed that the antioxidant defense system of the host is not efficient enough under severe stress conditions to maintain both growth performance and health without additional nutrients (Surai, 2018). Thus, modulation of the antioxidant response via several nutritional practices becomes a topic of great interest. In this context, we hypothesized that excess dietary Vit E (250 mg/kg) plus organic Se (1 mg/kg) could make a valuable contribution to this integrated antioxidant system by reducing the negative impact of HS and improving broiler performance and mRNA abundance of nutrient transporters.

In the present study, dietary supplementation of Vit E/Se significantly improved broiler BWG and FI under TN conditions on d 0 to 27. The current recommended levels for Vit E and Se are accepted as sufficient, and excess levels are generally not needed under standard production practices. However, there are contradicting results reporting either an enhancement (Swain et al., 2000; Dalia et al., 2018) or no change (Kim et al., 2010; Habibian et al., 2016) in broiler growth performance due to supplemental Vit E and/or Se under standard conditions. The increase in BWG and FI starting from the first week of this study might be attributable to the antioxidant effect of Vit E and Se against transport and handling stress or other possible stressors during standard husbandry practices. Nevertheless, this finding was not the main goal of the study.

The elevated environmental temperature between d 28 and d 35 markedly increased the birds' body temperature, and altered their behavior (panting, elevated wings, diminished feed intake) indicating that the birds were experiencing HS. As expected, the heat challenge retarded growth performance and increased mortality rates between d 28 and d 35 as well as during the overall experimental period. The negative effects of elevated environmental temperature on broiler performance and health were also documented in previous studies

(Dai et al., 2009; Niu et al., 2009; Quinteiro-Filho et al., 2010; Habibian et al., 2016). More recently, Emami et al. (2021) suggested that impaired performance during cyclic HS (35°C for 8 h) not only correlated with lower FI but also altered gut integrity and dysregulated immune response in HS-birds. Results from the current study indicated that dietary Vit E/Se supplementation had no effect on broiler BWG, FI, and FCR during the HS period (d 28−35). However, as an important finding, the mortality rate was significantly reduced in birds receiving a Vit E/Se (1.92%) supplement compared to birds in the control group (7.01%) during the HS period. Even though neither BWG nor FCR were statistically influenced during the HS period (d 28−35), the reduced mortality rate due to the dietary Vit E/Se intervention would raise the profitability rate in industrial practices by directly influencing flock performance. Moreover, dietary supplementation of Vit E/Se alleviated the growth suppression effect of the heat challenge by increasing BWG, FI, and EPEF during the overall experimental period (d 0−35). In agreement with these findings, Sahin and Kucuk (2001) reported that a combined use of 250 mg/kg Vit E and 0.2 mg/kg Se improved performance of Japanese quails under constant HS due to a possible synergistic mode of action between these nutrients in alleviating the negative impact of oxidative damage. In addition, dietary addition of organic Se (0.4 mg/kg) with or without ginger resulted in improved broiler performance as evidenced by a significant increase in BWG and FI (Safiullah et al., 2019). However, contradictory results were also reported concerning the efficiency of Vit E and/or Se in improving growth performance of broilers under HS conditions Habibian et al. (2016) did not observe any significant differences in performance between control and dietary Vit E (0, 125, or 250 mg/kg) and Se (0, 0.5, or 1 mg/kg) supplemented birds under 37°C cyclic HS for 8 h. Yet, they reported an increase of Se content in breast muscle accompanied with reduced malondialdehyde (**MDA**) content due to Vit E/Se supplementation. Similarly, Harsini et al. (2012) reported that single or combined (125 mg/kg Vit E and 0.5 mg/kg Se) use of Vit E and/or Se had no effect on broiler performance when birds were exposed to 37°C cyclic HS for 8 h.

In addition to the performance impairment, elevated environmental temperatures also induce changes in broiler body composition (De Antonio et al., 2017) such as increased fat deposition (Emami et al., 2021), reduced carcass protein content (Zhang et al., 2012), and reduced bone mineralization (Hosseini-Vashan et al., 2016). Our DEXA findings revealed that carcass, tissue, and lean weights were significantly reduced due to the HS; however, dietary supplementation of Vit E and Se markedly increased these values and alleviated the negative effects of HS in accordance with growth performance parameters. Since this technique is relatively new in poultry-focused studies, there are not enough published data to discuss the DEXA outputs of the current study. However, these results agree with published work whereby dietary addition of 250 mg/kg Vit E and

0.2 mg/kg Se increased the carcass characteristics of quails subjected to constant HS as determined by conventional methods (Sahin and Kucuk, 2001). In contrast, Habibian et al. (2016) showed that cyclic heat challenge (37°C for 8 h) and/or dietary supplemental Vit E and Se did not influence broiler carcass, breast, and thigh yields and these findings coincided with their performance results. It should be noted that the degree of environmental temperature and the stress duration experienced by the birds influence the efficiency of these antioxidants (Lara and Rostagno, 2013). Improvements in broiler growth performance and carcass parameters (carcass, tissue, and lean weights) in the present study are presumably related to the modulatory effects of Vit E and/or Se on antioxidant defense mechanisms (Khan et al., 2018; Kumbhar et al., 2018) and immune responses (Swain et al., 2000; Dalia et al., 2018).

Bone health issues present a serious problem in the broiler industry that leads to significant economic losses and reduced welfare (Shim et al., 2012). Elevated temperatures cause bone loss due to the increased corticosterone levels (Xu et al., 2018), which stimulates osteoclast activity (Hirayama et al., 2002) and reduces mineral consumption and absorption (Yan et al., 2019). Moreover, HS-related excessive rapid breathing causes respiratory alkalosis accompanied by increased blood pH and reduced ionized Ca concentration in the blood, which also contribute to reduced bone mineralization and strength (Yan et al., 2020). BMC and BMD, are important variables that provide valuable information regarding bone health and are strongly correlated with bone strength (Schreiweis et al., 2005). According to our findings, the HS challenge did not influence BMC or BMD, most likely due to the degree and duration of the implemented HS, but dietary addition of Vit E/Se improved BMC and BMD of those birds Talaty et al. (2009) suggested that BMD was not correlated with broiler body weight after 4 wk of age. Therefore, we can assume that increased BMC and BMD in Vit E/Se birds were directly related to dietary treatment, but not to improved weight gain. Similar to our findings, Vakili et al. (2010) reported that dietary Zn and Vit E supplementation improved the bone strength of heat-stressed broilers. An oxidative stress-related increase in ROS levels inhibits bone mineralization by elevating the osteoclast activity and inducing apoptosis of osteoblasts and osteocytes (Domazetovic et al., 2017). Conversely, antioxidants have an important role in maintaining osteoblast/osteoclast activity and bone health (Domazetovic et al., 2017). Therefore, the observed improvement in BMC and BMD values might be associated with the increased feed consumption and antioxidant properties of Vit E/Se. These results may indicate greater structural stiffness of leg bones; thus, dietary Vit E/Se supplementation may be useful under HS conditions to support bone health.

Absorption of nutrients through the intestinal tract is mediated by several transport proteins expressed on the enterocytes (Gilbert et al., 2008). Various factors and conditions including age, genetic selection, dietary nutrients, intestinal pathogens, and environmental stress regulate the expression levels of these transporters (Gilbert et al., 2008; Su et al., 2014; Habashy et al., 2017). Regardless of the dietary treatments, HS challenge significantly influenced mRNA levels of these nutrient transporters, especially on d 28. PepT1 is a member of the solute carrier 15 (**SLC15**) family of peptide transporters, capable of transporting di- and tripeptides (Daniel and Kottra, 2004; Madsen and Wong, 2011). The current study revealed that exposure to high temperature significantly increased mRNA abundance of PepT1 in the jejunum on d 28. Moreover, there was a tendency for upregulation of jejunal PepT1 on d 35. Increased expression of PepT1 was also demonstrated in response to elevated ambient temperature (Habashy et al., 2017) and fasting conditions in broiler chicks (Madsen and Wong, 2011). Thus, the HS-related reduction in feed intake might induce mRNA abundance of PepT1 to maximize protein uptake under stress conditions. SGLT1 transports glucose and galactose across the apical side of the intestinal epithelial cells (Moran et al., 2010). Glucose sensors located on the enterocytes sense the luminal presence of monosaccharides, which further induces a signaling cascade leading to upregulation of SGLT1 (Dyer et al., 2003). Therefore, downregulation of SGLT1 in the present study might be associated with the lower luminal glucose level caused by reduced feed intake in HS birds. Another possible explanation could be that the heat stressed birds try to promote gluconeogenesis and glycogenolysis by reducing the mRNA level of intestinal SGLT1 (Habashy et al., 2017). HS-related downregulation of intestinal SGLT1 in chickens was also evidenced in previous studies (Faseleh Jahromi et al., 2016; Habashy et al., 2017). However, there are also contradictory results indicating no differences in SGLT mRNA level in the jejunum of birds exposed to 32 ± 1°C cyclic HS for 10 h (Sun et al., 2015) and growing pigs subjected to natural high ambient temperature (Cervantes et al., 2016). NaPi-IIb is an important transport protein for the absorption of phosphorus in the small intestine. Previous studies revealed that expression of NaPi-IIb in the small intestine is primarily regulated by vitamin D_3 (Xu et al., 2002) and dietary P levels (Hattenhauer et al., 1999). In the present study, increased mRNA abundance of NaPi-IIb in HS birds on d 28 was likely an adaptation strategy to maintain P balance in response to reduced feed intake during HS Li et al. (2012) revealed that reduction in the dietary P level significantly induced NaPi-IIb mRNA abundance. However, NaPi-IIb mRNA abundance was not influenced by either Vit E/Se supplementation or HS challenge on d 35. Reduction in P demand (Waldroup et al., 2000) and downregulation of NaPi-IIb mRNA abundance (Li et al., 2017) with the growth of broilers could be the reason for the variation observed between d 28 and d 35. To the best of our knowledge, published data on the response of intestinal nutrient transporters to dietary Vit E/Se are very limited. Contrary to our expectations, we did not observe any additional modulation in PepT1, SGLT1, and NaPi-IIb

mRNA abundance in the jejunum due to dietary Vit E/Se supplementation. However, there are studies suggesting positive effects of several antioxidants, such as chromium (Orhan et al., 2019), on intestinal nutrient transporters of chickens subjected to HS. Therefore, further studies applying different environmental temperatures and duration are needed to extend our knowledge on the effects of Vit E/Se on intestinal nutrients transporters.

The results of this study revealed that growth performance, livability and carcass composition of broilers were adversely affected during a HS challenge. However, we showed that dietary supplementation of Vit E/Se might be a useful nutritional strategy to alleviate the negative impacts of elevated environmental temperatures on broiler production performance. Moreover, the observed improvement in BMC and BMD is an important finding regarding bone health in heat-stressed broilers. However, dietary Vit E/Se did not influence the levels of intestinal nutrient transporters measured in this experiment. Collectively, the present study revealed that dietary supplementation of Vit E and Se significantly improved broiler performance, carcass characteristics, and bone health, and reduced mortality rate under HS conditions likely due to the synergistic antioxidant action of Vit E and Se in alleviating the negative impact of oxidative damage.

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DISCLOSURES

The authors declare no conflict of interest.

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