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ARTICLE

Effects of Dietary Selenium and Vitamin E Supplementation on Growth Performance, Blood Biochemistry, Antioxidant Status, Immunity and Semen Quality of Male Turkeys Reared Under Chronic Heat Stress

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ABSTRACT

Heat stress impairs growth and fertility in male turkeys, largely through oxidative damage to metabolically active tissues and to spermatozoa. This study evaluated whether graded, supranutritional combinations of selenium (Se) and vitamin E could protect the productive, metabolic, antioxidant, immune and reproductive functions of male turkeys exposed to chronic cyclic heat stress. Fifty-four 30-week-old male turkeys were allotted to six dietary treatments (three replicates of three birds each): a basal diet (T1, control) or the basal diet supplemented with 0.3, 0.5, 0.7, 1.0 or 1.2 mg/kg Se combined with 100, 200, 300, 400 or 500 mg/kg vitamin E (T2–T6, respectively) for eight weeks, during which the birds were exposed to 36 ± 2 °C for 8 h daily. Body weight, feed intake, feed conversion ratio (FCR), serum biochemical indices, superoxide dismutase (SOD) activity, malondialdehyde (MDA), reduced glutathione (GSH), immunoglobulin G (IgG), plasma testosterone and semen characteristics were determined. Moderate supplementation (T2 and T3) produced the highest body weight at 34 weeks and the lowest FCR ($P < 0.05$), whereas the highest inclusion levels (T5 and T6) depressed growth and increased FCR relative to the control. Supplemented birds, particularly T3–T5, showed higher SOD activity and lower MDA, and T2–T3 had the lowest serum glucose, total lipids and cholesterol together with the highest total protein, albumin and IgG. Plasma testosterone, sperm concentration and progressive motility were greatest, and the percentage of abnormal spermatozoa lowest, in T3 and T4 ($P < 0.05$), whereas T6 was not superior to the control for any reproductive trait. Combined supplementation with 0.5–0.7 mg/kg Se and 200–300 mg/kg vitamin E therefore represents an effective nutritional strategy for protecting antioxidant status and semen quality in heat-stressed male turkeys, with no additional benefit, and possible adverse effects, at higher inclusion levels.

Keywords: *Heat Stress; Male Turkey; Selenium; Vitamin E; Semen Quality; Oxidative Stress; Testosterone.*

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INTRODUCTION

Reproductive efficiency of male breeders is a persistent constraint in commercial turkey production. Modern male turkeys have been selected intensively for rapid growth and large muscle mass, and their reproductive performance is physiologically fragile: testis weight and sperm output peak early in the reproductive season and then decline progressively, and plasma luteinising hormone and testosterone are poor predictors of semen production (Noirault *et al*., 2006). These inherent limitations are aggravated by environmental stressors, most notably heat stress, which is recognised as one of the most important environmental challenges to poultry production worldwide, reducing growth, feed efficiency, immune competence and welfare (Lara and Rostagno, 2013).

At the cellular level, the link between heat stress and impaired performance is a disturbance of the oxidant–antioxidant balance. Elevated ambient temperature increases mitochondrial electron leakage and the generation of reactive oxygen species (ROS), which overwhelm the endogenous antioxidant network and lead to lipid peroxidation, protein oxidation and DNA damage (Bottje, 2019; Surai *et al*., 2019a). The testis and spermatozoa are especially vulnerable. Heat stress induces apoptosis of germ cells, particularly pachytene spermatocytes and spermatids, and ROS-mediated damage to sperm membranes and DNA, resulting in reduced motility, abnormal morphology and lower fertilising ability (Shahat *et al*., 2020). Avian spermatozoa are unusually rich in long-chain polyunsaturated fatty acids, which account for 46 % of total fatty acids in turkey sperm phospholipids, and are therefore highly susceptible to peroxidative attack unless protected by the glutathione peroxidase and superoxide dismutase systems of the sperm and seminal plasma (Surai *et al*., 1998).

Selenium (Se) is an essential trace element whose biological functions are mediated through selenoproteins. The glutathione peroxidase (GPx) family reduces hydrogen peroxide and lipid hydroperoxides at the expense of reduced glutathione, with tissue-specific isoenzymes operating in the cytosol, plasma and membranes (Brigelius-Flohé, 1999). Phospholipid hydroperoxide glutathione peroxidase (GPx4) has a dual role in spermatogenesis, acting as a soluble peroxidase in spermatids and then as an enzymatically inactive structural protein of the mitochondrial capsule in the sperm midpiece, which explains the midpiece instability and infertility associated with Se deficiency (Ursini *et al*., 1999). Turkeys have a markedly higher Se requirement than rodents and chickens, with liver GPx4 activity indicating a minimum requirement of about 0.3 mg Se/kg diet (Sunde and Hadley, 2010; Taylor and Sunde, 2016), and they tolerate dietary inorganic Se up to 5 mg/kg without adverse effects on growth (Taylor *et al*., 2019). In broilers, however, the response to Se is dose-dependent and non-linear: 0.3–0.5 mg/kg nano-Se produced the best antioxidant indices whereas 2.0 mg/kg produced the poorest (Cai *et al*., 2012), and 5 mg/kg from sodium selenite depressed body weight and feed intake (Briens *et al*., 2014). This U-shaped relationship between Se status and biological outcome is well documented (Rayman, 2012). Organic Se sources are retained more efficiently in tissues than sodium selenite (Briens *et al*., 2014), and selenium-enriched yeast or Se nanoparticles improve semen quality, seminiferous epithelium thickness and fertility in ageing broiler breeder roosters (Sabzian-Melei *et al*., 2022; Asghari-Moghadam and Mehri, 2025).

Vitamin E (α-tocopherol) is the principal lipid-soluble, chain-breaking antioxidant of biological membranes, and its major, if not sole, physiological function is to scavenge peroxyl radicals and so preserve the integrity of membrane polyunsaturated fatty acids (Traber and Atkinson, 2007). In poultry males, increased dietary vitamin E raises the α-tocopherol concentration of semen and increases its resistance to oxidative stress imposed by heat and other stressors (Surai *et al*., 2019b). A meta-analysis of 19 studies confirmed that dietary vitamin E improves sperm concentration, motility, viability and fertility in chickens (Hayanti *et al*., 2022), and 300 mg/kg vitamin E increased sperm concentration, progressive motility and plasma testosterone while lowering MDA in grandparent roosters (Shabani *et al*., 2022). Selenium and vitamin E act cooperatively: vitamin E interrupts lipid peroxidation within the membrane while Se-dependent GPx removes the resulting hydroperoxides, and their combination (200 mg/kg vitamin E with 0.3 mg/kg organic Se) reduced seminal plasma lipid peroxidation to about 28 % of control values and doubled seminal GPx activity in cockerels kept at 33–36 °C, with corresponding increases in sperm count and motility (Ebeid, 2009, 2012). In heat-stressed broilers, the combination improved antibody responses, reduced the heterophil-to-lymphocyte ratio and reduced lipid oxidation in breast meat (Habibian *et al*., 2014; Habibian *et al*., 2016).

Although the nutrient requirements of turkeys for growth are well established, the amounts of Se and vitamin E needed to sustain antioxidant defences and semen quality in mature male turkeys under chronic heat stress have not been defined, and most of the available evidence derives from chickens. Since turkeys have a higher Se requirement and a high tolerance to Se,

the optimal level for heat-stressed males may differ from that of other poultry species. This study was therefore designed to evaluate the effects of graded combinations of supranutritional Se and vitamin E on growth performance, serum biochemistry, antioxidant and immune indices, plasma testosterone and semen quality in male turkeys reared under chronic cyclic heat stress, and to identify the inclusion range that maximises benefit without adverse effects.

MATERIALS AND METHODS

Birds, housing and experimental design

Fifty-four healthy male turkeys, 30 weeks of age, were weighed individually and randomly assigned to six dietary treatments in a completely randomised design, with nine birds per treatment distributed into three replicate pens of three birds each. The initial body weight did not differ among treatments (Table 2). Before the experiment, the poultry house and equipment were thoroughly cleaned and disinfected. Birds were kept on deep litter and had *ad libitum* access to feed and clean drinking water throughout the eight-week experimental period (30–38 weeks of age). Drinkers were cleaned daily and feeders were sanitised weekly.

Heat stress protocol

To induce chronic cyclic heat stress, the ambient temperature in the house was raised to 36±2 °C for 8 consecutive hours each day (10:00–18:00 h) throughout the experiment, after which the temperature was allowed to return to the moderate ambient range for the remainder of the day. Temperature was recorded continuously with a digital thermometer placed at bird height.

Experimental diets

A maize–wheat–soybean meal basal diet (BD) containing 20 % crude protein and 3150.6 kcal/kg metabolisable energy was formulated to meet the nutrient requirements of adult male turkeys (Table 1) (NRC, 1994). The six treatments were: T1, BD without supplementation (control); T2, BD + 0.3 mg/kg Se + 100 mg/kg vitamin E; T3, BD + 0.5 mg/kg Se + 200 mg/kg vitamin E; T4, BD + 0.7 mg/kg Se + 300 mg/kg vitamin E; T5, BD + 1.0 mg/kg Se + 400 mg/kg vitamin E; and T6, BD + 1.2 mg/kg Se + 500 mg/kg vitamin E.

Growth performance

Birds were weighed individually at 30 (initial), 34 and 38 weeks of age, and body weight gain was calculated as the difference between final and initial weights. Feed offered and refused was recorded per replicate pen, and daily feed intake (g/bird/day) was calculated for the periods ending at 34 and 38 weeks. Feed conversion ratio (FCR) was expressed as kg feed consumed per kg body weight gain.

Blood sampling and serum biochemistry

At the end of the experiment (38 weeks), blood samples were collected from the wing (brachial) vein of three birds per replicate into plain tubes without anticoagulant. Serum was separated by centrifugation at 3000 × *g* for 15 min and stored at −20 °C until analysis. Serum glucose (Trinder, 1969), total protein (Henry et al., 1974), (Doumas *et al*., 1971), cholesterol (Watson, 1960) were determined with commercial colorimetric kits using a spectrophotometer, following the manufacturers' instructions. Globulin was calculated as the difference between total protein and albumin.

Table (1) Ingredients and calculated chemical composition of the basal diet

Ingredient	Inclusion (%)
Yellow maize	46.25
Wheat	16.20
Soybean meal (48 % CP)	29.60
Vegetable oil	4.15
Limestone	1.00
Salt (NaCl)	0.30
Vitamin–mineral premix[1]	2.50
Total	**100.00**
Calculated composition	
Crude protein (%)	20.00
Metabolisable energy (kcal/kg)	3150.6
Crude fibre (%)	3.60
Lysine (%)	1.00
Methionine (%)	0.44
Methionine + cystine (%)	0.80
Calcium (%)	1.04
Available phosphorus (%)	0.40

[1] BROMIX 2.5W (WAFI B.V.). Composition per kg of premix as declared by the manufacturer: vitamin A, 1,400,000 IU; vitamin D_3, 300,000 IU; vitamin E, 50 g; vitamin K_3, 4 g; vitamin B_1, 3 g; vitamin B_2, 15 g; vitamin B_6, 6 g; vitamin B_{12}, 0.04 g; niacin, 60 g; pantothenic acid, 20 g; folic acid, 0.20 g; Fe, 150 g; Zn, 4.8 g; P, 3.18 g; Mn, 100 g; Cu, 50 g; I, 80 g; Co, 10 g; Se, 1.5 g; antioxidant, 0.20 g; carrier to 1 kg.

Antioxidant and immune indices

Serum superoxide dismutase (SOD) activity was measured with a commercial assay kit and expressed as U/mL. Lipid peroxidation was assessed as serum malondialdehyde (MDA) concentration by the thiobarbituric acid reactive substances method and expressed as µmol/L, and reduced glutathione (GSH) was determined as an index of the non-enzymatic antioxidant pool and expressed as µmol/L. Serum immunoglobulin G (IgG) concentration was quantified using a commercial turkey-specific ELISA kit according to the manufacturer's protocol and expressed as ng/mL [authors to insert kit manufacturers and catalogue numbers].

Semen quality and plasma testosterone

Semen was collected at the end of the experiment from each male by the abdominal massage technique. Sperm concentration (10^6 spermatozoa /mL) was determined with a hemocytometer after appropriate dilution, progressive motility (%) was estimated subjectively under a phase-contrast microscope at 37 °C, and the percentage of morphologically abnormal spermatozoa was determined on eosin–nigrosin-stained smears by counting at least 200 spermatozoa per sample. Plasma testosterone concentration was measured by a commercial ELISA kit [authors to insert manufacturer] and expressed as ng/mL.

Statistical analysis

The experiment was arranged as a completely randomised design with the replicate pen as the experimental unit for performance traits and the individual bird for blood and semen traits. Data were analysed by one-way analysis of variance using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC, USA). When the treatment

effect was significant, means were separated by Duncan's multiple range tests. Results are presented as means ± standard deviation, and differences were considered significant at $P < 0.05$.

RESULTS

The effects of the dietary treatments on body weight, weight gain, feed intake and FCR are presented in Table 2. Initial body weight was similar across treatments. At 34 weeks of age, body weight was highest in T2 (7498.0 ± 58.43 g) and T3 (7456.3 ± 47.34 g), both significantly greater than the control (7181.5 ± 44.21 g) and then T5 and T6 ($P < 0.05$); T6 recorded the lowest weight (6803.2 ± 44.45 g). At 38 weeks, T2 (9844.0 ± 86.56 g) and T3 (9848.4 ± 83.12 g) remained numerically the heaviest and were significantly heavier than T6 (9265.6 ± 79.24 g), whereas T1, T4 and T5 occupied an intermediate position that did not differ significantly from either extreme. Total body weight gain over the eight weeks followed the same pattern, ranging from 5032.0 g in T2 and 4992.1 g in T3 to 4592.4 g in T6.

Daily feed intake during the first period (34 weeks) did not differ significantly among treatments (288.1–304.2 g/bird/day). During the second period (38 weeks), feed intake was significantly lower in T2 (250.8 ± 14.80 g) and T3 (255.1 ± 15.63 g) and highest in T5 (308.9 ± 16.90 g) and T6 (311.4 ± 22.40 g) ($P < 0.05$). Consequently, FCR was most favourable in T2 and T3 at both 34 weeks (3.02 and 3.10) and 38 weeks (2.99 and 2.98), and least favourable in T5 and T6 (3.83 and 3.96 at 34 weeks; 3.44 and 3.54 at 38 weeks), with the control and T4 intermediate ($P < 0.05$).

Table (2) Effect of dietary selenium and vitamin E on body weight, weight gain, feed intake and feed conversion ratio of male turkeys reared under chronic heat stress (means ± SD).

Item	Treatments					
	T1	T2	T3	T4	T5	T6
Body weight (g)						
30 wk (initial)	4761.5	4812.0	4856.3	4861.3	4767.2	4673.2
34 wk	7181.5 ± 44.21^{b}	7498.0 ± 58.43^{a}	7456.3 ± 47.34^{a}	7281.3 ± 54.23^{ab}	6987.2 ± 59.30^{c}	6803.2 ± 44.45^{c}
38 wk	9733.2 ± 67.32^{ab}	9844.0 ± 86.56^{a}	9848.4 ± 83.12^{a}	9793.2 ± 74.43^{ab}	9499.6 ± 79.12^{ab}	9265.6 ± 79.24^{b}
Total gain, 30–38 wk	4971.7	5032.0	4992.1	4931.9	4732.4	4592.4
Feed intake (g/bird/day)						
34 wk	292.8 ± 9.90	290.0 ± 12.90	288.1 ± 16.30	301.0 ± 8.97	304.2 ± 13.90	301.4 ± 14.20
38 wk	285.8 ± 20.20^{ab}	250.8 ± 14.80^{c}	255.1 ± 15.63^{c}	281.9 ± 13.80^{b}	308.9 ± 16.90^{a}	311.4 ± 22.40^{a}
Feed conversion ratio (kg feed/kg gain)						
34 wk	3.38 ± 0.26^{b}	3.02 ± 0.13^{c}	3.10 ± 0.42^{bc}	3.48 ± 0.18^{ab}	3.83 ± 0.51^{a}	3.96 ± 0.14^{a}
38 wk	3.13 ± 0.33^{ab}	2.99 ± 0.45^{b}	2.98 ± 0.18^{b}	3.14 ± 0.28^{ab}	3.44 ± 0.43^{a}	3.54 ± 0.61^{a}

$^{a–e}$ Means within a row with different superscript letters differ significantly ($P < 0.05$)
T1, basal diet (control); T2, 0.3 mg/kg Se + 100 mg/kg vitamin E; T3, 0.5 mg/kg Se + 200 mg/kg vitamin E; T4, 0.7 mg/kg Se + 300 mg/kg vitamin E; T5, 1.0 mg/kg Se + 400 mg/kg vitamin E; T6, 1.2 mg/kg Se + 500 mg/kg vitamin E.

Serum biochemical indices are shown in Table 3. Serum glucose was lowest in T2 (234.23 ± 21.13 mg/dL) and T3 (229.12 ± 28.09 mg/dL), intermediate in the control and T4, and highest in T6 (305.12 ± 24.89 mg/dL) ($P < 0.05$). Total protein and albumin were highest in T2 and T3 and lowest in T5 and T6, which fell below the control ($P < 0.05$); globulin did not differ among treatments. Total lipids were markedly reduced in T2 (1.79 ± 0.18 g/dL), T3 (1.82 ± 0.36 g/dL) and T4 (2.08 ± 0.27 g/dL) compared with the control (3.26 ± 0.48 g/dL), but were elevated in T5 (3.73 ± 0.54 g/dL) and T6 (3.98 ± 0.36 g/dL). Similarly, total cholesterol was lowest in T2–T4 (102.7–124.2 mg/dL), did not differ

from the control (132.4 ± 33.63 mg/dL), and was significantly increased in T5 (164.6 ± 43.12 mg/dL) and T6 (186.4 ± 35.63 mg/dL) ($P < 0.05$).

Table (3) Effect of dietary selenium and vitamin E on serum biochemical parameters of male turkeys reared under chronic heat stress (means ± SD).

Parameter	Treatments					
	T1	T2	T3	T4	T5	T6
Glucose (mg/dL)	258.34 ± 28.18^{b}	234.23 ± 21.13^{c}	229.12 ± 28.09^{c}	264.53 ± 18.66^{b}	288.55 ± 34.77^{ab}	305.12 ± 24.89^{a}
Total protein (g/dL)	4.63 ± 0.45^{ab}	4.91 ± 0.33^{a}	4.97 ± 0.21^{a}	4.53 ± 0.39^{ab}	4.21 ± 0.24^{c}	4.04 ± 0.61^{d}
Albumin (g/dL)	2.34 ± 0.09^{b}	2.68 ± 0.64^{a}	2.65 ± 0.43^{a}	2.42 ± 0.23^{ab}	2.11 ± 0.31^{c}	2.12 ± 0.12^{c}
Globulin (g/dL)	2.29 ± 0.43	2.23 ± 0.53	2.32 ± 0.19	2.11 ± 0.47	2.10 ± 0.32	1.92 ± 0.45
Total lipids (g/dL)	3.26 ± 0.48^{b}	1.79 ± 0.18^{d}	1.82 ± 0.36^{cd}	2.08 ± 0.27^{c}	3.73 ± 0.54^{a}	3.98 ± 0.36^{a}
Total cholesterol (mg/dL)	132.4 ± 33.63^{c}	109.3 ± 21.07^{c}	102.7 ± 26.14^{c}	124.2 ± 24.67^{cd}	164.6 ± 43.12^{b}	186.4 ± 35.63^{a}

$^{a-e}$ Means within a row with different superscript letters differ significantly ($P < 0.05$)

T1, basal diet (control); T2, 0.3 mg/kg Se + 100 mg/kg vitamin E; T3, 0.5 mg/kg Se + 200 mg/kg vitamin E; T4, 0.7 mg/kg Se + 300 mg/kg vitamin E; T5, 1.0 mg/kg Se + 400 mg/kg vitamin E; T6, 1.2 mg/kg Se + 500 mg/kg vitamin E.

Dietary Se and vitamin E significantly influenced the antioxidant and immune indices (Table 4). Serum SOD activity was lowest in the control (71.26 U/mL) and was increased by all supplemented diets, reaching its highest values in T3 (142.12 U/mL) and T4 (137.32 U/mL) ($P < 0.05$). Serum MDA, the index of lipid peroxidation, decreased progressively from the control (3.64 µmol/L) through T2 (2.10), T3 (1.96) and T4 (1.75) to the lowest value in T5 (1.60 µmol/L), but rose again in T6 (2.98 µmol/L), which nevertheless remained below the control ($P < 0.05$). In contrast, reduced glutathione showed the opposite pattern: the highest concentration was observed in the control (103.23 µmol/L) and the lowest in T3 (58.33 µmol/L), with intermediate values in the other supplemented groups. Serum IgG was highest in T2 (274.54 ng/mL), followed by the control (251.12 ng/mL) and T3 (243.62 ng/mL), and was significantly reduced in T5 (221 ng/mL), T6 (180 ng/mL) and particularly T4 (171.48 ng/mL) ($P < 0.05$).

Table (4) Effect of dietary selenium and vitamin E on serum antioxidant indices and immunoglobulin G of male turkeys reared under chronic heat stress.

Parameter	Treatment					
	T1	T2	T3	T4	T5	T6
SOD (U/mL)	71.26^{c}	122.54^{ab}	142.12^{a}	137.32^{a}	110.34^{b}	95.37^{ab}
MDA (µmol/L)	3.64^{a}	2.10^{c}	1.96^{cd}	1.75^{d}	1.60^{e}	2.98^{b}
GSH (µmol/L)	103.23^{a}	69.29^{b}	58.33^{c}	87.27^{b}	70.63^{b}	77.46^{ab}
IgG (ng/mL)	251.12^{ab}	274.54^{a}	243.62^{b}	171.48^{e}	221.00^{c}	180.00^{d}

$^{a-e}$ Means within a row with different superscript letters differ significantly ($P < 0.05$)

SOD, superoxide dismutase; MDA, malondialdehyde; GSH, reduced glutathione; IgG, immunoglobulin G. T1, basal diet (control); T2, 0.3 mg/kg Se + 100 mg/kg vitamin E; T3, 0.5 mg/kg Se + 200 mg/kg vitamin E; T4, 0.7 mg/kg Se + 300 mg/kg vitamin E; T5, 1.0 mg/kg Se + 400 mg/kg vitamin E; T6, 1.2 mg/kg Se + 500 mg/kg vitamin E.

The reproductive responses are summarised in Table 5. Plasma testosterone was significantly higher in T3 (4.2 ± 0.62 ng/mL) and T4 (4.0 ± 0.43 ng/mL) than in the control (3.1 ± 0.45 ng/mL) and T6 (2.9 ± 0.22 ng/mL), with T2 and T5 intermediate ($P < 0.05$). Sperm concentration increased from 280 ± 35.13 × 10^{6}/mL in the control to 350 ± 42.09 and 340 ± 18.32 × 10^{6}/mL in T3 and T4, respectively, whereas T6 had the lowest concentration (260 ± 36.14 × 10^{6}/mL) ($P < 0.05$). Progressive motility was likewise

greatest in T3 (78 ± 13.11 %) and T4 (75 ± 18.66 %) and lowest in T6 (60 ± 17.26 %), which was significantly below the control (65 ± 9.12 %). The percentage of morphologically abnormal spermatozoa was reduced from 18 ± 3.32 % in the control to 10 ± 2.65 % in T3 and 11 ± 3.14 % in T4, while T6 (20 ± 4.37 %) did not differ from the control ($P < 0.05$).

Table (5) Effect of dietary selenium and vitamin E on plasma testosterone and semen quality of male turkeys reared under chronic heat stress (means ± SD).

Parameter	Treatments					
	T1	T2	T3	T4	T5	T6
Testosterone (ng/mL)	3.1 ± 0.45^{b}	3.5 ± 0.36^{ab}	4.2 ± 0.62^{a}	4.0 ± 0.43^{a}	3.6 ± 0.45^{ab}	2.9 ± 0.22^{b}
Sperm concentration (10^6/mL)	280 ± 35.13^{c}	310 ± 23.67^{b}	350 ± 42.09^{a}	340 ± 18.32^{a}	320 ± 27.22^{ab}	260 ± 36.14^{d}
Progressive motility (%)	65 ± 9.12^{b}	70 ± 20.08^{ab}	78 ± 13.11^{a}	75 ± 18.66^{a}	72 ± 24.68^{ab}	60 ± 17.26^{c}
Abnormal spermatozoa (%)	18 ± 3.32^{a}	14 ± 3.07^{b}	10 ± 2.65^{c}	11 ± 3.14^{bc}	13 ± 2.37^{b}	20 ± 4.37^{a}

$^{a–e}$ Means within a row with different superscript letters differ significantly ($P < 0.05$; Duncan's multiple range test).
T1, basal diet (control); T2, 0.3 mg/kg Se + 100 mg/kg vitamin E; T3, 0.5 mg/kg Se + 200 mg/kg vitamin E; T4, 0.7 mg/kg Se + 300 mg/kg vitamin E; T5, 1.0 mg/kg Se + 400 mg/kg vitamin E; T6, 1.2 mg/kg Se + 500 mg/kg vitamin E.

DISCUSSION

The present study shows that combined dietary supplementation with Se and vitamin E modulates growth, metabolism, antioxidant status, humoral immunity and semen quality of male turkeys exposed to chronic cyclic heat stress, and that the response is clearly dose-dependent. Low-to-moderate inclusion levels (0.3–0.7 mg/kg Se with 100–300 mg/kg vitamin E) were beneficial, whereas the two highest levels (1.0–1.2 mg/kg Se with 400–500 mg/kg vitamin E) abolished these benefits and, for several traits, produced values inferior to those of the unsupplemented control. This biphasic pattern is consistent with the well-recognised U-shaped relationship between Se status and biological outcome (Rayman, 2012).

Heat stress depresses body weight and feed intake and worsens FCR in poultry (Lara and Rostagno, 2013; Habibian *et al*., 2014), and part of this loss of efficiency reflects the diversion of energy and nutrients towards repairing oxidative damage and maintaining redox balance in mitochondria (Bottje, 2019). The superior weight gain and lower FCR observed in T2 and T3 are therefore consistent with a reduction in the oxidative burden, as reflected by their higher SOD activity and lower MDA, allowing a greater proportion of absorbed nutrients to be used for tissue accretion. Habibian *et al*. (2016) similarly reported that Se and vitamin E improved feed efficiency and reduced breast-meat lipid oxidation in heat-stressed broilers but not in thermoneutral birds, indicating that the growth response to these antioxidants becomes apparent mainly when oxidative stress is imposed.

In contrast, T5 and T6 consumed more feed during the second period yet gained less weight, so that their FCR was the poorest of all groups. A depression of body weight and feed intake at high dietary Se has been described in broilers given 5 mg/kg Se, as sodium selenite (Briens *et al*., 2014), and 2.0 mg/kg nano-Se produced the poorest antioxidant and immune indices in the same species (Cai *et al*., 2012). Although turkey poults appear to tolerate up to 5 mg/kg inorganic Se without growth depression over 28 days (Taylor *et al*., 2019), the present birds were mature males exposed simultaneously to heat stress for eight weeks, and the combination of very high Se with very high vitamin E (500 mg/kg) may have disturbed redox signalling rather than supported it; vitamin E at high concentration can act as a pro-oxidant when the regenerating antioxidant network is overwhelmed (Surai *et al*., 2019a). The increased feed intake

with reduced gain in T5 and T6 suggests a metabolic inefficiency, possibly reflecting a greater energetic cost of handling excess Se, rather than a reduction in appetite.

Chronic heat stress elevates plasma glucose, triglycerides and cholesterol and enhances hepatic lipogenesis in broilers (Habibian *et al*., 2014; Lu *et al*., 2019). The lower serum glucose, total lipids and cholesterol together with the higher total protein and albumin in T2–T4 therefore indicate an attenuation of the metabolic disturbance induced by heat, which may be explained by improved hepatic function when hepatocyte membranes are protected against lipid peroxidation, and by reduced glucocorticoid-driven gluconeogenesis and proteolysis in less stressed birds. Albumin is synthesised exclusively by the liver and is a sensitive index of hepatic synthetic capacity and nutritional status, and its increase in the moderately supplemented groups supports an improved protein metabolism. The hyperglycaemia, hyperlipidaemia and hypoproteinaemia in T5 and T6, which exceeded the values of the control, are consistent with the growth depression in these groups and point to a metabolic cost of excess supplementation. Serum globulin did not differ among treatments, suggesting that the changes in total protein were driven mainly by albumin.

The increase in SOD activity and the decrease in MDA in supplemented birds demonstrate that the Se–vitamin E combination strengthened the antioxidant defence network under heat stress. Selenium is required for the synthesis of GPx isoenzymes that remove hydrogen peroxide and lipid hydroperoxides (Brigelius-Flohé, 1999), whereas α-tocopherol terminates the propagation of lipid peroxidation within membranes (Traber and Atkinson, 2007); their joint action reduces the steady-state concentration of lipid peroxides and hence the formation of MDA. Comparable reductions in MDA and increases in antioxidant enzyme activities have been reported with Se and vitamin E in heat-stressed cockerels (Ebeid, 2012) and broilers (Habibian *et al*., 2016). The lowest MDA in T5 and the rebound in T6 indicate that the antioxidant benefit reached a plateau around 1.0 mg/kg Se and was partly lost at the highest inclusion.

The inverse relationship between dietary supplementation and reduced glutathione deserves comment. The highest GSH concentration was recorded in the unsupplemented control, with the lowest value in T3. Glutathione is both the substrate consumed by GPx during peroxide reduction and an inducible component of the Nrf2-regulated adaptive response to oxidative stress (Surai *et al*., 2019a). Two non-exclusive explanations are therefore possible: in the supplemented groups, the greater GPx-dependent flux through the glutathione cycle may have increased GSH utilisation, whereas in the control the persistent oxidative challenge may have induced compensatory upregulation of glutathione synthesis. Because oxidised glutathione and the GSH:GSSG ratio were not measured, the present data cannot distinguish between these mechanisms, and the GSH result should be interpreted with caution.

Selenium and vitamin E are both immunomodulatory; selenoproteins regulate ROS and redox status in immune cells and influence antibody production and inflammatory signalling (Hoffmann and Berry, 2008), and vitamin E improved primary and secondary antibody responses in heat-stressed broilers, with an additional effect of Se on secondary responses (Habibian *et al*., 2014). The higher IgG in T2 is in agreement with these findings. However, IgG was not increased in T3 and was significantly reduced in T4–T6, which argues against a simple dose-related immunostimulation and is consistent with the observation that high Se intake may not always be beneficial for immunity (Hoffmann and Berry, 2008). Measurement of specific antibody titres after antigen challenge would be required to establish the functional significance of these changes.

The most important finding of this study is the improvement in plasma testosterone, sperm concentration and progressive motility, and the reduction in abnormal spermatozoa, in birds receiving 0.5–0.7 mg/kg Se with 200–300 mg/kg vitamin E. Heat stress impairs spermatogenesis through germ-cell apoptosis and ROS-mediated damage to sperm membranes and DNA (Shahat *et al*., 2020), and avian spermatozoa, including those of the turkey, are

particularly susceptible because of their high content of long-chain polyunsaturated fatty acids (Surai *et al*., 1998). Protection of the sperm membrane by α-tocopherol and of the cytosol and mitochondria by Se-dependent GPx would preserve membrane fluidity and the mitochondrial function required for motility, while GPx4 contributes structurally to the stability of the midpiece (Ursini *et al*., 1999). The results agree closely with those obtained in cockerels at 33–36 °C, in which 200 mg/kg vitamin E with 0.3 mg/kg organic Se increased sperm count and motility and reduced dead spermatozoa while lowering seminal lipid peroxidation (Ebeid, 2009, 2012), and with the improvement in sperm quality, fertility and seminiferous epithelium thickness obtained with 0.45 mg/kg selenium-enriched yeast in ageing broiler breeder roosters (Sabzian-Melei *et al*., 2022). The increase in plasma testosterone in T3 and T4 suggests that the antioxidant protection extended to Leydig cells, whose steroidogenic capacity is sensitive to oxidative stress; a similar rise in testosterone with 300 mg/kg vitamin E has been reported in grandparent roosters (Shabani *et al*., 2022). Although a meta-analysis did not detect a consistent effect of vitamin E alone on testosterone in chickens (Hayanti *et al*., 2022), suggesting that the response observed here may depend on the combination with Se and on the presence of heat stress.

Semen quality in T6 was no better than in the control and, for motility and sperm concentration, was significantly worse. The sensitivity of the testis to Se excess is biologically plausible, since testicular selenoprotein synthesis is tightly regulated and excessive Se may disturb redox-sensitive signalling in germ cells (Rayman, 2012). The optimum for semen quality in the present study (0.5–0.7 mg/kg supplemental Se) is above the 0.3 mg/kg requirement estimated for growth and GPx activity in turkey poults (Sunde and Hadley, 2010; Taylor and Sunde, 2016), which supports the hypothesis that the reproductive and antioxidant demands of mature, heat-stressed males exceed the requirements defined for growth.

Several limitations should be acknowledged. First, Se and vitamin E were increased in parallel, so the contribution of each nutrient and their interaction cannot be separated; a factorial design is needed to define the independent dose–response of each. Second, no thermoneutral control group was included, so the magnitude of the heat-stress effect itself could not be quantified. Third, the number of replicates was small (three pens of three birds), the trial lasted eight weeks, and the Se content of the basal diet and of the premix was not analysed. Fourth, oxidised glutathione, GPx and catalase activities, sperm viability and DNA integrity, and fertility after insemination were not measured, and the immune response was assessed only as total IgG. Future studies should compare inorganic, organic and nano-Se sources in factorial combination with vitamin E, include thermoneutral controls, extend the observation period over a full reproductive season, and relate semen quality to fertility and hatchability after artificial insemination.

CONCLUSIONS

Supplementing the diet of mature male turkeys with 0.3–0.7 mg/kg Se and 100–300 mg/kg vitamin E during chronic cyclic heat stress improved growth and feed efficiency, normalised serum glucose, lipid and protein profiles, increased SOD activity, reduced lipid peroxidation and, at 0.5–0.7 mg/kg Se with 200–300 mg/kg vitamin E, increased plasma testosterone, sperm concentration and progressive motility while reducing sperm abnormalities. Higher inclusion levels (1.0–1.2 mg/kg Se with 400–500 mg/kg vitamin E) provided no additional benefit and impaired growth, metabolic and reproductive indices. A combination of about 0.5–0.7 mg/kg Se with 200–300 mg/kg vitamin E can therefore be recommended as a practical nutritional strategy to protect the antioxidant status and semen quality of male turkeys in hot climates, with the caution that supplementation above these levels may be counterproductive.

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