

Effects of selenium and vitamin E on performance, physiological response, and selenium balance in heat-stressed sheep¹

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ABSTRACT: Forty-two 7-mo-old Australian Merino wethers were used in a 50-d trial to investigate the effects of Se and vitamin E on the performance and physiological responses of heat-stressed sheep. Sheep were exposed to thermoneutral conditions (maximum = 24°C and minimum = 20°C) for 28 d followed by heat (maximum = 38°C and minimum = 28°C) for 22 d. Hot conditions were imposed between 0700 and 1800 h. Sheep were randomly allocated to diets containing 0.8 mg/kg Se (Sel-Plex), 150 mg/kg vitamin E, or 0.8 mg/kg Se and 150 mg/kg vitamin E for either the duration of the study (50 d) or from d 1 of the hot period until the end of the study. A control group that received no supplemental Se and vitamin E for the duration of the study was included. Feed intake was measured daily and sheep were weighed weekly. Blood samples were collected from all sheep

before feeding on d 1, 21, and 49 for measurement of biochemical and enzymatic variables. The concentration of Se was determined in offered and refused feed, feces, urine, water, plasma, liver, and kidneys. Exposure to heat reduced ($P < 0.05$) DMI by 11.9%, ADG by 198 g, serum concentration of urea nitrogen and Se by 17.8%, and plasma total antioxidant status by 26.4%. During hot conditions, sheep receiving Se and vitamin E supplements for 50 d had reduced ($P < 0.05$) BW loss and elevated G:F compared to control sheep. Serum Se concentration and the plasma total antioxidant status were greatest in sheep receiving Se and vitamin E supplements for 50 d ($P < 0.05$). These results indicate that dietary supplementation with Se and vitamin E reduces the adverse effects of a high heat load. Additional studies are warranted to elucidate the mechanisms responsible for these effects.

Key words: heat stress, Merino sheep, physiological response, selenium, vitamin E

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INTRODUCTION

Heat stress induces oxidative stress and reduces antioxidant status by promoting overproduction of free radicals and reactive oxygen species and by impairing the antioxidant defense system (Miller et al., 1993; Altan et al., 2000). Hot environmental conditions

markedly decrease blood concentration of antioxidant micronutrients (Zn, Se, and vitamin E) in ruminants (Bernabucci et al., 2002; Saker et al., 2004; Burke et al., 2007) and poultry (Altan et al., 2003; Saini et al., 2007). Decreases in blood concentration of these micronutrients have been associated with decreases in their uptake, which occur as a result of the depressive effect of heat stress on feed intake (Siegel, 1995). However, decreased uptake may also result from endogenous depletion because these micronutrients are important components of the antioxidant defense system. Therefore, it is possible that requirements for antioxidant micronutrients are increased by exposure to hot conditions.

As integral parts of the antioxidant defense system, Se and vitamin E play important roles in the growth and health of humans and animals (Underwood, 1977; McDowell, 2003; Surai, 2006). These micronutrients are often added to diets to improve livestock production

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and enhance immune competence. Sheep provided with dietary Se and vitamin E showed a 5.7% increase in BW (Celi et al., 2010). Moreover, the addition of Se and vitamin E improved glutathione peroxidase (**GSH-Px**) activity in blood and increases plasma concentrations of total antioxidant capacity and Se in dairy cattle (Calamari et al., 2011) and goats (Katamoto et al., 1998).

The effects of Se and vitamin E supplementation on the productivity and physiological responses of sheep exposed to heat stress are not known. Therefore, the objectives of this study were to determine whether inclusion of Se and vitamin E in sheep diets before or during exposure to hot conditions improves antioxidant status and ameliorates the adverse effects of thermal stress.

MATERIALS AND METHODS

The study was conducted at the Queensland Animal Science Precinct, The University of Queensland, Gatton Campus, Australia (27°34' S, 152°20' E), with the approval of The University of Queensland Animal Ethics Committee.

Animals and Management Practices

Forty-two 7-mo-old Australian Merino wethers (21.5 ± 3.2 kg mean BW; approximately 30 mm wool length) were individually housed for 50 d in elevated (approximately 1 m above the floor) metabolism pens (1.5 m long by 0.5 m wide) in an environmental chamber. Each metabolism pen was fitted with a feed trough and a 10-L plastic water bucket. Sheep were given a 1-wk adaptation period in the metabolism pens immediately before commencement of the study and were monitored daily when inside the environmental chamber. Sheep were removed from the pens every 7 d for weighing and remained out of their pens for 3 h thereafter, by the policy of The University of Queensland Animal Ethics Committee.

All sheep were fed the same basal diet, which consisted of mainly chopped alfalfa hay (*Medicago sativa*) and barley (Table 1) and was formulated to meet the nutritional requirements (NRC, 1985) of sheep. Feed was offered at 2.5% of BW once daily at 0730 h and fresh water was offered ad libitum. Feed troughs and water buckets were cleaned daily at 0700 h, and feed and water intake were measured by recording refusals at 0700 h. During the study, representative samples (5%) of feed offered and feed refusals were collected daily and pooled on a weekly basis for analysis.

Experimental Design and Diets

The experiment was conducted over a 50-d period subdivided into 2 continuous periods with different envi-

Table 1. Ingredients and chemical composition of basal diet

| Item | Dietary content |
|-----------------------------------------|-----------------|
| Ingredient, % of dietary DM | |
| Barley grain | 67 |
| Lucerne hay | 30 |
| Sodium bicarbonate | 1.0 |
| Dicalcium phosphate | 0.5 |
| Limestone | 0.9 |
| Salt | 0.4 |
| Mineral and vitamin premix ¹ | 0.2 |
| Nutrient composition, DM basis | |
| NEg, Mcal/kg | 1.02 |
| CP, % | 12.24 |
| NDF, % | 34.47 |
| ADF, % | 14.37 |
| Se, mg/kg | 0.03 |
| Vitamin E, mg/kg | 30.5 |

¹Mineral and vitamin premix contained (per kg) 1,000 mg Co as CoSO₄, 500 mg I as CoI₂, 30,000 mg Fe as FeSO₄, 40,000 mg Mn as MnO, 40,000 mg Zn as ZnSO₄, 200 g Mg as MgO, 8.00 mega-IU vitamin A, and 1.60 mega-IU vitamin D.

ronmental conditions. All sheep were subjected to 28 d at thermoneutral conditions (**TNC**; maximum temperature [**T_{MAX}**] of 24°C and minimum temperature [**T_{MIN}**] of 20°C) followed by 22 d at elevated ambient temperature (**T_a**; **HOT**; **T_{MAX}** = 38°C and **T_{MIN}** = 28°C). During the HOT period, **T_a** was increased at a rate of 5.0°C/h from 0800 h, reaching **T_{MAX}** at approximately 1000 h, and maintained at **T_{MAX}** until 1800 h. From 1800 h, **T_a** was then decreased by 5.0°C/h, reaching **T_{MIN}** by approximately 2000 h, and maintained at **T_{MIN}** until 0800 h. This protocol was designed to simulate the summer diurnal temperature pattern in South East Queensland, Australia. Relative humidity (**RH**) was maintained at >50% in both the TNC and HOT periods. Room temperature and humidity were controlled using an industrial building system designed by East Coast Mechanical Services, Northgate, Australia. Sheep were exposed to a lighting regimen of 13 h on (0500 to 1800 h) and 11 h off with light intensity at 580 lux.

Four diets were used in this study, consisting of the same basal diet with or without Se and vitamin E supplementation, as follows: 1) basal diet without Se or vitamin E, 2) basal diet with 0.8 mg Se/kg DM (in the form of Sel-Plex [Alltech, Nicholasville, KY]; 2,000 mg Se/kg DM), 3) basal diet with 150 mg vitamin E/kg DM (in the form of E50 [Rabar Pty. Ltd., Beaudesert, Australia]; 500 g vitamin E/kg DM), and 4) basal diet with 0.8 mg Se/kg DM and 150 mg vitamin E/kg DM. Weighed aliquots of Se and vitamin E were mixed with 100 g finely ground barley and added to the diet in place of barley. During the month preceding the study, all sheep were fed the basal diet without supplemental Se or vitamin E.

At the beginning of the experiment (d 1), sheep were randomly assigned to 1 of 7 treatments (6 animals in each treatment on the basis of weight) that differed in the dietary supplementation (Se, vitamin E, or both) and in the period over which the supplements were fed (for the duration of the experiment [50 d] or during heat exposure alone [22 d]); the treatments were 1) basal diet alone for 50 d (control; **CON**), 2) basal diet plus Se for 50 d (**Se50**), 3) basal diet plus vitamin E for 50 d (**E50**), 4) basal diet plus Se and vitamin E for 50 d (**Se+E50**), 5) basal diet during the TNC period (d 1–28) and then basal diet plus Se during the HOT period (d 29–50; **Se22**), 6) basal diet during the TNC period and then basal diet plus vitamin E during the HOT period (**E22**), and 7) basal diet during the TNC period and then basal diet plus Se and vitamin E during the HOT period (**Se+E22**).

At the termination of the study (d 50), all sheep were deprived of feed and water for 16 h and then slaughtered by captive-bolt stunning (CASH; Accles & Shelvoke, West Midlands, UK). Livers and kidneys were collected immediately after slaughter and weighed. Subsamples of livers (left lobes) and whole kidneys were frozen at -20°C until analysis.

Climatic Measurements

Ambient temperature and RH were measured at 10-min intervals using temperature/RH sensors and measurements were stored using a data logger (Metasys; Johnson Controls Inc., Milwaukee, WI) until downloaded. Sensors were calibrated before the study and checked for accuracy at the end of the study. Ambient temperature and RH were recorded every 10 min and hourly means were calculated, and an hourly temperature–humidity index (**THI**) was calculated using the following equation (adapted from Thom, 1959): $\text{THI} = (0.8 \times T_a) + [(\text{RH}/100) \times (T_a - 14.3)] + 46.4$.

Feed Analyses

Feed was sampled before the study commenced (1 wk) and weekly during the study, and samples were frozen at -20°C . At the end of the experiment, samples were pooled and analyzed for nutrient composition, including total Se and vitamin E content, at The University of Queensland laboratories (Gatton, QLD, Australia) using Association of Official Analytical Chemists (1990) procedures. Dry matter content was determined by drying samples in an oven at 100°C for 24 h and ash content was determined by incinerating samples at 550°C for 3 h in a muffle furnace. Crude protein content was measured using an elemental analyzer (MACRO CHNS; Elementar Analysensysteme GmbH, Hanau, Germany).

Neutral detergent fiber and ADF were determined according to methods described by Van Soest et al. (1991) and the Association of Official Analytical Chemists (method number 973.18 C; AOAC, 1990), respectively. Total Se and vitamin E content was determined for each dietary ingredient. Selenium concentration was determined by inductively coupled plasma mass spectrometry (Vista-PRO CCD; Varian Inc., Walnut Creek, CA). Vitamin E concentration was determined by HPLC (Agilent 1100; Agilent Technologies Inc., Santa Clara, CA) according to the method of Manz and Philip (1981). The average nutrient composition and total Se and vitamin E content of the basal diet are presented in Table 1.

Dry Matter Intake, BW, and Feed Efficiency

The weights of offered feed and feed refusals were measured daily at 0700 h and feed intake was calculated on a DM basis. Sheep were weighed at 0730 h, before feeding, on d 1, and weekly thereafter until the end of the study. Body weight gain per kilogram DMI was calculated for each sheep.

Blood Biochemical and Enzymatic Variables

Blood samples were collected by jugular venipuncture from all sheep before feeding on d 1, 28, and 49. At each collection, two 10-mL aliquots of blood were taken into Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). To obtain serum, blood samples were collected in tubes without additives and allowed to clot for at least 2 h at room temperature (25°C) before they were centrifuged. To obtain plasma, blood samples were collected in tubes containing lithium heparin and stored on ice for approximately 3 h after collection. In both cases, whole blood was centrifuged at $2,400 \times g$ for 15 min at 4°C , and serum and plasma were frozen at -20°C until analysis. Serum concentrations of glucose, NEFA, creatinine, total protein, urea, alkaline phosphatase (**ALP**), alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), and creatine kinase (**CK**) were analyzed using an autoanalyzer (AU2700; Olympus, Center Valley, PA) according to manufacturer's recommendations. Plasma total antioxidant status (**TAS**) was measured using a trolox-equivalent Antioxidant Capacity kit (Cayman Chemical, Ann Arbor, MI).

Determination of Se Status

On d 42, 4 sheep were randomly selected from each treatment for use in a digestion trial. During a 5-d collection period, feed offered and refused, water offered and refused, and fecal and urine mass were measured

daily. Representative samples were collected during this 5-d period, pooled (5% of feed offered and refused, 10% of urine, and 20% of feces), and frozen at -20°C for determination of the apparent digestibility of Se. Urine was separated from feces using a separator attached to the floor of the metabolism cage. Before determination of Se concentrations, samples of feed offered and refused, feces, and tissue (liver and kidney) were dried for 48 h at -40°C using a freeze dryer (Beta 1-8LD plus; Martin Christ, Osterode am Harz, Germany) and then ground through a 1-mm screen. Aliquots (approximately 300 mg) of dried samples were weighed and transferred into clean 20-mL digestion tubes (Thomas Scientific, Swedesboro, NJ). Samples were first pre-digested by adding 6 mL nitric acid to each tube and incubating overnight. Next, 2 mL perchloric acid was added and the tubes were placed into a temperature-controlled digestion block (AIM 600 Digestion Block System; AIM Lap Automatic Technologies, Clontarf, Australia) set to maintain 80°C for 45 min, maintain 160°C for 120 min, then increase $1^{\circ}\text{C}/\text{min}$ up to 185°C , and maintain 185°C for 30 min. After completion of digestion, samples were diluted with deionized water (calibrated to 20 mL) and transferred to polypropylene tubes (Gilson Medical Electronics, Middleton, WI) for analysis according to Association of Official Analytical Chemists (1990; method 966.16) procedures. Total Se contents of serum, urine, and water were assayed using the method described by Delves and Sieniawska (1997). A 200- μL aliquot of sample was diluted with 5 mL high-purity nitric acid (UBE Industries Ltd, Tokyo, Japan) containing 1% butan, 0.66% Triton-X 100, 1% ammonia, and 0.33% ammonium phosphate. The mixture was digested using a CEM microwave digestion system (MDS 2000; CEM Corp., Matthews, NC) and incubated in 3 steps: 600 W for 2 min, 0 W for 2 min, and 450 W for 45 min. After completion of digestion, samples were diluted with deionized water and transferred to polypropylene tubes for analysis. All Se concentrations were determined by inductively coupled plasma mass spectrometry and the average for isotopes with masses of 78 and 80 was recorded.

Statistical Analyses

Data were analyzed using repeated measures and the PROC MIXED model (SAS Inst. Inc., Cary, NC). Dietary treatment (diets), sheep within treatment, and climate period (TNC and HOT) were included in the model. Productive, biochemical, and enzymatic data were included as main effects, and treatment \times climate period was included as the interaction term. Concentrations of Se in liver and kidneys were analyzed as a completely randomized design the PROC

Table 2. Mean ambient temperature (T_a), relative humidity (RH), and temperature–humidity index (THI) in climate facility during 28 d at thermoneutral conditions (TNC) and 22 d at elevated T_a (HOT)

| Item | Period | |
|------------------------------------|--------|------|
| | TNC | HOT |
| Mean T_a , $^{\circ}\text{C}$ | 22.2 | 35.3 |
| Maximum T_a , $^{\circ}\text{C}$ | 27.4 | 38.5 |
| Minimum T_a , $^{\circ}\text{C}$ | 15.5 | 26.6 |
| RH, % | 75.5 | 59.5 |
| THI ¹ | 70.0 | 84.1 |

¹THI was calculated as $(0.8 \times T_a) + [(RH/100) \times (T_a - 14.3)] + 46.4$.

MIXED model (SAS Inst. Inc.) including dietary treatment and sheep within treatment. The random variable (error term) was sheep within treatment. Data are presented as least square mean and differences were considered significant at $P < 0.05$.

RESULTS

Climate Conditions

Mean climatic conditions are presented in Table 2 and Fig. 1. During the TNC period (d 1–28), T_a ranged from 15.5 to 27.4°C with a mean of 22.2°C , RH ranged from 58.8% to 84.6% with a mean of 75.5%, and THI ranged from 62.3 to 74.6 with a mean of 70.0. During the HOT period (d 29–50), T_a ranged from 26.6 to 38.5°C with a mean of 35.3°C , RH ranged from 56.8% to 63.2% with a mean of 59.5%, and THI ranged from 81.4 to 90.7 with a mean of 84.1.

Dry Matter Intake, BW, and Feed Efficiency

The effects of Se and vitamin E supplementation on performance of Merino sheep during the experiment are presented in Table 3. There was an effect ($P < 0.001$) of climate period on DMI. The mean DMI for all treatments during the HOT period was 91 g/d (11.9%) less than that during the TNC period (673 vs. 764 g/d, respectively). Neither Se nor vitamin E affected DMI ($P > 0.05$), and there were no interactions between dietary treatment and climate period ($P > 0.05$) for DMI.

There was an effect of climate period on ADG ($P < 0.001$). The mean ADG for all treatments decreased from 149.4 g/d during the TNC period to -26.4 g/d during the HOT period. There was no difference ($P > 0.05$) in ADG between the control and treatment diets over the experimental period (whole duration of the study) or during the TNC period. However, during the HOT period, the Se50 and Se+E50 groups had less average BW loss (ADG = -16.7 and -15.1 g/d, respectively; Table 3)

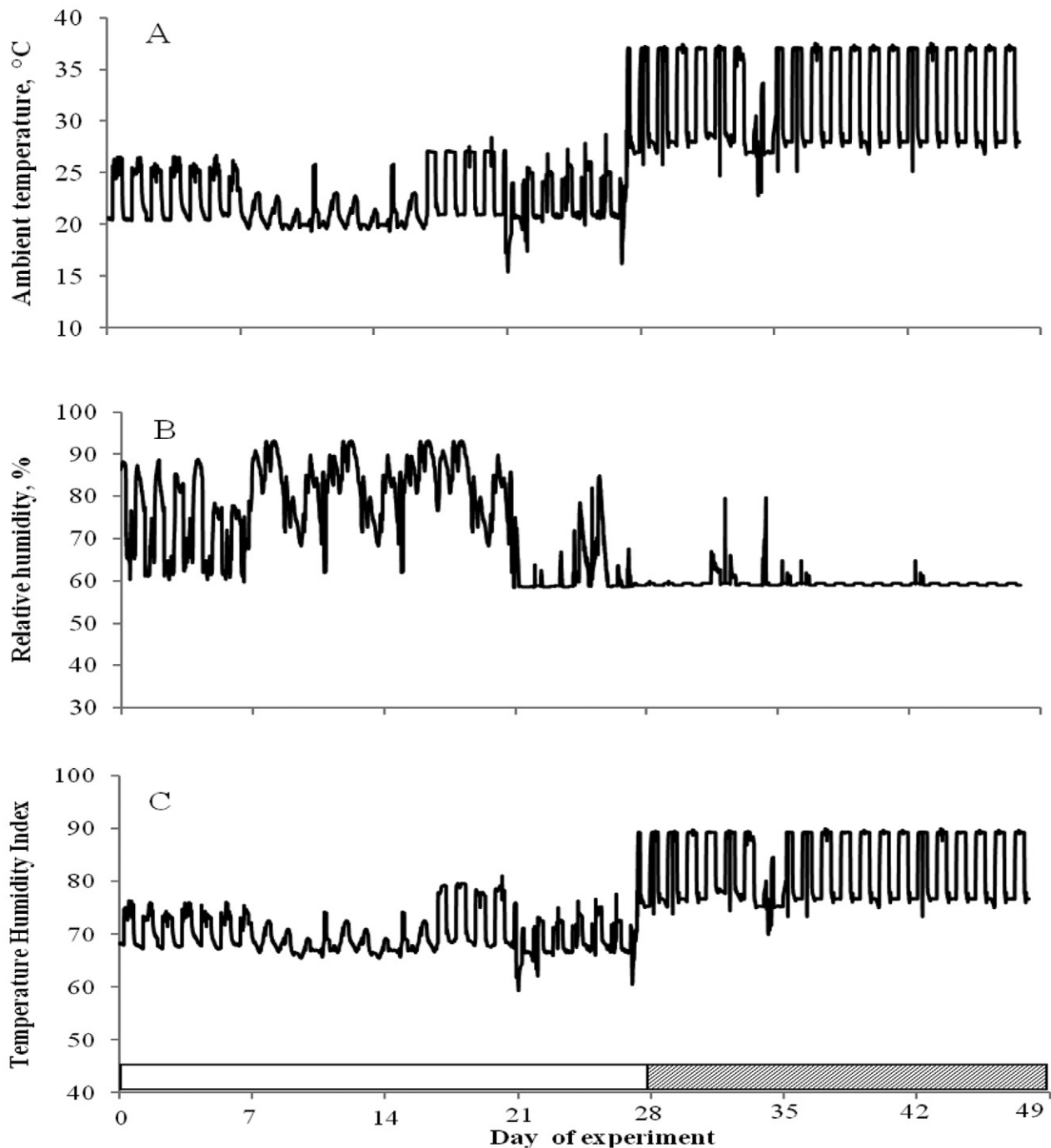


Figure 1. Mean ambient temperature (A), relative humidity (B), and temperature–humidity index (C) in the climate facility during thermoneutral (ambient temperature [T_a] = 20–24°C; 28 d; unshaded bar) and hot (T_a = 28–38°C; 22 d; shaded bar) conditions. Measurements were made every 10 min for 50 d.

than the other groups (all $P < 0.05$), and BW loss was similar across the other groups during this period (ADG = –24.6 to –30.2 g/d). There were diet \times climate period interactions ($P < 0.05$) for ADG for the Se and Se+E diets when the 50- and 22-d periods were compared, whereas there were no differences ($P > 0.05$) in ADG between sheep fed vitamin E for either 50 or 22 d.

Gain-to-feed ratio was also affected ($P < 0.001$) by climate period, decreasing from 0.194 during the TNC period to –0.004 during the HOT period. However,

there were no effects ($P > 0.05$) of dietary treatment and interaction between dietary treatment and climate period on G:F (Table 3).

Serum Biochemical and Enzymatic Variables

The effects of Se and vitamin E supplementation on serum biochemical variables are presented in Table 4. There was no dietary treatment \times climate period interaction ($P > 0.05$) for serum biochemical profile measured.

Table 3. Effects of Se and vitamin E supplementation on DMI, ADG, and G:F¹

| Item | Dietary treatment ² | | | | | | | SE |
|-------------------------|--------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|
| | CON | Se50 | E50 | Se+E50 | Se22 | E22 | Se+E22 | |
| DMI, g/d ³ | | | | | | | | |
| d 1–28 | 766 | 750 | 758 | 781 | 771 | 765 | 759 | 37.2 |
| d 29–50 | 666 | 663 | 668 | 693 | 688 | 690 | 674 | 32.6 |
| d 1–50 | 716 | 707 | 713 | 738 | 724 | 712 | 718 | 34.6 |
| ADG, g/d ^{3,4} | | | | | | | | |
| d 1–28 | 148.2 | 151.2 | 147.6 | 150.0 | 151.4 | 150.7 | 147.0 | 13.7 |
| d 29–50 | –28.5 ^b | –16.7 ^a | –24.6 ^b | –15.1 ^a | –30.2 ^b | –31.4 ^b | –28.6 ^b | 3.83 |
| d 1–50 | 58.8 | 67.2 | 61.5 | 67.4 | 60.5 | 59.6 | 59.2 | 6.99 |
| G:F ³ | | | | | | | | |
| d 1–28 | 0.19 | 0.20 | 0.20 | 0.19 | 0.20 | 0.19 | 0.20 | 0.01 |
| d 29–50 | –0.06 | –0.03 | –0.04 | –0.03 | –0.04 | –0.05 | –0.04 | 0.02 |
| d 1–50 | 0.07 | 0.09 | 0.08 | 0.8 | 0.08 | 0.09 | 0.08 | 0.01 |

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

¹Values are for sheep ($n = 42$) exposed to 28 d thermoneutral conditions (d 1 to 28) followed by 22 d hot conditions (d 29 to 50).

²CON = control (basal diet alone for 50 d); Se50 = basal diet plus Se for 50 d; E50 = basal diet plus vitamin E for 50 d; Se+E50 = basal diet plus Se and vitamin E for 50 d; Se22 = basal diet during the TNC period (d 1–28) and then basal diet plus Se during the HOT period (d 29–50); E22 = basal diet during the TNC period and then basal diet plus vitamin E during the HOT period; Se+E22 = basal diet during the TNC period and then basal diet plus Se and vitamin E during the HOT period.

³Climate period effect ($P < 0.001$).

⁴Diet \times climate period interaction ($P < 0.05$).

Exposing sheep to hot environmental conditions resulted in ($P < 0.05$) reductions in serum concentrations of NEFA (from 0.24 to 0.19 mM; data not shown) and urea (from 4.79 to 3.71 mM; data not shown) when compared to those sheep exposed to thermoneutral conditions. However, there was no effect of climate period on serum concentrations of glucose, creatinine, or total protein ($P > 0.05$; data not shown). There was no effect of dietary treatment on serum levels of NEFA, total protein, or urea during the TNC and HOT periods ($P > 0.05$; Table 4). However, sheep fed the Se+E50 diet had greater serum concentrations of glucose (3.13 mM; data not shown) and creatinine (83.47 μ M; data not shown) compared with sheep fed other diets ($P < 0.007$ and $P < 0.001$, respectively).

The effects of Se and vitamin E supplementation on serum concentrations of enzymatic variables are presented in Table 5. Exposure of sheep to hot conditions resulted in an increase in the serum concentration of AST (from 95.6 to 109.0 units/L; $P < 0.001$) and decreases in serum concentrations of ALP (from 282.5 to 215.8 units/L; $P < 0.001$) and ALT (from 10.19 to 11.13 units/L; $P < 0.03$). However, the mean serum CK concentration was not affected ($P > 0.05$) by climate period.

There were no differences ($P > 0.05$) between the control and treatment diets in serum concentrations of enzymes measured over the experiment or during the TNC and HOT conditions, with the exception of concentrations of ALT (Table 5). Sheep fed treatment diets (Se, E, or both) for 50 d had the lowest ($P < 0.01$) mean serum concentrations of ALT compared with sheep fed

the control or treatment diets for 22 d. Sheep fed supplements for 50 d had ($P < 0.04$) a 15.8% lower serum ALT concentration than sheep fed supplements for 22 d (9.64 vs. 11.48 units/L; data not shown). There were no dietary treatment \times climate period interactions for any serum enzymes measured ($P > 0.05$; Table 5).

Oxidative Status and Se in Blood

The effects of Se and vitamin E supplementation on plasma antioxidant status and serum Se concentration are presented in Table 6. Total antioxidant status and serum Se concentration on d 1 were similar across treatments (4.07 mM trolox/L and 57.35 μ g/L, respectively). There was an effect of climate period on TAS, which was 1.22 mM trolox/L (26.24%) lower during the HOT period than during the TNC period (3.43 vs. 4.65 mM trolox/L; $P < 0.001$; data not shown). There was also an effect of dietary treatment on TAS ($P < 0.05$) and differences between sheep in the control and treatment diets were also observed ($P < 0.05$) for TAS according to environment. Sheep fed the Se50, E50, or Se+E50 diet had a higher TAS than sheep fed the control, Se22, E22, or Se+E22 diet in both the TNC and HOT periods ($P < 0.01$; Table 6).

The mean concentration of Se in serum for all sheep was not affected ($P > 0.05$) by climate period and was similar during thermoneutral and hot conditions. However, in the CON group (no supplemental Se or vitamin E), serum Se concentration during the HOT period was ($P < 0.05$) numerically less (17.7%) than during the TNC period (55.5 vs. 67.4 μ g/L; Table 6). The

Table 4. Effects of Se and vitamin E supplementation on serum concentrations of biochemical variables¹

| Analyte | Dietary treatment ² | | | | | | | SE | <i>P</i> -value ⁵ |
|-----------------------------|--------------------------------|-------|-------|--------|-------|-------|--------|------|------------------------------|
| | CON | Se50 | E50 | Se+E50 | Se22 | E22 | Se+E22 | | |
| Glucose, mM ³ | | | | | | | | | |
| TNC period | 2.83 | 2.87 | 2.91 | 2.84 | 3.13 | 3.08 | 2.95 | 0.29 | 0.11 |
| HOT period | 3.00 | 2.79 | 2.83 | 2.98 | 2.86 | 2.98 | 2.93 | 0.22 | 0.17 |
| NEFA, mM ⁴ | | | | | | | | | |
| TNC period | 0.22 | 0.25 | 0.24 | 0.26 | 0.23 | 0.21 | 0.24 | 0.02 | 0.56 |
| HOT period | 0.24 | 0.26 | 0.23 | 0.25 | 0.25 | 0.22 | 0.21 | 0.03 | 0.74 |
| Creatinine, μM ³ | | | | | | | | | |
| TNC period | 75.83 | 78.28 | 78.48 | 75.60 | 75.07 | 76.48 | 78.75 | 1.91 | 0.39 |
| HOT period | 74.98 | 77.70 | 78.93 | 75.00 | 75.88 | 75.46 | 76.82 | 1.74 | 0.60 |
| Total protein, g/L | | | | | | | | | |
| TNC period | 58.75 | 58.66 | 57.78 | 59.71 | 59.35 | 58.38 | 59.83 | 0.69 | 0.51 |
| HOT period | 59.33 | 58.07 | 58.25 | 59.43 | 58.07 | 58.67 | 59.40 | 0.67 | 0.64 |
| Urea, mM ⁴ | | | | | | | | | |
| TNC period | 4.20 | 4.13 | 4.05 | 4.17 | 3.96 | 4.07 | 3.93 | 0.45 | 0.37 |
| HOT period | 3.87 | 4.21 | 3.67 | 3.72 | 3.77 | 3.82 | 3.76 | 0.41 | 0.52 |

¹Values are for sheep ($n = 42$) exposed to 28 d at thermoneutral conditions (TNC; ambient temperature [T_a] = 20–24°C) followed by 22 d at elevated T_a (HOT; $T_a = 28$ –38°C). Blood samples were collected on d 28 and 49.

²CON = control (basal diet alone for 50 d); Se50 = basal diet plus Se for 50 d; E50 = basal diet plus vitamin E for 50 d; Se+E50 = basal diet plus Se and vitamin E for 50 d; Se22 = basal diet during the TNC period (d 1–28) and then basal diet plus Se during the HOT period (d 29–50); E22 = basal diet during the TNC period and then basal diet plus vitamin E during the HOT period; Se+E22 = basal diet during the TNC period and then basal diet plus Se and vitamin E during the HOT period.

³Main effect of treatment.

⁴Diet effect ($P < 0.05$).

⁵Climate period effect ($P < 0.05$).

serum concentration of Se was affected ($P < 0.01$) by dietary treatment (Table 6). The addition of Se (alone or in combination with vitamin E) to diets for 50 d resulted in an increase ($P < 0.05$) in Se concentration in serum over the experiment. Moreover, sheep fed the Se22 and Se+E22 diets had greater ($P < 0.05$) Se concentrations compared with sheep in the CON, E50, and E22 groups (Table 6). Supplemental vitamin E for 50 d increased the serum concentration of Se during the HOT period and over the experiment compared with the CON diet (Table 6). Interaction between diet and climate period ($P < 0.01$) was observed for Se concentration in serum (Table 6).

Selenium Digestibility, Excretion, and Balance

The effects of Se and vitamin E supplementation on Se digestibility, excretion, and balance during the HOT period are presented in Table 7. Over the 5-d collection period (d 42–46), daily Se intake was affected ($P = 0.0001$) by dietary treatment, as expected. All groups with supplemental Se (Se50, Se+E50, Se22, and Se+E22) had greater total and dietary Se intake than those without (control, E50, and E22). Total Se excretion averaged 199.4 μg/d for all sheep across treatments, and the mean fecal and urinary Se concentrations were 119.7 (59%) and 79.7 μg/d (41%), respectively. Supplemental Se for 50 d increased ($P = 0.0001$) total

Se excretion when compared to other groups. Sheep fed the Se50 or Se+E50 diet had greater total Se excretion and fecal Se concentration than sheep fed any other diet (all $P = 0.0001$). The average total Se output and fecal Se excretion were similar across all other dietary treatments. Groups with supplemental Se (Se50, Se+E50, Se22, and Se+E22) had greater ($P = 0.0002$) urinary Se concentration than those without (control, E50, and E22; Table 7). Consequently, the mean absorption and retention of Se were greater ($P = 0.0001$) in sheep with supplemental Se (Se50, Se+E50, Se22, and Se+E22) than those without (control, E50, and E22; Table 7).

The mean concentration of Se in liver and kidneys over all treatments was 1.26 and 3.65 mg/kg DM, respectively. There was an effect of dietary treatment on Se concentration in both organs (both $P < 0.01$; Table 7). Sheep fed the Se50 or Se+E50 diet had greater Se concentration in liver and kidneys than sheep fed the Se22 or Se+E22 diet ($P < 0.01$), and sheep fed the Se22 or Se+E22 diet had greater ($P < 0.01$) Se concentration in liver and kidneys than sheep without supplemental Se (control, E50, and E22).

DISCUSSION

Selenium and vitamin E are involved in the antioxidant defense system, through which they play important

Table 5. Effects of Se and vitamin E supplementation on serum concentrations of enzymatic variables¹

| Enzyme ² | Dietary treatment ³ | | | | | | | SE | <i>P</i> -value ⁴ |
|------------------------------|--------------------------------|-------|-------|--------|-------|-------|--------|------|------------------------------|
| | CON | Se50 | E50 | Se+E50 | Se22 | E22 | Se+E22 | | |
| AST, units/L ⁵ | | | | | | | | | |
| TNC period | 90.8 | 90.4 | 90.1 | 94.7 | 100.9 | 92.1 | 93.7 | 6.72 | 0.32 |
| HOT period | 107.1 | 98.3 | 111.1 | 106.6 | 112.9 | 113.3 | 104.7 | 7.36 | 0.19 |
| ALT, units/L ^{5, 6} | | | | | | | | | |
| TNC period | 12.4 | 10.5 | 10.5 | 11.7 | 11.2 | 11.4 | 10.9 | 1.17 | 0.24 |
| HOT period | 11.4 | 10.1 | 9.8 | 10.2 | 10.4 | 10.9 | 10.2 | 0.97 | 0.37 |
| ALP, units/L ⁶ | | | | | | | | | |
| TNC period | 251.6 | 271.9 | 255.0 | 270.6 | 263.7 | 258.3 | 268.7 | 46.6 | 0.46 |
| HOT period | 216.2 | 218.7 | 208.5 | 222.1 | 215.2 | 220.4 | 219.6 | 32.8 | 0.63 |
| CK, units/L | | | | | | | | | |
| TNC period | 159.8 | 139.9 | 132.4 | 164.5 | 144.5 | 162.3 | 146.0 | 35.5 | 0.31 |
| HOT period | 123.1 | 122.9 | 119.2 | 135.2 | 127.1 | 128.0 | 134.6 | 32.4 | 0.59 |

¹Values are for sheep ($n = 42$) exposed to 28 d at thermoneutral conditions (TNC; ambient temperature [T_a] = 20–24°C) followed by 22 d at elevated T_a (HOT; $T_a = 28$ –38°C). Blood samples were collected on d 28, and 49.

²ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase.

³CON = control (basal diet alone for 50 d); Se50 = basal diet plus Se for 50 d; E50 = basal diet plus vitamin E for 50 d; Se+E50 = basal diet plus Se and vitamin E for 50 d; Se22 = basal diet during the TNC period (d 1–28) and then basal diet plus Se during the HOT period (d 29–50); E22 = basal diet during the TNC period and then basal diet plus vitamin E during the HOT period; Se+E22 = basal diet during the TNC period and then basal diet plus Se and vitamin E during the HOT period.

⁴Main effect of treatment.

⁵Climate period effect ($P < 0.01$).

⁶Diet effect ($P < 0.01$).

roles in the growth and health of humans and animals (Underwood, 1977; McDowell, 2003; Surai, 2006). However, the concentrations of these 2 micronutrients required to maintain the productivity and health of sheep exposed to high heat load are challenged because of reduced feed intake under such these conditions (Siegel, 1995). The Se and vitamin E requirements of heat stressed sheep are largely unknown because existing recommendations (0.1 and 15 mg/d for Se and vitamin E, respectively; NRC, 1985) were estimated based on prevention of white muscle disease in sheep (McDowell, 1992). Therefore, the concentration of Se used in this study was up to 8 times the NRC (1985) recommendation and was calculated based on results obtained by Alhidary et al. (2012) whereas the concentration of vitamin E used in the current study was recommended by Kott et al. (1998).

It is generally accepted that heat stress exerts adverse effects when the THI exceeds 78 (Fuquay, 1981). In the present study, the mean THI over the HOT period was 84.1, indicating that sheep were subjected to moderate heat stress (THI between 78 and 89; Fuquay, 1981). Although the sheep were not exposed to severe or extreme climatic conditions, the conditions were harsh enough to invoke a heat-stress response. The 12% reduction in DMI observed in response to heat load is consistent with a previous study in which the mean DMI of lambs decreased by 13% when the dry-bulb temperature (T_{db}) was increased from 25 to 35°C (Nardon et al., 1991).

In the current study, supplementation with Se or vitamin E had no effect on DMI. This is consistent with previous studies indicating that there is no beneficial effect of dietary Se and vitamin E on feed intake in sheep (Rock et al., 2001; Qin et al., 2007; Kumar et al., 2009) or dairy cattle (Lee et al., 2007; Ebrahimi et al., 2009; Covey et al., 2010) under TNC. However, the interaction between dietary Se plus vitamin E and high environmental temperature on feed consumption in sheep has not previously been investigated.

Previous studies on sheep productivity indicated that growth rate is increased by dietary Se alone or in combination with vitamin E (Spears et al., 1986; Langlands et al., 1991; Kumar et al., 2009; Celi et al., 2010). Similarly, supplementation with Se plus vitamin E in the current study resulted in an 11.5% reduction in BW loss during exposure to heat load compared with sheep that did not receive Se and vitamin E supplementation (–165.1 vs. –186.7 g/d). The mechanism by which Se and vitamin E improve growth rate has not been documented but could involve improvement of thyroid hormone activity.

An increase in the serum concentrations of glucose was observed in sheep fed diets supplemented with Se and vitamin E for 50 d in the current study. This effect of Se and vitamin E on blood glucose concentration in heat-stressed ruminants has not yet been documented. However, there is evidence of a positive relationship between blood Se and glucose concentrations in

Table 6. Effects of Se and vitamin E supplementation on total antioxidant status (TAS) in plasma and Se concentration in serum¹

| Item | Dietary treatment ² | | | | | | | SE |
|-----------------------------------|--------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|
| | CON | Se50 | E50 | Se+E50 | Se22 | E22 | Se+E22 | |
| TAS, mM trolox/L ^{3,4,5} | | | | | | | | |
| TNC period | 4.09 ^b | 5.32 ^a | 5.36 ^a | 5.40 ^a | 4.17 ^b | 4.03 ^b | 4.12 ^b | 0.17 |
| HOT period | 2.97 ^b | 3.92 ^a | 4.00 ^a | 3.95 ^a | 3.05 ^b | 3.14 ^b | 3.03 ^b | 0.11 |
| Overall | 3.45 ^b | 4.62 ^a | 4.68 ^a | 4.63 ^a | 3.57 ^b | 3.64 ^b | 3.60 ^b | 0.47 |
| Se, µg/L ^{3,5} | | | | | | | | |
| TNC period | 67.4 ^b | 128.9 ^a | 80.3 ^b | 136.3 ^a | 63.9 ^b | 74.1 ^b | 76.9 ^b | 7.04 |
| HOT period | 55.5 ^d | 119.7 ^a | 75.7 ^c | 123.6 ^a | 98.4 ^b | 66.5 ^{cd} | 101.5 ^b | 4.36 |
| Overall | 61.4 ^d | 124.3 ^a | 78.0 ^{bc} | 129.9 ^a | 81.1 ^{bc} | 70.3 ^{cd} | 89.2 ^b | 4.12 |

^{a-d}Within a row, means without a common superscript differ ($P < 0.05$).

¹Values are for sheep ($n = 42$) exposed to 28 d at thermoneutral conditions (TNC; ambient temperature [T_a] = 20–24°C) followed by 22 d at elevated T_a (HOT; $T_a = 28$ –38°C). Blood samples were collected on d 28 and 49.

²CON = control (basal diet alone for 50 d); Se50 = basal diet plus Se for 50 d; E50 = basal diet plus vitamin E for 50 d; Se+E50 = basal diet plus Se and vitamin E for 50 d; Se22 = basal diet during the TNC period (d 1–28) and then basal diet plus Se during the HOT period (d 29–50); E22 = basal diet during the TNC period and then basal diet plus vitamin E during the HOT period; Se+E22 = basal diet during the TNC period and then basal diet plus Se and vitamin E during the HOT period.

³Diet effect ($P < 0.01$).

⁴Climate period effect ($P < 0.01$).

⁵Diet × climate period interaction ($P < 0.01$).

adult humans (Bleys et al., 2007). However, the level to which glucose concentration was increased in the present study was within the reference range of serum glucose concentrations for sheep (2.8–5.6 mM; Aiello, 1998). Moreover, the results of previous studies are conflicting. A decrease in plasma glucose concentration from 3.09 to 2.76 mM as result of supplementing the diets of calves with Se (0.3 mg Sel-Plex per kg DM) has been reported (Ebrahimi et al., 2009).

A 5.7% increase in serum creatinine concentration was associated with Se and vitamin E supplementation in the current study and the mean creatinine concentration was within the normal range for sheep (64–177 µM; Aiello, 1998). Previous studies have suggested that the increase in creatinine concentration during hot environmental conditions could be attributed to an increase in muscle activity in the respiratory system and thoracic cavity caused by an increased respiratory rate and increased oxygen consumption as a part of the thermoregulatory processes to maintain homeostasis (Mendel et al., 2005; Brosnan and Brosnan, 2010).

In the current study, there were increases in serum concentrations of ALT and AST and a decrease in serum concentration of ALP after exposure sheep to an T_a of 38°C. These changes in the blood concentrations of these enzymes would be caused by reduced thyroid hormone activity during high heat load (Collins and Weiner, 1968; Magdub et al., 1982). It is reported that there is a strong correlation between thyroid hormone activity and blood concentrations of the above enzymes (Khan et al., 2010). Furthermore, an elevated THI is associated with increased protein oxidation,

which impairs tissue function and increases the blood concentrations of oxidative enzymes such as AST, ALT, and CK (Sandre et al., 2004; Juniper et al., 2008).

Previous studies have demonstrated significant associations between Se plus vitamin E and blood concentrations of CK, AST, ALP, and ALT in sheep (Whanger et al., 1977; Cronje et al., 2006; Surai, 2006; Braun et al., 2010). Concentrations of AST and CK in sheep injected daily with 1.6 mg Se were less than in untreated sheep (48 vs. 96 and 40 vs. 87 units/L, respectively; Cronje et al., 2006). Moreover, Surai (2006) indicated that increases in ALT and CK activities in the blood of sheep and cattle were associated with a diet deficient in Se and vitamin E. This suggests that the reduced concentration of ALT in the sera of sheep supplemented with Se and vitamin E in the current study was likely caused by improved antioxidant status and thyroid hormone activity during the HOT period.

Evidence from previous studies indicates that hot environmental conditions promote oxidative stress and reduce antioxidant status as a result of an increase in the production of reactive oxygen species or impairment of the antioxidant defense system, which markedly decreases blood concentrations of markers of antioxidant capacity. Furthermore, studies in rats exposed to high environmental temperatures have reported increases in peroxidation in the liver and in activities of enzymes involved in free radical production, including xanthine oxidase (Skibba et al., 1989; Ando et al., 1997). The effect of heat load on TAS in the current study is consistent with these previous studies in that high heat load had a negative effect on antioxidant defense systems.

Table 7. Effects of Se and vitamin E supplementation on Se digestibility, excretion, and balance¹

| Item | Dietary treatment ² | | | | | | | SE | P-value |
|----------------------|--------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------|---------|
| | CON | Se50 | E50 | Se+E50 | Se22 | E22 | Se+E22 | | |
| Se intake, µg/d | | | | | | | | | |
| Diet | 18.3 ^b | 578.2 ^a | 18.4 ^b | 561.3 ^a | 563.3 ^a | 20.4 ^b | 555.3 ^a | 26.53 | 0.0001 |
| Water | 108.5 | 110.3 | 107.6 | 109.4 | 106.8 | 109.2 | 109.4 | 6.94 | 0.99 |
| Total | 126.8 ^b | 688.4 ^a | 126.0 ^b | 670.6 ^a | 670.0 ^a | 129.6 ^b | 664.7 ^a | 28.86 | 0.0001 |
| Se output, µg/d | | | | | | | | | |
| Fecal | 63.9 ^c | 199.1 ^a | 67.0 ^c | 194.7 ^a | 124.9 ^b | 66.5 ^c | 121.5 ^b | 17.88 | 0.0001 |
| Urinary | 56.2 ^b | 135.2 ^a | 50.0 ^b | 126.9 ^a | 72.0 ^b | 52.0 ^b | 65.7 ^b | 14.27 | 0.0002 |
| Total | 120.1 ^b | 334.3 ^a | 117.0 ^b | 321.6 ^a | 196.9 ^b | 118.5 ^b | 187.2 ^b | 27.71 | 0.0001 |
| Absorbed Se, µg/d | 62.9 ^b | 489.3 ^a | 58.1 ^b | 475.9 ^a | 545.1 ^a | 63.0 ^b | 543.1 ^a | 29.57 | 0.0001 |
| Retained Se, µg/d | 6.7 ^b | 354.2 ^a | 11.1 ^b | 348.9 ^a | 473.1 ^a | 8.1 ^b | 477.5 ^a | 30.44 | 0.0001 |
| Se liver, mg/kg DM | 0.81 ^c | 1.73 ^a | 0.94 ^c | 1.92 ^a | 1.28 ^b | 0.87 ^c | 1.24 ^b | 0.22 | 0.01 |
| Se kidneys, mg/kg DM | 3.41 ^c | 3.96 ^a | 3.34 ^c | 3.99 ^a | 3.67 ^b | 3.36 ^c | 3.81 ^b | 0.16 | 0.01 |

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹Sheep were subjected to 28 d thermoneutral conditions (ambient temperature = 20–24°C) followed by 22 d hot conditions (ambient temperature = 28–38°C). Samples were collected on d 42 through 47.

²CON = control (basal diet alone for 50 d); Se50 = basal diet plus Se for 50 d; E50 = basal diet plus vitamin E for 50 d; Se+E50 = basal diet plus Se and vitamin E for 50 d; Se22 = basal diet during the TNC period (d 1–28) and then basal diet plus Se during the HOT period (d 29–50); E22 = basal diet during the TNC period and then basal diet plus vitamin E during the HOT period; Se+E22 = basal diet during the TNC period and then basal diet plus Se and vitamin E during the HOT period.

Selenium, as a component of GSH-Px and thio-redoxin reductase, and vitamin E, as a main lipid peroxidation chain breaker, play essential roles in controlling antioxidant activity by balancing the pro- and antioxidant status of the body (Arthur, 2000; Wang and Quinn, 2000; Burke et al., 2007). Their protective effects involve all 3 types of antioxidant defense systems in animal cells: 1) prevention of radical formation, 2) prevention and restriction of chain formation and propagation, and 3) excision and repair of damaged parts of molecules (Surai, 2006). The improvement in TAS observed in the current study when sheep were supplemented with Se, vitamin E, or both for 50 d is similar to results obtained by Sathya et al. (2007) showing a reduction in plasma concentrations of lipid peroxidation products in buffaloes after injection of Se (30 mg) and vitamin E (1,000 mg). Similarly, dairy cows subjected to moderate heat stress (THI 72–78) and supplemented with Se had lower plasma concentrations of reactive oxygen species than non-supplemented cows subjected to the same heat stress (Calamari et al., 2011).

Previous studies on Se bioavailability have often used the activity of GSH-Px in erythrocytes as an indicator of Se status, as 75 to 85% of erythrocyte Se is incorporated with GSH-Px in ruminants (Rotruck et al., 1973; Beilstein and Whanger, 1983; Surai, 2006). However, the concentration of Se in serum was used in the present study to assess the Se status of the sheep over 50 d because the activity of GSH-Px in erythrocytes reflects long-term Se status (90–120 d) whereas serum or plasma Se concentration reflects short-

term Se status (Ullrey, 1987; Thomson et al., 2004). Furthermore, studies with sheep established that the reference range for serum Se concentration in sheep is between 60 and 160 µg/L (Stowe and Herdt, 1992), whereas a baseline serum Se of <50 µg/L may indicate that sheep are deemed to be Se deficient (Puls, 1994; Davis et al., 2006). With regard to this point, the average initial serum concentration of Se in the current study was 57.35 µg/L, which is above the threshold for Se deficiency and is considered marginal (between 30 and 60 µg/L; Puls, 1994).

The reduction in serum Se concentration observed in nonsupplemented sheep in the HOT period in the current study was similar to the 17.6% reduction reported in Japanese quail exposed to hot conditions (1.78 vs. 2.16 µg/L at Tdb = 34 vs. 25°C, respectively; Sahin et al., 2008). Moreover, it has been reported that serum Se concentration in Angus heifers is less in August than in June (75 vs. 110 µg/L at Tdb = 39.1 vs. 38.6°C, respectively; Burke et al., 2007). The reduction in Se concentration in hot environmental conditions may be caused by an increase in Se metabolism to minimize the effects of oxidative stress, which increases under hot environmental conditions. However, in the current study, the increases in serum concentrations of Se in sheep supplemented with 0.8 mg/kg of Se are similar to the results of Cristaldi et al. (2005) and Davis et al. (2008).

Previous studies have revealed a strong correlation between Se concentrations in urine and the diet. A relative increase in Se urinary excretion occurs with increasing dietary Se to decrease Se retention and

maintain Se homeostasis (NRC, 1983; Koenig et al., 1997; Weiss, 2005). In the current study, supplementation of the diet with 0.8 mg Se/kg DM, alone or in combination with vitamin E, increased the urinary excretion of Se. The percentage of total Se intake excreted (27.4%) was similar to that reported previously for dairy cattle (30%; Ivancic and Weiss, 2001) and sheep (23–33%; Koenig et al., 1997).

In the present study, Se concentration was higher in kidneys than liver, which is consistent with results from previous studies on sheep (Smith and Isopenko, 1997; Qin et al., 2007) and dairy cattle (Combs and Combs, 1986; Pavlata et al., 2001). Conflicting reports have demonstrated higher Se concentrations in liver than kidneys in sheep (Cristaldi et al., 2005; Davis et al., 2008) and calves (Lawler et al., 2004; Skrivanova et al., 2007). Langlands et al. (1984) concluded that the percentage of total-body Se is similar in the liver and kidneys of Merino sheep (4%); however, the percentage of total-body Se in the liver was increased by up to 9% by dietary Se supplementation. In addition, in lambs supplemented for 42 d with 6.30 mg Se/kg DM, the Se clearance rate was greater in kidneys than in the liver (43 vs. 20%; Juniper et al., 2008).

In conclusion, productive, biochemical, and enzymatic variables indicated that the Australian Merino sheep used in this study were exposed to moderate heat stress. The results of this study show that the addition of Se, alone or in combination with vitamin E, at levels in excess of dietary recommendations before exposure to hot conditions improved the ability of sheep to tolerate thermal stress in terms of BW, enzymatic profile, and antioxidant status. Therefore, the current dietary recommendations for Se and vitamin E may be inadequate for sheep exposed to hot conditions and warrant reconsideration. Furthermore, several of the protective effects of Se and vitamin E on growth and physiological responses observed in this study have not been previously reported and warrant further investigation.

Further studies are required to define the effects of Se and vitamin E supplementation and to quantify interactions between these antioxidant micronutrients and the responses of sheep and other species to hot conditions. The level, source, and duration of Se and vitamin E supplementation should be considered when determining the most beneficial effect for productive performance and the health of sheep exposed to a high heat load.

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