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Growth performance and meat quality of finishing pigs fed diets supplemented with antioxidants and organic acids in late summer

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ABSTRACT

Context. Heat stress compromises growth performance and meat quality and results in economic losses in pork production. **Aims.** We investigated the effects of supranutritional levels of selenium (Se) and vitamin E (VitE), along with organic acid blends, on the growth performance and meat quality of finishing pigs over a period of weeks during late summer to early autumn in Westbrook, Queensland, Australia. **Methods.** A total of 264 crossbred pigs (25.8 ± 2.4 kg, mean \pm s.d.) at 11 weeks of age were randomly assigned in a $2 \times 2 \times 2$ factorial design with two aging times (2 or 5 days) nested within each pig. The factors included antioxidants (Se/E, with recommended or supranutritional doses of Se and VitE), an organic acids (OA) blend added to drinking water (control vs supplemented), and sex (female vs male). **Key results.** Between 16 and 18 weeks of age, high Se/E decreased daily feed intake ($P = 0.010$) but had no effects on average daily gain or feed conversion efficiency (FCE). Male pigs grew faster ($P = 0.040$) and had a higher FCE than females ($P = 0.050$). Supplementation with OA increased FCE in males but not females (OA \times Sex interaction, $P = 0.035$). Between Weeks 16 and 20, male pigs grew faster ($P < 0.001$), tended to eat more ($P = 0.057$), and had higher FCE ($P = 0.002$) than females ($P < 0.001$). There were no main effects of Se/E or OA on meat quality, except protein oxidation was reduced by high Se/E ($P = 0.047$). Sex impacted only Warner-Bratzler shear force (WBSF), with male pigs having lower WBSF than females ($P = 0.053$). Meat aging decreased WBSF ($P < 0.001$), but it increased cooking loss ($P = 0.036$), myofibrillar fragmentation index ($P < 0.001$), lipid oxidation ($P < 0.001$) and colour parameters ($P < 0.001$ for all). **Conclusions.** Supplementation with Se/E for up to 10 weeks and OA for 5 weeks did not influence production parameters or pork quality in late summer, except that high Se/E decreased protein oxidation, and significant heat stress conditions were not experienced as expected. **Implications.** Supplementation with Se/E and OA may be effective when environmental temperatures are higher.

Keywords: antioxidant, growth performance, heat stress, meat quality, organic acids, pigs, selenium, vitamin E.

Introduction

High environmental temperature is responsible for reducing growth performance, reproduction, meat quality, and productivity, resulting in economic loss in pig production (Montilla *et al.* 2014; Rauw *et al.* 2020). Global warming is increasing the frequency and severity of extreme heatwave events, and there is growing concern about the impacts of heat stress (HS) on pork production during summer (Renaudeau and Dourmad 2022). Heat stress is associated with respiratory alkalosis, oxidative stress and digestive dysfunction, and compromised productive parameters (Cui *et al.* 2016; Liu *et al.* 2016, 2022). Antioxidant enzymes and vitamins remove excess reactive oxygen species, and these pathways can be augmented by supplementation of selenium and vitamin E, respectively. Selenium (Se) is an integral element of glutathione peroxidase (GPx), an antioxidant enzyme that neutralises lipid peroxidation and protects the cell membrane damage (Hoekstra 1975). Previous studies reported that feeding a diet supplemented with Se

improved antioxidant capacity in muscle tissue and positively affected the meat quality of finishing pigs (Chen *et al.* 2019; Bobček *et al.* 2004). Similarly, as a chain-breaking antioxidant, vitamin E (VitE) supplementation has been shown to reduce the occurrence of PSE meat (pale soft and exudative) and improve the oxidative stability of pork (Cheah *et al.* 1995; Hastly *et al.* 2002). The PSE pork is characterised by meat that has a pale colour and soft texture and is exudative, causing increased drip loss and cooking loss, reduced colour, and negatively affecting consumer acceptability (Lee and Choi 1999; Adzitey and Nurul 2011). Organic acids, alternative dietary supplements, are also widely used in pig production to enhance health, growth performance and meat quality (Chen *et al.* 2017; Morel *et al.* 2019). As an acidifier, organic acid could prevent respiratory alkalosis, which can happen in hot summer conditions (Nørgaard *et al.* 2010). Further, 2-hydroxy-4-(methylthio)butanoic acid (HMTBa), a unique source of methionine, has demonstrated the potential to alleviate stress in pigs by improving antioxidant and detoxification ability (Martín-Venegas *et al.* 2006; Wang *et al.* 2019). Dietary supplementation with HMTBa improves nutrient digestibility and intestinal microbial populations and protects against increased intestinal damage (Li *et al.* 2008; Martín-Venegas *et al.* 2013). In addition, feeding a diet with HMTBa above the recommendations over 14 days before slaughter improved pork quality by increasing glutathione content and decreasing thiobarbituric acid reactive substances (TBARS) levels in muscle (Lebret *et al.* 2018). The intrinsic differences between male and female pigs can independently influence (Xia *et al.* 2023) or interact with nutrition and the environment on productive traits, carcass characteristics and meat quality (Sundrum *et al.* 2011; Martins *et al.* 2023). However, immunocastration of male pigs is considered an alternative procedure performed during the finishing period to control boar taint in pork and improve the acceptability of customers (Marjeta *et al.* 2017; Werner *et al.* 2021). Ageing has improved pork quality by enhancing tenderness, flavour, juiciness and overall liking (Channon *et al.* 2004) but could also increase meat oxidation (Rant *et al.* 2019; Ribeiro *et al.* 2021). In Australia, pork raw materials are typically hung in a chilled room at an abattoir for 2 days before being delivered to supermarkets or butchers where pork can be available for sale, but its shelf life is no more than 5 days.

Therefore, the hypotheses being tested in this experiment were that antioxidants (Se and VitE) and blended organic mixture supplementation over late summer to early autumn, when the critical temperature for pigs is likely to be exceeded, would increase growth performance and improve the meat quality of growing-finishing pigs.

Materials and methods

The conduct of the project followed the Animal Care and Protection Act 2001, the Australian Code for the Care and

Use of Animals for Scientific Purposes, 8th Edition 2013 (the Code) and all other relevant Commonwealth and State legislation and was approved by the Animal Ethics Committee of CHM Alliance Pty Ltd., Qld, Australia (Protocol no. CHM PP 116/18).

Environmental weather data

The experiment was conducted in a research facility located in Westbrook, Queensland, Australia. Daily weather observations during the experimental period were collected at Toowoomba Airport weather station (site number: 041529), located about 10 km from the experimental site. The meteorological data collected from the Australian Bureau of Meteorology included daily maximum temperature, temperature, and relative humidity at 9 am and 3 pm. The temperature–humidity index (THI) was calculated based on the equation described by Vashi *et al.* (2018) as follows:

$$\text{THI} = 0.8 \times T + \left(\frac{\text{RH} \times (T - 14.4)}{100} \right) + 46.4,$$

where T is the environmental temperature (°C), and RH is the relative humidity (%).

Based on THI, the levels of heat stress were classified as follows: normal (THI < 74), mild heat stress (74 ≤ THI < 78), moderate or dangerous (78 ≤ THI < 82) and severe (THI > 82) (Mellado *et al.* 2018).

Growth performance trial

The experiment was conducted in a commercial research facility from February to May 2019 and consisted of three replicates with 24 pens ($n = 11/\text{pen}$) and 6 pens/treatment group. A total of 264 crossbred pigs at 11 weeks of age were randomly assigned in a $2 \times 2 \times 2$ factorial design with the factors being antioxidants (Control vs supranutritional levels of Se and VitE), organic acids (control vs supplemented) and sex (female vs male). In this study, pigs were fed a single diet through the entire experimental period (19.4% crude protein and 14 MJ digestible energy/kg, Table 1) containing the normal inclusion of Se and VitE for this farm (–Se/E, 0.1 g/tonne Se plus 40 g/tonne VitE) or high supranutritional levels of Se and VitE (+Se/E, 0.5 g/tonne Se plus 100 g/tonne VitE). In the last 5 weeks (from Week 16 to 20), pigs were fed the same diets as the previous stage, but half of the pigs from each diet were supplemented with organic acids (Activate[®], Novus International, Inc. Mascot, NSW) in drinking water at 1 L/1000 L (+OA), whereas the other half were not supplemented (–OA). The bodyweight was recorded individually at the beginning of Week 11 and at the end of Weeks 15, 18 and 20. The average daily gain (ADG) was calculated from each pig, and daily feed intake (ADFI) and feed conversion efficiency (FCE) were calculated from each pen during experimental periods. The FCE was expressed as kg of ADG/kg of

Table 1. Ingredient and nutritional compositions of the control diet (as fresh as-fed basis).

Items	Amount
Ingredient	%
Wheat	39.2
Sorghum	25.0
Canola meal 37%	11.8
Millrun 16%	5.0
Soybean meal 46%	7.75
Blood meal 90%	1.0
Meat meal 51%	5.0
Canola oil	2.0
Molasses	2.0
Limestone	0.15
Salt	0.25
Choline chloride 60%	0.01
Lysine-HCl	0.40
DL-Methionine	0.045
L-Threonine	0.03
L-Tryptophan	0.005
Rovabio excel 10%	0.05
Hi-Phos	0.0075
Deodorase Farm pack	0.1
Premix ^A	0.2
Calculated values	
Dry matter (%)	90.0
Digestible energy (MJ/kg)	14.0
Crude protein (%)	19.4
Crude fibre (%)	3.7
SID lysine/digestible energy (%/MJ)	0.07

SID lysine, standardised ileal digestible lysine.

^ASupplied per kg of diet: vitamin A, 8000 IU; vitamin D₃, 1600 IU; vitamin K, 1.5 mg; vitamin B₁, 1.5 mg; vitamin B₂, 4 mg; vitamin B₆, 1 mg; vitamin B₁₂, 10 mg; pantothenate, 13 mg; niacin, 17 mg; biotin, 100 µg; folic acid, 0.2 mg; iron, 45 mg; zinc1000 mg; manganese, 40 mg; copper, 20 mg, chromium picolinate, 0.4 mg; cobalt, 0.5 mg, iodine, 1.5 mg.

ADFI. As was normal practice on this farm, the male pigs were immunocastrated by vaccination with Improvac (Zoetis Australia Pty Ltd, Rhodes, NSW, Australia) at 13 and 17 weeks of age.

Meat quality measurement

At the end of the experiment, 72 pigs (three pigs/pen of six pens/treatment) were randomly selected for meat quality measurement upon reaching market weight. These pigs were slaughtered at an abattoir, and *longissimus dorsi* muscles were collected and transported to the laboratory for meat quality analysis the following day (approximately 24 h postslaughter).

Upon arrival at the laboratory, the meat samples were divided equally into two parts, and then randomly assigned to an aging period of either 2 or 5 days postslaughter at 4°C. All samples were frozen after aging at -20°C for further analysis.

The ultimate pH of the pork samples was measured after the pigs were slaughtered for about 24 h. The pH of the meat sample was measured using a spear-head electric pH probe (IJ44C, Ionode Pty. Ltd., Tennyson Queensland, Australia) attached to a waterproof pH-mV-temperature meter (WP80, TPS Pty. Ltd., Brisbane, Queensland, Australia) by inserting the pH probe approximately 2 cm inside meat for 30 s. The pH meter was calibrated using pH 4 and pH 7 buffer solutions (Hanna Instruments, Keysborough, Victoria, Australia) before the measurement. The pH value of each sample was the average result of five measurements (four around and one in the middle).

Colour measurement

The surface colour of meat was measured on Day 2 and Day 5 postslaughter using a Hunterlab Miniscan EZ (Hunter Assoc. Labs Inc., VA, USA). The machine was calibrated against black and white tie references before measurement. Samples were allowed to bloom at 4°C for 20 min before measurement. Triplicates of surface colour were measured in the CIE system, and the value of *L** (lightness), *a** (redness) and *b** (yellowness) was obtained from the average values of three readings.

Water-holding capacity

The water-holding capacity (WHC) of meat samples was determined as expressed juice using the centrifugation method described by Laakkonen *et al.* (1970) and modified by Choi *et al.* (2014). *Longissimus* muscle samples (0.5 ± 0.05 g) were placed in a centrifugation tube (Centrifugal Filter Units, Merck Millipore, Tullagreen, Carrigtwohill, Co. Cork, Ireland) with filter units and then heated for 20 min at 80°C using a digital block heater (DBH40D, Ratek Instruments, Boronia, Victoria, Australia). After being cooled for 10 min at room temperature, samples were centrifuged at 2000g for 10 min at 4°C. The expressed juice was calculated as the difference of sample weight before and after heating and expressed as a percent of the original sample weight. A higher value of released juice will indicate a lower WHC.

Drip loss

The drip loss was assessed according to the EZ-DripLoss procedure described by Christensen (2003). Briefly, the *longissimus dorsi* muscles were cut into slices of 2.5 cm thickness. Three cylindrical muscle cores were taken in each slice by a fixed blade knife (25-mm diameter). Each core sample was placed into two special EZ-DripLoss containers with a funnel shape (Christensen Aps Industrivaengetand, Hilleroed, Denmark). All empty containers and samples were weighed using a precision laboratory scale (Model 1419 MP8-I; Sartorius, Gottingen, Germany). After storing at 4°C for 48 h,

the container with meat and juice was weighed, then the meat was taken out to weigh the container with juice.

Cooking loss

Cooking loss of pork samples was conducted as per the method of Channon *et al.* (2014) with some modifications. Pork samples were prepared in 3-cm thick slices with an approximate weight of 100 ± 5 g and placed into individual plastic bags. Then, the bags were suspended in a metal rack and cooked in a water bath (F38-ME, Julabo, Seelbach, Germany) preheated to 70°C. Samples were cooked to an internal temperature of 70°C (approximately 35 min). After cooking, samples were cooled in ice water for 30 min to prevent further cooking. Samples were gently dried with paper towels to remove the remaining moisture on the meat surface before they were reweighed to calculate cooking loss, presented as percentage weight loss resulting from cooking.

Warner–Bratzler shear force

The Warner–Bratzler shear force (WBSF) was measured according to the method of Fang *et al.* (2018). After cooking loss measurement, the pork samples were wrapped in a plastic bag and chilled at 4°C overnight prior to texture analysis. Six rectangular strips of $1 \text{ cm}^2 \times 4 \text{ cm}$ were obtained from each sample by cutting parallel to the direction of muscle fibres. Warner–Bratzler Shear Force was measured using a shear blade (V-shaped) adapted to a texture analyser (Lloyd Instruments Ltd., Largo, FL, USA) with a 500N load cell, and the shearing speed was set at 300 mm/min. The peak of the shear force was recorded, and the mean WBSF (N) value for each sample was calculated from six strips.

Myofibrillar fragmentation index

Myofibrillar fragmentation index (MFI) was measured according to the method of Culler *et al.* (1978), as modified by Ha *et al.* (2019) with some modifications. Briefly, frozen meat samples (4 g) were homogenised in 7 mL of cold extraction buffer (50 mM Tris-HCl, 10 mM EDTA, pH 8.3) at 13,000 rpm for 10 s using a homogeniser (IKA Ultra Turrax® T 25 digital, Staufen, Germany). Homogenates were centrifuged at 1500g for 10 min at 2°C, and the supernatants were discarded. The sample was resuspended in 25 mL of the extraction buffer, and this process was repeated twice. After the third wash, the pellet was resuspended in 5 mL of extraction buffer and vortexed to mix well. The suspension was filtered through a tea strainer to remove fat and connective tissue, and then another 5 mL of extraction buffer was added to wash the strainer. Protein concentration was determined using a Biuret assay with bovine serum albumin as standard. The myofibrillar protein suspension was diluted to a final protein concentration of 0.5 mg/mL with the extraction buffer, and 1 mL of the sample was transferred to a cuvette and then mixed well before reading the absorbance at 540 nm (A_{540}) with a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) warmed for

20 min before assaying. The myofibrillar fragmentation index was calculated as $A_{540} \times 200$.

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) assay was used to quantify lipid oxidation for the 2 and 5-day aged samples following methods described by Ha *et al.* (2019) with minor modification. Briefly, 4 g of pork samples were homogenised in 7.5 mL of 10% (w/v) trichloroacetic acid (TCA) solution containing 0.1% ethylenediaminetetraacetic acid (EDTA) and 0.1% propyl gallate (PG) using a homogeniser (IKA Ultra Turrax® T 25 digital, Staufen, Germany) at 19,000 rpm for 60 s. The homogenate was centrifuged at 2000g for 8 min at 4°C in a Rotina 380R Centrifuge (Hettich Instruments, Beverly, Massachusetts, United States). Afterwards, the supernatant was filtered through Grade 1 Whatman filter paper (Cat no. WHA1001-110, Whatman International Ltd, Maidstone, United Kingdom). An equal amount of filtrate (1 mL) and 0.02 M thiobarbituric acid solution (1 mL) were mixed in a screw-cap tube and incubated in the water bath at 95°C for 60 min before cooling in ice. The absorbance at 532 nm was measured with a spectrophotometer (Multiskan Spectrum, Thermofisher) and subtracted by the absorbance at 600 nm to correct nonspecific turbidity.

The standards were prepared from a 2 mM 1,1,3,3-tetraethoxypropane solution (TEP) with serial dilution using Milli-Q water. The final concentration of TEP ranges from 2 µM to 10 µM. Then 1 mL of standard solutions was mixed with 1 mL of 0.02 M TBA solution, the same as for the pork filtrate mentioned above. Results were expressed as mg malondialdehyde (MDA)/kg of meat using a calibration curve of TEP.

Protein oxidation

Protein carbonyl content was measured according to a method developed by Fagan *et al.* (1999) and with some modifications by Shakeri *et al.* (2020). Briefly, pork *longissimus* muscle (1 ± 0.01 g) was cut into small pieces and then homogenised in 5 mL of pyrophosphate buffer (2 mM sodium pyrophosphate, 10 mM tris maleate, 100 mM potassium chloride and 2 mM ethylene glycol tetraacetic acid, pH = 7.4) using an IKA homogeniser (T25 digital Ultra-Turrax®, Selangor, Malaysia) at 17,000 rpm for 40 s. The sample was then split into two equal aliquots of 1 mL. The aliquots were washed by adding 9 mL of HCl:acetone (3:100) (v/v) and centrifuged at 5000g for 5 min at 4°C. Then, samples were rewashed with 1 mL of HCl:acetone (3:100) (v/v) as mentioned above. The pellets were washed twice by adding 1 mL 10% (w/v) TCA, vortexed and centrifuged 5000g at 4°C for 5 min. One identical pellet was mixed with 0.5 mL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) dissolved in 2 M HCl to determine carbonyl. Another pellet was mixed with 0.5 mL of 2 M HCl for protein measurement. Both samples were placed in the dark for 30 min with 10 s vortexes done every 10 min. Then, 0.5 mL of 20% TCA was added to samples,

vortexed and left on ice for 10 min, then centrifuged at 5000g for 5 min at 4°C, and the supernatant was decanted. Samples were washed once more with 1 mL of 20% TCA before being washed three times with 5 mL of ethanol:ethyl acetate (1:1) (v/v). All tubes were centrifuged at 5000g for 5 min at 4°C to remove liquid in each wash. After that, tubes received 1 mL of 6 M guanidine hydrochloride solution (dissolved in 20 mM potassium dihydrogen phosphate, pH 2.3) and were shaken overnight at 4°C. The absorbance of supernatant from the tube containing DNPH was read at 370 nm to calculate carbonyl concentration. In comparison, that of the other tube was read at 280 nm to determine protein concentration. The protein content was calculated and expressed as nmol carbonyl/mg protein.

Statistical analyses

Since the hypothesis was that antioxidants would increase growth performance during the initial phase of the study, these data were analysed by *t*-test using Genstat Ver. 22. Data for growth performance for the main study was analysed by ANOVA with a fixed effect of antioxidant (Se/E), organic acids (OA) and sex, and replicate was included as a random effect. For meat quality analysis, the refrigerated storage time (T, days after postmortem) was an additional fixed effect in the model using Genstat 22nd version (VSN International, Hemel Hempstead, UK). It is important to note that the aging time for quality measurements was nested within each animal as a repeated measure. In the text, data were presented as the adjusted means of the main effects of antioxidants (–Se/E vs +Se/E), organic acids (–OA vs +OA), sex (female vs male) and meat aging (2 days vs 5 days). Data were expressed as adjusted means and standard error of the difference (sed) for the full interactions (Se/E × OA × Sex for production data and Se/E × OA × sex × T for meat quality). Fisher's least significant difference test was applied to compare multiple means that were statistically significant. Differences were considered significant if the *P*-value ≤ 0.05, and a trend was considered if the *P*-value ≤ 0.10.

Results

Environmental conditions

The daily temperatures and THI over the experimental period were expressed as the maximum and minimum values at 09:00 and 15:00 hours in Figs 1 and 2. According to Fig. 1, half time of the experiment had a maximum temperature higher than 23°C, known as the upper limit of the thermal neutral zone for growing-finishing pigs (Liu *et al.* 2022), and most of these days were distributed in late February and March. However, when it comes to the THI value, it illustrated that most of the experimental time, pigs were under suitable

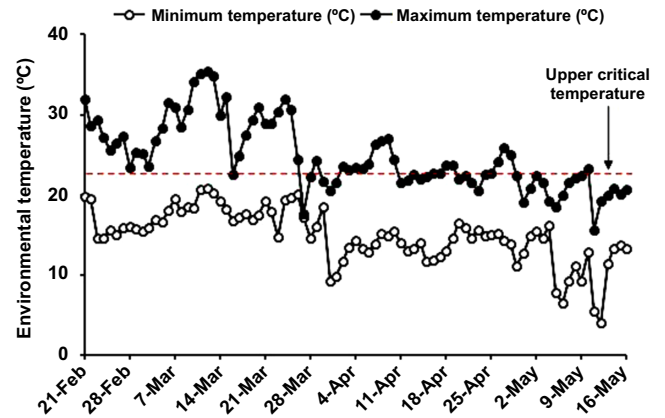


Fig. 1. Daily maximum and minimum temperature during the experiment. The data were observed from Toowoomba airport weather station, Queensland, Australia (27.54°S, 151.91°E, site number: 041529), located approximately 9.8 km away from the experimental site. Replicate 1 lasted from 21 February to 2 May, Replicate 2 from 28 February to 9 May, and Replicate 3 from 7 March to 16 May.

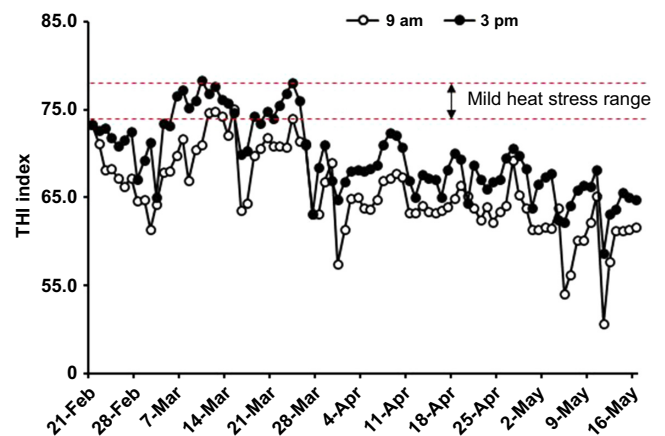


Fig. 2. The temperature–humidity index during the experiment. The data were observed from Toowoomba airport weather station, Queensland, Australia (27.54°S, 151.91°E, site number: 041529), located approximately 9.8 km away from the experimental site. Replicate 1 lasted from 21 February to 2 May, Replicate 2 from 28 February to 9 May, and Replicate 3 from 7 March to 16 May.

conditions because the THI values were below the heat stress threshold (THI < 74) (Fig. 2).

Growth performance

During the initial grower phase of the study before the introduction of the OA (Weeks 11–15), supplementation with Se/E tended to increase ADG (0.886 vs 0.923 kg/day, *P* = 0.063) but did not affect ADFI (*P* = 0.17) or FCE (*P* = 0.15) (data not shown). The effects of sex, Se/E and OA supplementation on the growth performance of finishing pigs during the finishing phase are presented in Table 2.

Table 2. Effects of sex (S), antioxidants (Se/E) and organic acid (OA) supplementation on growth performance of finishing pigs over late summer–early autumn.

Parameters	Sex, S	–Se/E		+Se/E		sed	P-value ^A			
		–OA	+OA	–OA	+OA		S	Se/E	OA	S.OA
Number of pigs ^B		66	63	64	66					
Week 15–18		66	63	64	66					
ADG (kg/day)	F	1.16	1.10	1.16	1.10	0.078	0.040	0.71	0.32	0.024
	IM	1.18	1.30	1.13	1.29					
ADFI (kg/day)	F	2.62	2.56	2.56	2.46	0.094	0.52	0.010	0.32	0.51
	IM	2.72	2.64	2.46	2.52					
FCE	F	0.442	0.433	0.456	0.447	0.028	0.050	0.17	0.11	0.035
	IM	0.433	0.493	0.464	0.513					
Week 18–20										
ADG (kg/day)	F	1.04	1.02	0.88	1.08	0.123	0.042	0.30	0.45	0.46
	IM	1.19	1.17	1.09	1.10					
ADFI (kg/day)	F	2.70	2.55	2.75	2.77	0.268	0.14	0.44	0.89	0.52
	IM	2.84	2.89	2.85	3.02					
FCE	F	0.381	0.398	0.324	0.389	0.057	0.35	0.17	0.39	0.61
	IM	0.420	0.428	0.372	0.381					
Week 15–20										
ADG (kg/day)	F	1.09	1.08	1.04	1.09	0.042	0.001	0.18	0.75	0.81
	IM	1.22	1.23	1.19	1.18					
ADFI (kg/day)	F	2.60	2.58	2.57	2.63	0.118	0.057	0.45	0.96	0.77
	IM	2.78	2.75	2.67	2.67					
FCE	F	0.418	0.412	0.409	0.405	0.017	0.002	0.63	1.00	0.58
	IM	0.444	0.453	0.446	0.448					
Final weight (kg)	F	96.4	92.7	94.6	96.9	2.23	0.001	0.80	0.98	0.47
	IM	100	101	100	101					

ADG, average daily gain; ADFI, average daily feed intake; FCE, feed conversion efficiency; F, female; IM, immunocastrated male; sed, standard error of the difference for the interaction between S, Se/E and OA.

^ANo other interactions were significant ($P < 0.05$).

^BThree pigs from –Se/E*+OA group (pigs fed a normal level of Se and Vit E and supplemented with organic acids) and two pigs from +Se/E*–OA group (pigs fed a supranutritional level of Se and Vit E, without organic acids supplementation) were lost between Week 11 and Week 15 of age. The ADFI and FCE were calculated based on pen averages with 11 pigs/pen (six experimental units/dietary treatment).

Between 16 and 18 weeks of age, male pigs grew faster than female pigs (1.13 vs 1.22 kg/day, $P = 0.040$) (Table 2). Although there were no main effects of dietary supplements on ADG, there was a significant interaction ($P = 0.024$) such that OA supplementation increased ADG in males (1.16 vs 1.29 kg/day) but not in females (1.16 vs 1.0 kg/day). There was no effect of sex or OA on ADFI, whereas dietary Se/E supplementation decreased ADFI (2.64 vs 2.50 kg/day, $P = 0.010$) between 16 and 18 weeks. Between 16 and 18 weeks of age, male pigs had a higher FCE than female pigs (0.444 vs 0.476, $P = 0.050$) (Table 2). Although there were no main effects of dietary supplements on FCE, there was a significant interaction ($P = 0.035$) such that OA supplementation increased FCE in males (0.448 vs 0.503 kg/day) but not in females (0.449 vs 0.440). Between Weeks 18 and 20, the only significant effect was that male pigs grew more

quickly than females (1.00 vs 1.14 kg/day, $P = 0.042$). Over the entire finisher phase between 16 and 20 weeks, male pigs grew faster (1.08 vs 1.20 kg/day, $P = 0.001$), tended to eat more (2.60 vs 2.72 kg/day, $P = 0.057$) had higher FCE (0.411 vs 0.447, $P = 0.002$) and were heavier at slaughter (95.2 vs 100.4 kg, $P < 0.001$) than females. There were no main or interactive effects of dietary supplementation over the full finishing period.

Meat quality

There were no effects of sex, Se/E or OA on pork loin pH, water holding capacity or drip loss ($P > 0.05$ for all, Table 3). WBSF was lower in males than in females (25.9 vs 28.1 N, $P = 0.053$), but there was no significant effect of Se/E ($P = 0.13$) or OA ($P = 0.17$). WBSF declined between Day 2 and Day 5 of aging (31.3 vs 22.7 N, $P < 0.001$). The

Table 3. Effects of sex (S), antioxidants (Se/E), organic acid (OA) supplementation over late summer-early autumn and day of aging (Day) on objective measures of the *longissimus dorsi* of finishing pigs.

Parameters	Sex, S	-Se/E		+Se/E		sed	P-value ^A			
		-OA	+OA	-OA	+OA		S	Se/E	OA	Day
pH ^B	F	5.66	5.65	5.64	5.65	0.029	0.50	0.65	0.72	–
	IM	5.68	5.64	5.65	5.66					
Expressed juice (%) ^C	F	64.8	64.1	63.7	65.7	1.74	0.38	0.62	0.26	–
	IM	62.8	64.3	63.5	64.7					
Drip loss (%) ^C	F	4.53	3.64	3.34	4.03	0.869	0.22	0.64	0.71	–
	IM	4.29	4.58	4.14	4.64					
WBSF (N) ^D										
Day 2	F	1.429 (26.9)	1.461 (28.9)	1.493 (31.1)	1.503 (31.8)	0.052	0.053	0.13	0.17	0.001
	IM	1.494 (31.2)	1.546 (35.2)	1.509 (32.3)	1.522 (33.3)					
Day 5	F	1.361 (23.0)	1.288 (19.4)	1.296 (19.8)	1.418 (26.2)					
	IM	1.346 (22.2)	1.36 (22.9)	1.366 (23.2)	1.393 (24.7)					
Cooking loss (%)										
Day 2	F	23.0	23.6	23.2	23.3	0.90	0.53	0.82	0.63	0.036
	IM	22.7	22.8	22.4	23.2					
Day 5	F	23.9	22.4	23.8	23.4					
	IM	24.1	26.1	23.8	24.0					

Expressed juice was calculated as the difference of sample weight before and after heating and expressed as a percent of the original sample weight. A higher value of released juice will indicate a lower WHC.

WBSF, Warner–Bratzler Shear Force; F, female; IM, immunocastrated male; sed, standard error of the difference for the interaction between S, Se/E, OA and Day.

^AThere were no significant interactions ($P < 0.05$).

^BpH values were measured at 24 h post mortem.

^CAnalyses were performed on pork at 2 days of aging.

^DData were \log_{10} transformed for statistical analysis due to heterogeneity of variances. Back-transformed means are in parentheses. A total of 72 pigs (18 pigs per treatment with nine males and nine females) were selected from the 259 pigs at the end of the experiment.

cooking loss increased between Day 2 and Day 5 of aging (23.0 vs 23.9%, $P = 0.036$) but was not affected by sex ($P = 0.53$), Se/E ($P = 0.82$) or OA ($P = 0.63$) (Table 3).

Protein oxidation, as measured by carbonyl units, was lower in pork from pigs that received the +Se/E diet during the finishing stage (2.60 vs 2.28 nmol/mg protein, $P = 0.047$), but there was no effect of sex, OA or day postslaughter (Table 4). There was no effect of sex ($P = 0.22$), AOX ($P = 0.76$) or OA ($P = 0.51$) on the myofibrillar fragmentation index. However, MFI increased between Day 2 and Day 5 postslaughter (45.2 vs 70.0, $P < 0.001$) (Table 4). There was no effect of sex, Se/E or OA on TBARS. However, TBARS increased between Day 2 and Day 5 postslaughter (0.099 vs 0.140 mg MDA/kg, $P < 0.001$) (Table 4). There were no effects of sex, Se/E or OA on any measures of colour (Table 4). However, L^* (52.8 vs 54.7, $P < 0.001$), a^* (7.34 vs 8.26, $P < 0.001$) and b^* (14.7 vs 16.2, $P < 0.001$) all increased between 2 and 5 days of aging (Table 4).

Discussion

This experiment aimed to investigate whether supplementation with Se/E and OA can ameliorate symptoms associated

with heat stress in finisher pigs during the late summer. The major findings of this study were that supplementation, either Se/E and OA or their combination above the amounts normally fed, had only subtle effects on feed intake, growth rate and feed efficiency of finishing pigs in the late summer. Furthermore, dietary supplements did not markedly improve objective meat quality and oxidative meat stability, although aging decreased shear force and increased cooking loss, L^* , a^* , b^* values and lipid oxidation. This study could provide evidence that the recommended requirements for Se/E and OA are sufficient to avoid oxidative stress in pigs during cool weather, such as late summer. Therefore, these supranutritional supplements do not need to be added to grower-finisher pigs' diets during periods where the THI is below the mild heat stress threshold. Nevertheless, there were some subtle positive effects of Se/E and OA supplementation that are worth exploring.

This experiment was originally planned to be conducted during summer conditions. However, for logistical reasons, the study was conducted later in the summer than anticipated. In the initial grower phase of the study, which was conducted earlier in the summer when the THI was close to the upper critical value, Se/E supplementation tended to increase

Table 4. Effects of sex (S), antioxidants (Se/E), organic acid (OA) supplementation over late summer–early autumn and day of aging (Day) on measures of oxidation and colour of the *longissimus dorsi* of finishing pigs.

Parameters	Sex, S	–Se/E		+Se/E		sed	P-value ^A			
		–OA	+OA	–OA	+OA		S	Se/E	OA	Day
Carbonyl (nmol/mg protein)										
Day 2	F	2.98	2.78	2.23	2.04	0.507	0.46	0.047	0.34	0.34
	IM	2.50	3.17	2.56	2.22					
Day 5	F	2.62	2.12	2.05	2.23					
	IM	2.45	2.14	2.86	2.10					
MFI										
Day 2	F	40.1	49.9	47.6	52.1	11.63	0.22	0.76	0.51	0.001
	IM	49.1	41.5	40.9	40.2					
Day 5	F	57.2	80.8	81.4	71.8					
	IM	71.4	64.0	62.5	71.2					
TBARS (mg MDA/kg)										
Day 2	F	0.135	0.115	0.110	0.082	0.0232	0.36	0.35	0.68	0.001
	IM	0.083	0.087	0.091	0.085					
Day 5	F	0.112	0.143	0.162	0.121					
	IM	0.150	0.161	0.127	0.143					
L*										
Day 2	F	53.5	51.3	52.3	53.3	1.63	0.79	0.81	0.47	0.001
	IM	52.4	53.6	53.1	52.6					
Day 5	F	56.2	54.8	55.9	53.1					
	IM	53.5	54.8	54.4	54.6					
a*										
Day 2	F	7.04	7.40	7.42	7.59	0.678	0.24	0.95	0.88	0.001
	IM	7.98	7.30	7.02	7.00					
Day 5	F	7.47	8.29	8.15	7.95					
	IM	8.55	8.50	8.91	8.27					
b*										
Day 2	F	14.7	14.7	14.6	15.0	0.532	0.57	0.71	0.31	0.001
	IM	14.7	14.8	14.6	14.3					
Day 5	F	16.4	16.6	16.0	16.0					
	IM	15.6	16.3	16.1	16.6					

MFI, myofibrillar fragmentation index; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; F, female; IM, immunocastrated male; sed, standard error of the difference for the interaction between S, Se/E, OA and Day.

^AThere were no significant interactions ($P < 0.05$). A total of 72 pigs (18 pigs per treatment with nine males and nine females) were selected from the 259 pigs at the end of the experiment.

ADG by approximately 4% with no change in ADFI or FCE. However, supplementation with Se/E and OA did not affect the growth performance of pigs in the finisher period. The lack of observed effects of dietary supplements on productive traits during the finisher phase may be attributed to the environmental conditions, as indicated by THI values, suggesting that pigs were mostly under normal conditions throughout the experimental duration. Interestingly, there was a positive effect of OA on ADG and FCE in the male pigs over the first 3 weeks of the finisher period. A previous

study has demonstrated that male pigs can exhibit reduced performance in the transition period after dietary or housing changes (Dunshea 2001), so this finding suggests that OA supplementation may have assisted the male pigs with dealing with these stressors after this dietary change at 15 weeks of age. Additionally, it was also during this period when the immunocastration vaccine was administered to the male pigs, which can cause reduced ADFI and performance in the immediate period after the second vaccination (Dunshea *et al.* 2011). Biologically, male pigs

are more vulnerable to stressors and diseases than females. For example, a greater mortality in barrows compared with gilts was observed on a commercial farm that had experienced porcine circovirus-associated disease in its history (Nevrkla *et al.* 2017). The improved growth rate and feed efficiency in male pigs fed OA suggest that the OA may have a more significant impact on the gut health of males by acidifying intestinal digesta (Tugnoli *et al.* 2020), reducing levels of pathogens such as pathogenic bacteria (De Busser *et al.* 2011; Roldan-Henao *et al.* 2023) and improving nutrient digestibility (Franco *et al.* 2005) compared with females. Environmental stressors, such as high summer temperatures, compromise pigs' productive traits due to physiological responses (Oliveira *et al.* 2018; Pouillet *et al.* 2022). Reduced feed intake is a common response to reduce heat production, and it is a major factor contributing to the poor growth rate of grower-finisher pigs in the hot season. It was estimated that the ADFI decreased by about 55 g for each degree above the upper limit of the thermoneutral zone (Le Bellego *et al.* 2002), and the reduction was greater at 100 g/degree when pigs were housed at 32.2°C (White *et al.* 2008). Dietary supplementation with AOX, such as Se and VitE could potentially improve growth performance by reducing the concentration of reactive oxygen species (ROS) and maintaining a balance of redox status (Gao *et al.* 2010; Liu *et al.* 2021; Sharaf *et al.* 2021). Similarly, organic acids, such as HMTBa, can be metabolised to become a source of methionine for animal requirements. In pigs, a diet supplemented with HMTBa improved growth performance, reduced pathogenic bacteria and increased the beneficial bacterial population in the gastrointestinal tract (Li *et al.* 2008; Wang *et al.* 2019; Qin *et al.* 2022). Although feeding Se and VitE has been shown to improve antioxidant capacity and reduce oxidative stress in heat-stressed pigs (Liu *et al.* 2016), the environmental conditions of this study might not have triggered oxidative stress; thus, dietary supplements might not exhibit their biological activities and affected production traits.

It has been reported that meat quality might be influenced by environmental factors, such as seasonal temperature. Under hot conditions, pigs might suffer the risk of oxidative stress in skeletal muscle (Montilla *et al.* 2014), which can impair meat quality (Pardo *et al.* 2021; Ponnampalam *et al.* 2022). For example, rearing pigs in summer conditions or high ambient temperature increases the occurrence of PSE meat (Gregory 2010; Čobanović *et al.* 2020), which is partly attributed to oxidative stress (Bernabucci *et al.* 2002; Ganesan *et al.* 2018). Reactive oxygen species can be scavenged by endogenous antioxidant enzymes or nutritional vitamins (Ponnampalam *et al.* 2022). By serving as a cofactor of glutathione peroxidase (an endogenous antioxidant enzyme), supplementation with Se reduced drip loss of broiler carcass (Downs *et al.* 2000) and improved the loin eye area in finishing pigs (Wolter *et al.* 1999). Similarly, feeding VitE (500 mg/kg) has been reported to reduce drip loss and PSE carcasses, coupled with inhibition of phospholipase in the

longissimus thoracis of pigs (Cheah *et al.* 1995). Another feed additive, HMBTa, a source of methionine, has demonstrated a positive influence on meat quality by improving protein deposition and carcass composition (Lemme *et al.* 2020). However, the result of the present study shows that supplementation with either Se/E or OA or their combination did not impact meat quality parameters, such as pH, drip loss and WHC. Additionally, diets supplemented with Se/E and OA also did not exhibit improvements in the oxidative status of the meat, except that dietary Se/E reduced protein oxidation, as evidenced by reduced pork muscle carbonyl concentrations. As mentioned earlier, during only 4–6 weeks of the experimental duration, daily maximum temperatures were above the upper critical temperature threshold, and the THI showed mild heat stress. Exposure to such environments might not trigger oxidative stress, which could be the main reason for the lack of effects of Se/E and OA on meat quality. Similar to the effects observed for Se/E and OA, no significant differences were found in any measurements of *longissimus* muscle of finisher pigs between males and females, except for a tendency for WBSF to be higher in males than females.

Cooking loss is associated with water loss during the cooking process and represents the water-holding capacity of meat at high temperatures (Tang *et al.* 2013; Fan *et al.* 2020). Muscle water has three forms: bound, immobilised, and free water. Under high-temperature treatment (cooking), water can be converted from immobilised to free form, resulting in water loss (Tang *et al.* 2013). In the current study, the increase in cooking loss over the storage period illustrated that aging increased the immobilised and free water content and agreed with previous studies (Tang *et al.* 2013; Babür *et al.* 2019; Fan *et al.* 2020). The meat colour is an important parameter associated with the pH and water-holding capacity and might affect consumers' acceptance (Tapp *et al.* 2011; Warner 2016). The form of myoglobin, the primary pigment in meat on the surface of the meat, is responsible for the colour of meat (Warner 2016). During the storage, meat colour changes from purple (reduced deoxymyoglobin) to bright red (oxymyoglobin) and brown (metmyoglobin). In this study, the higher L^* values, a^* values, and b^* values on Day 5 indicated that pork samples became brighter and yellower with more intense redness. These results were consistent with the previous study, which reported that 7-day aging increased L^* values, a^* values and b^* values of beef samples (Fan *et al.* 2020). Another study reported that storage time increased L^* values and b^* values but decreased a^* values of dry-cured pork neck (Kim *et al.* 2014).

The reduced shear force on Day 5 compared to Day 2 of refrigerated storage samples indicates substantial myofibrillar proteolysis associated with aging. These results were supported by a nearly 30% reduction in WBSF, showing improvements in instrumental tenderness between Days 2 and 5 for stored pork. Postmortem changes occur in biochemistry and the activity of endogenous proteolytic enzymes such as calpain, lysosomal proteases, and cathepsins (Bhat *et al.* 2018). These proteases

are responsible for the degradation of myofibrillar proteins and the tenderisation of meat during aging. These results were consistent with Kim *et al.* (2007) and Cho *et al.* (2016), who reported that aging time increased meat tenderness by increasing MFI and decreased WBSF. Recent studies have found little effect of aging on the WBSF of pork. For example, no improvement in WBSF and tenderness was observed in both the pork loin (*M. longissimus thoracis*) and silverside muscles (*M. biceps femoris*) between a 2-day and 7-day aging period (Channon *et al.* 2018a). Furthermore, there was no improvement in the WBSF of the pork loin between a 7-day and 28-day post-slaughter period, although a decreased WBSF was observed in silverside muscles (Channon *et al.* 2018b). In this study, the findings that a 5-day aging period resulted in reduced WBSF indicate that this may be the optimal time for obtaining the highest meat tenderness and consumer palatability. This result is particularly relevant in Australia, where pork carcasses are typically delivered to retail stores within 24–48 h after slaughter, allowing for an additional 3–5 days of retail sales. The higher concentration of TBARS observed on Day 5 compared to Day 2 of refrigerated storage indicated that meat aging was associated with increased lipid oxidation. This agreement with previous studies reported that lipid oxidation increased over storage time in broiler meat (Shakeri *et al.* 2019), pork (Kim *et al.* 2014) and lamb (Rant *et al.* 2019). The increased lipid oxidation coupled with the changes in colour values agrees with Akamittath *et al.* (1990), who reported that lipid oxidation had a close relationship with the discolouration of meat because the oxidation of muscle pigment catalysed this process. Furthermore, lipid oxidation can result in the development of rancid off-flavours, as well as the loss of the desirable characteristic flavour notes and palatability (Cheng and MacDonald 2019; Dhakal *et al.* 2022).

Conclusions

The results of this experiment showed immune-castrated male pigs had higher growth performance than females and only subtle beneficial effects of Se, VitE or OA on growth performance and meat quality under the experimental conditions. This study began during summer, but the pigs entered the finisher period in late summer and early autumn, when conditions had moderated, and significant heat stress conditions were not experienced as expected. Due to climatic conditions, the effectiveness of the treatments under heat stress could not be evaluated.

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Data availability. The data presented in this study are available on request from the corresponding author.

Conflicts of interest. Robyn Warner and Frank Dunshea are Associate Editors of *Animal Production Science*. To mitigate this potential conflict of interest they had no editor-level access to this manuscript during peer review.

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