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Effect of antioxidant and osmolyte enriched or energy-dense diet on heat –stressed fattening pigs

L. De Prekel [a,*], D. Maes [a], A. Van den Broeke [b], M. Aluwé [b]

[a] Unit of Porcine Health Management, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[b] Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Scheldeweg 68, 9090 Melle, Belgium

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ABSTRACT

Heat stress negatively affects pig welfare, health and production. Osmolytes and antioxidants are considered potential mitigators of heat stress-induced damage. Modification of feed composition, specifically lower CP, also aims to reduce internal metabolic heat production. This study investigated the effect of an enriched or energy-dense (**E-dense**) diet on heat-stressed fattening pigs (n = 192 in total). Dietary treatments were administered (*ad libitum*) when pigs reached ± 80 kg. The control diet comprised 15% CP, 3.6% crude fat, 9.1 MJ/kg net energy, 0.4 mg/kg inorganic selenium (**Se**), and 100 ppm vitamin E; the enriched diet contained the same chemical composition but was supplemented with 0.2 mg/kg inorganic Se, 0.2 mg/kg selenomethionine, 200 ppm vitamin E, 200 ppm vitamin C and 0.1% betaine; the E-dense diet featured 13.6% CP, 6.6% crude fat, and increased energy (9.7 MJ/kg) and lysine content. The lysine:energy ratio of all three diets was the same. A 1-week heat wave (± 30 °C and Temperature-Humidity Index of ± 78.4) was induced 2 times when pigs were 20 and 22 weeks old. Physiological parameters and performance parameters were assessed weekly. At the end of the trial, carcass and meat quality were evaluated. Additive enrichment of the diet resulted in a numerically increased daily gain over the 6-week trial compared to the control group (925 vs 891 g/day), $P = 0.090$). The E-dense group had a higher increase in rectal temperature during heat load compared to the control group (0.38 vs 0.28 °C, $P = 0.018$). Over the entire trial, the E-dense group had a higher feed conversion ratio than the control group (2.95 vs 2.67, $P = 0.006$). Carcass traits revealed increased fat thickness of 0.9 mm in the E-dense group ($P = 0.035$), along with lower lean meat content (–1.1%, $P = 0.002$). The meat of the enriched group displayed elevated vitamin E and Se levels ($P < 0.001$), which may be beneficial for the consumer. Overall, the nutritional strategies did not significantly prevent physiological heat stress or enhance performance, but the supplementation of antioxidants and osmolytes tended to ameliorate daily gain over the entire trial.

Implications

This study investigated the effectiveness of feed additives and modifications in feed composition to mitigate the harmful effects of high heat loads in fattening pigs. The tested nutritional strategies did not significantly reduce physiological heat stress or improve performance. However, the supplementation of antioxidants and osmolytes tended to improve overall daily gain. While management strategies such as feed adjustments may provide short-term solutions at a lower cost compared to climate adaptations, our specific study did not confirm their efficacy.

Introduction

Heat stress in pigs can cause major economic (St-Pierre et al., 2003) and health problems. Heat stress can have consequences at every level, such as reduced performance (Renaudeau et al., 2011), an alteration in physiological parameters, e.g. increased rectal temperature and respiration rate (Huynh et al., 2005), the occurrence of respiratory alkalosis which increases the blood pH (Liu et al., 2018a), and increased fat in carcass composition compared to thermoneutral pair-fed conditions (Le Bellego et al., 2002; Pearce et al., 2013a). Measures to mitigate heat stress are therefore essential. Although climate adaptation systems such as cooling pads or earth-air heat exchangers are highly effective in reducing heat stress (Schauberger et al., 2019), the installation cost can be quite high. Simple, low-cost, and easy-to-implement

* Corresponding author.
E-mail address: Lotte.deprekel@ugent.be (L. De Prekel).

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solutions are therefore needed to address heat stress on an *ad hoc* basis. Dietary changes can be a viable option in this regard, especially since they can be implemented quickly and easily in existing pig barns. Nutritional strategies to mitigate heat stress can be implemented either by altering the amount of protein, fibre and fat or by adding antioxidants and osmolytes (Cottrell et al., 2015). The heat increment is higher when protein is used as an energy source than when carbohydrates or fats are used (Musharaf and Latshaw, 1999). An alteration in chemical composition by reducing the protein content can therefore result in lower internal metabolic heat production (Morales et al., 2019). However, it is important to maintain nutritional values, and therefore, crystalline amino acids should be added to reach the pigs' requirements (Kerr et al., 2003). Antioxidants and osmolytes can help mitigate the negative effects that occur during heat stress (Burg, 1995; Cottrell et al., 2015). Betaine, for example, has osmotic properties known to enhance intestinal health and nutrient digestibility (Ratriyanto et al., 2009). By increasing cytoplasmic volume and cellular free water content in response to high osmolarity, betaine facilitates cell proliferation during stressful conditions (Csonka, 1989). Moreover, it reduces the energy requirement for maintenance (Siljander-Rasi et al., 2003) and enhances the water-binding capacity of intestinal cells (Kettunen et al., 2001). Given that osmotic stress (when normal cell volume cannot be maintained (Burg, 1995) due to the changing cell environment) often accompanies heat stress (Pearce et al., 2013b), betaine may mitigate heat-related challenges and promote nutrient absorption through its protective effects on intestinal cells (Ratriyanto et al., 2009). Feed additives such as selenium (**Se**) and vitamin E, known to act synergistically, are commonly used during heat stress conditions to combat oxidative stress caused by an imbalance between free radicals, oxidants, and antioxidant capacity (Cottrell et al., 2015). Oxidative stress typically arises from blood redistribution to peripheral tissues for optimal heat dissipation at the expense of gastrointestinal blood supply (Collin et al., 2001). These antioxidants alleviate heat-induced oxidative stress by neutralising oxidants (Cottrell et al., 2015), thereby potentially enhancing overall performance. Studies have shown some promising results in mitigating heat stress in pigs through diet adaptations (Ratriyanto et al., 2009; Gabler et al., 2013; Morales et al., 2018; Pathak et al., 2018), but disagreement and contradictory results are also reported, especially regarding the effect of simultaneous supplementation of various feed additives, their quantities and form (Cottrell et al., 2015; Liu et al., 2018a; Shakeri et al., 2018; Shakeri et al., 2019). Studies on non-synthetic forms of feed additives and/or high doses frequently find positive outcomes on physiology and performance parameters (Attia et al., 2009; Chauhan et al., 2014; Le et al., 2020; Quisirumbay-Gaibor et al., 2020; Shakeri et al., 2020). However, the application of feed additives is constrained by legally permitted doses, and natural forms are often costlier or unavailable. In the present study, we investigated the effect of an additive-enriched as well as a composition-altered diet on heat stress-related parameters in fattening pigs during high heat loads. The aim was to assess whether a practical and legally permissible dietary intervention with a mix of natural and synthetic forms could alleviate heat stress in fattening pigs. The impact of the different diets during two consecutive periods with high heat loads was evaluated by physiological parameters, blood gas parameters, performances, carcass, and meat quality parameters of fattening pigs.

Material and methods

The Ethics Committee of Flanders Research Institute for Agricultural, Fisheries and Food Research (ILVO) approved all experimental procedures during the trial (number 2023/434).

Study design

A total of 32 pens of mixed-sex (three gilts and three barrows) fattening pigs (two consecutive batches of 96 pigs) (Topigs TN70 x Belgian Piétrain) were randomly divided into three dietary groups: control (n = 10); enriched (n = 11), a diet supplemented with antioxidants and osmolytes; and energy-dense (**E-dense**, n = 11) diet, which was a diet with altered chemical composition (Table 1). The trial started at 18 weeks of age (72.1 ± 11.9 kg) with a 1-week acclimatisation period and ended at slaughter at 25 or 26 weeks of age (116.4 ± 9.5 kg) (Fig. 1). Two artificial heat waves were induced for 7 days, i.e. the first heat wave at 20 weeks of age (87.4 ± 9.3 kg) and the second at 22 weeks of age (98.7 ± 9.4 kg).

Housing and management

The trial was performed in four compartments of the Pig Campus (the experimental pig housing from ILVO, Ghent University (UGent) and University College Ghent (HOGENT)) located in Melle, Belgium. Two consecutive weaning batches, with 3-week intervals between each batch, were divided into two compartments each. Each compartment consisted of eight pens of six pigs with a random distribution of the three dietary groups. A feeder was located in the right or left front corner of each pen, with one drinking nipple in the left or right back of the pen. All pens in the compartment had a partially slatted floor with a total pen surface of 4.88 m^2. The compartment was artificially lit from 0730 to 1530 h plus natural light from a window (2.07 m^2) on the south side for batch 1 (compartments 1 and 2) and on the north side for batch 2 (compartments 3 and 4).

Feed

The pigs were fed a standard grain-based first-phase pig diet up to 17 weeks of age. From week 18 onwards, pigs were fed with one of the three diets, all produced at the ILVO Feed Pilot (Melle, Belgium; Table 1). The control diet consisted of a standard grain-based second-phase diet with 15.0% CP, 3.5% crude fat, net energy of 9.1 MJ/kg, electrolyte balance of 182.5 meq/kg and standardised ileal digestible (**SID**) lysine of 7.8 g/kg. The enriched diet contained the same ingredients and analysed chemical composition, but another premix was added with an extra supplementation of 100 ppm vitamin E, 200 ppm vitamin C, 1 307 ppm betaine as well as 0.200 ppm organic Se (L-selenomethionine) instead of 0.200 ppm inorganic Se (sodium selenite). The E-dense diet contained a lower CP content of 13.6% and a higher crude fat content of 6.6%, net energy of 9.7 MJ/kg, electrolyte balance of 220 meq/kg and SID lysine of 8.4 g/kg. The E-dense diet was provided with extra synthetic amino acids according to the recommendations of CVB (2023) to compensate for the decrease in CP content (Table 1). The SID lysine: net energy ratio of all three diets was the same. Diets were formulated based on 1) literature to identify various feed additives/ strategies that could mitigate heat stress (Haydon et al., 1990; Ratriyanto et al., 2009; Gan et al., 2014; Cottrell et al., 2015; Lv et al., 2015; Stewart et al., 2015; Liu et al., 2016; Liu et al., 2017; Liu et al., 2018b; Tang et al., 2019; De Prekel et al., 2024a) and (2) the advice of the feed expert group of the research project Coolpigs, and were formulated to meet optimal nutrient requirements and amino acid levels of fattening pigs between 80 and 120 kg (CVB, 2023). Feed and water were provided *ad libitum* to all groups.

Climate control

The compartment was mechanically ventilated by channel ventilation, meaning that the incoming air entered the compartment

Table 1
Ingredients, premix composition and analysed chemical composition of the control, enriched and E-dense diet provided for fattening pigs starting at 18 weeks of age.

Ingredients and chemical composition		Control	Enriched	E-dense
Ingredients [%]				
Corn		20	20	22
Barley		20	20	20
Wheat		19	19	15
Wheat gluten		10	10	10
Soybean meal		7	7	3
Corn flakes		5	5	3
Palm kernel flakes (CF < 180)		5	5	0
Sunflower seed meal		3	3	6
Rapeseed meal		3	3	0
Cane molasses		3	3	3
Lignobond		1	1	1
Premix Control		1	0	1
Premix Enriched		0	1	0
Limestone		0.8	0.8	0.9
Barn wheat		0.4	0.4	4.8
Salt		0.4	0.4	0.2
Animal fat		0.3	0.3	3.5
Robisco pellets		0.0	0.0	2.5
Oat hulls		0.0	0.0	1.8
Sodium bicarbonate		0.0	0.0	0.6
Mono calcium phosphate		0.0	0.0	0.1
L-lysine HCL		0.40	0.40	0.60
DL-methionine		0.09	0.09	0.16
L-threonine		0.13	0.13	0.23
L-tryptophan		0.02	0.02	0.05
L-valine		0.00	0.00	0.03
L-histidine HCL		0.00	0.00	0.02
Isoleucine valine 50/50		0.00	0.00	0.14
Leucine valine 90/10		0.00	0.00	0.07
Analysed chemical composition[1]				
CP (N × 6.25) [g/kg]		150	150	136
Crude fat [g/kg]		35	34	66
Crude ash [g/kg]		44	44	45
Crude fibre [g/kg]		59	59	53
DM [g/kg]		890	890	894
Net energy [MJ/kg][2]		9.1	9.1	9.7
SID lysine: net energy ratio [g/MJ][2]		0.86	0.86	0.87
Electrolyte balance [meq/kg][2]		182.5	182.5	220
Composition premix[3]				
Vitamin E [mg/kg]	Calculated	100.0	200.0	100.0
	Analysed (DL-alfa-tocopherol)[4]	99	195	NA
Vitamin C [mg/kg]	Calculated	0.0	200.0	0.0
	Analysed (ascorbic acid)[5,6]	<5	51.7	NA
Betaine hydrochloride [mg/kg]	Calculated	0.0	1306.7	0.0
	Analysed (total)[6,7]	1 400	2 300	NA
Sodium selenite [mg/kg]	Calculated	0.4	0.2	0.4
L-Selenomethionine [mg/kg]	Calculated	0.0	0.2	0.0
	Analysed (total)[3]	0.5	0.6	NA
Amino acid profile [g/kg][8]				
Lysine	Calculated (SID)	7.8	7.8	8.4
	Calculated (total)	9.0	9.0	9.4
	Analysed (total)	8.8	8.8	9.5
Methionine	Calculated (SID)	2.9	2.9	3.3
	Calculated (total)	3.2	3.2	3.6
	Analysed (total)	3.3	3.2	3.8
Threonine	Calculated (SID)	5.4	5.4	5.7
	Calculated (total)	6.5	6.5	6.6
	Analysed (total)	6.3	6.3	6.4
Tryptophan	Calculated (SID)	1.6	1.6	1.7
	Calculated (total)	1.9	1.9	2.0
	Analysed (total)	1.9	2.0	2.0
Isoleucine	Calculated (SID)	4.4	4.4	4.3
	Calculated (total)	5.3	5.3	5.0
	Analysed (total)	5.5	5.3	5.2
Leucine	Calculated (SID)	8.9	8.9	8.4
	Calculated (total)	10.8	10.8	9.9
	Analysed (total)	10.9	10.6	9.8

(*continued on next page*)

Table 1 (*continued*)

Ingredients and chemical composition		Control	Enriched	E-dense
Valine	Calculated (SID)	5.4	5.4	5.6
	Calculated (total)	6.7	6.7	6.7
	Analysed (total)	7.0	6.6	6.9
Histidine	Calculated (SID)	2.9	2.9	2.6
	Calculated (total)	3.5	3.5	3.1
	Analysed (total)	3.5	3.5	3.3
Phenylalanine	Calculated (SID)	5.5	5.5	4.7
	Calculated (total)	6.6	6.6	5.6
	Analysed (total)	6.6	6.5	5.7
Methionine + Cysteine:Lysine	Calculated (SID)	0.62	0.62	0.60
	Calculated (total)	0.66	0.66	0.63
	Analysed (total)	0.68	0.66	0.65
Threonine:Lysine	Calculated (SID)	0.66	0.66	0.65
	Calculated (total)	0.72	0.72	0.70
	Analysed (total)	0.72	0.72	0.67
Tryptophan:Lysine	Calculated (SID)	0.20	0.20	0.19
	Calculated (total)	0.21	0.21	0.21
	Analysed (total)	0.22	0.23	0.21
Isoleucine:Lysine	Calculated (SID)	0.54	0.54	0.49
	Calculated (total)	0.59	0.59	0.53
	Analysed (total)	0.63	0.60	0.55
Leucine:Lysine	Calculated (SID)	1.15	1.15	0.99
	Calculated (total)	1.20	1.20	1.05
	Analysed (total)	1.24	1.20	1.03
Valine:Lysine	Calculated (SID)	0.66	0.66	0.64
	Calculated (total)	0.74	0.74	0.71
	Analysed (total)	0.80	0.75	0.73

SID: standardised ileal digestible, NA: not applicable.
[1] Analysed by the ILVO ANIMALAB, Melle, Belgium.
[2] Formulated composition
[3] Calculated and produced by DSM, Deinze, Belgium.
[4] Analysed by Ecca, Merelbeke, Belgium.
[5] Analysed by Eurofins Food Testing Belgium, Nazareth, Belgium.
[6] Analysed 1 year after production which can influence the concentration of vitamin C.
[7] Total betaine calculated as total betaine hydrochloride.
[8] Analysed amino acid by FFQ Laboratorium, Merksem, Belgium.

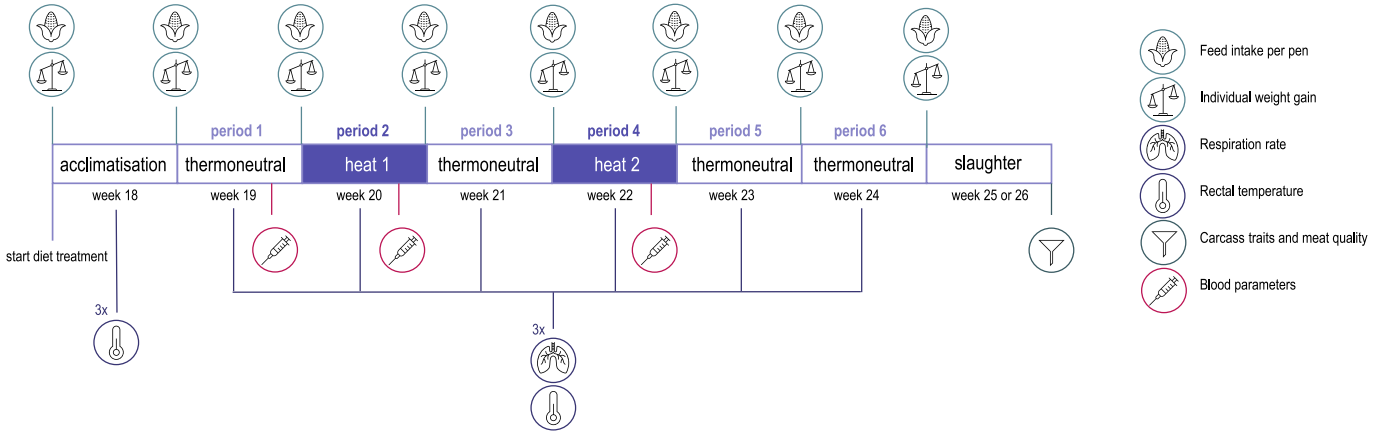

Fig. 1. Visual representation of the pig trial design.

via the slats of the compartment corridor. During the thermoneutral weeks, the indoor climate was automatically controlled by a climate computer (Hotraco Agri©, Hotraco Group, Hegelsom, The Netherlands). During the artificial heatwaves, the same validated heating protocol of De Prekel et al. (2024b) was used. During the first heatwave of batch 1, the temperature was increased in one step from 23.5 °C to 30.0 °C. This resulted in an abrupt and large temperature increase. The temperature of all subsequent artificial heat waves increased incrementally from 25 °C at 1200 h on the first day of the heat wave with increases of 2 °C per half-day, ending around 31 °C at 1800 h on the second day of the heatwave. During the artificial heatwave, the temperature remained constant during the day and night. Two sensors (HOBO MX2301A, Onset®, Bourne, MA, USA) were placed in the corridor at a height of 150 cm in every compartment. These sensors logged the relative humidity and ambient temperature in the compartment in 10-min intervals during the entire trial period. The data from the climate sensors were used to calculate the Temperature-Humidity Index (**THI**) (1):

$$THI = 0.72 * T_{DB} + 0.72 * T_{WB} + 40.6 \quad (1)$$

$$T_{WB} = T_{DB} * tan^{-1}\left(0.151977 * \sqrt[2]{(RH + 8.313659)}\right) + tan^{-1}(T_{DB} + RH) - tan^{-1}(RH - 1.676331) + 0.00391838 * RH^{\frac{3}{2}} * tan^{-1}(0.023101 * RH) - 4.686035$$

With THI: temperature-humidity index, T_{DB}: dry-bulb temperature [°C], T_{WB}: wet-bulb temperature [°C] and RH: relative humidity [%].

The THI values can be used to indicate the risk of heat stress in animals. The THI threshold values for potential heat stress in this trial were 75 ≤ THI < 79: warning for HS, 79 ≤ THI < 84: danger of HS, THI ≥ 84: great danger of HS (NWSCR, 1976). The target THI values during the heat wave period were set to exceed 75, while the THI values during thermoneutral weeks were maintained below 75 (Supplementary Fig. S1).

Description of critical methods

Physiological parameters

Three reference animals (two barrows and one gilt, or two gilts and one barrow) per pen were randomly selected to monitor the physiological parameters throughout the study: respiration rate (**RR**) and rectal temperature (**T_{rectal}**). The same reference animals were evaluated throughout the trial. The parameters were measured 3 times per week in the afternoon (between 1300 and 1500 h) starting at 19 until 24 weeks of age (Fig. 1). Respiration rate (breaths/min) was scored visually based on the number of flank movements per 30 s multiplied by 2. Respiration rate was only evaluated when a pig was lying in a resting position. The same observers performed all the evaluations. Rectal temperature (°C) was measured using a digital thermometer (Veterinär-thermometer SC12, Scala electronics GmbH, Stahnsdorf, Germany) for approximately 15 s. During the acclimatisation period (week 18), T_{rectal} was measured for 3 consecutive days. This allowed the animals to become accustomed to the presence of the observers in the pen as well as the temperature measurement procedure, which reduced the risk of a stress-induced increase in T_{rectal} during the first observations.

Blood (gas) parameters

From the group of reference animals monitored for physiological parameters, one gilt and one barrow per pen were selected for blood collection at 3 time points: in the week before the first heat wave and during the two heat waves (Fig. 1). Blood gas analysis to check the heat stress level was conducted on all these selected reference animals in each dietary group. Additionally, vitamin E and selenium levels in the blood were analysed for the same two reference animals per pen, but this analysis was limited to the control and enriched groups. For vitamin E and Se analysis, blood was collected via the vena jugularis (20 mL EDTA) and centrifuged for 2–6 h after sampling for 10 min at 4 °C and 1 500 g. After this, plasma was divided into four aliquots and stored at −80 °C. Per dietary group, sex, week and batch, aliquots were clotted and analysed by an external laboratory (Dierengezondheidszorg Vlaanderen, Lier, Belgium). The analysis for vitamin E was done by HPLC-UV and for selenium by IVP-MS. For the blood gas analysis, a lithium heparin blood tube of 2 mL was filled with 1 mL of venous blood and analysed via an i-STAT alinity system blood gas analyser (Zoetis©, Louvain-La-Neuve, Belgium). Blood (220 µl) was put on a CG8 + cartridge which analysed pH, ionised calcium, Sodium, Potassium, glucose, haematocrit, haemoglobin, oxygen partial pressure, carbon dioxide partial pressure, bicarbonate, total carbon dioxide, oxygen saturation and base excess. Performance parameters.

Individual animal weight and feed intake per pen were measured weekly. At the beginning of each week, feeders were filled manually and the provided feed was weighed. Every extra feed addition within the same week was also recorded. At the end of each week, residual feed was weighed. Based on these data, average daily feed intake (**ADFI**), average daily gain (**ADG**) and feed conversion ratio (**FCR**) were calculated per pen, per week and over the entire trial period. It is important to note that the pigs were not fasted prior to weight measurements. Consequently, the feeding status at the time of weighing (same time every week), along with increased drinking behaviour during hot periods, could possibly impact weight measurements.

Carcass traits and instrumental meat quality

The pigs were transported to a commercial slaughterhouse after a fasting period of a minimum of 16 h. Each batch of pigs was sent to the slaughterhouse in two groups: the first group, with an average pen weight of ≥ 110 kg at 24 weeks of age, was slaughtered in week 25, and the second group was slaughtered in week 26. Pigs were slaughtered by exsanguination after carbon dioxide stunning. The hot carcasses were weighed, and the 'AutoFOM III™' system (Frontmatec, Kolfing, Denmark) was used to measure backfat and muscle thickness [mm], ham fat thickness [mm] and estimate lean meat and ham meat content [%] as described by Kowalski et al. (2020).

At 35 min postmortem, the initial pH (Type HI98163 electrode FC2323, Hanna Instruments®, Temse, Belgium) of all reference animals was measured in the *Musculus longissimus thoracis et lumborum* next to the 7th rib (starting from the rostral side of the carcass). Approximately 22 h after slaughter, loin samples of the left side of all reference animals were collected in the slaughterhouse. Twenty-four hours postmortem, the ultimate pH was measured by three repeated measurements at the bottom, the middle and the top of the loin sample. Then, visible connective tissue and fat were removed from the *M. longissimus thoracis et lumborum* and the muscle was cut into meat slices of 2.5 cm. For each trait, the muscle slices (and duplicates) were taken at the same location. Starting 2.5 cm from caudal towards cranial end, slices were then taken for (1) colour and marbling, (2) drip loss and IMF (two slices), (3) cooking and shearing, (4) vitamin E, (5) colour and marbling, (6) selenium, (7) drip loss and IMF (two slices), and (8) colour and marbling determinations. Water holding capacity was evaluated according to the drip loss method of Honikel (1987) using two slices. Each meat slice was weighed and put on a hook that was part of the cover of a drip loss container, after which the drip loss containers were stored at 4 °C for 48 h. The sample was then weighed and the drip loss percentage was calculated. Cooking loss and Warner-Bratzler shear force were measured according to the methods of Boccard et al. (1981) and Honikel (1998). Two slices of 2.5 cm were put in a warm water bath of 75 °C for 60 min and cooled down for a minimum of 15 min by tap water. Then, five cylindrical samples (Ø 1.27 mm) per slice were cut parallel to the fibre direction and sheared perpendicular to the fibre direction using the Warner-Bratzler shear (Multitest 2.5-DV and AFG 250 N, Mecmesin, UK). Afterwards, the 2 most extreme values were removed from the dataset and the average of the remaining eight measurements per animal was calculated. Intramuscular fat content (**IMF**) was measured with the NIRS device (NIRS DS2500 L™, FOSS, Hilleroed, Denmark) according to a calibration model based on the reference method of Bligh and Dyer (Hanson and Olley, 1963). First, two meat slices of 2.5 cm thickness were homogenised using a meat grinder and a disperser (ULTRA-TURRAX®, IKA®-Werke GmbH & Co. KG, Staufen im Breisgau, Germany). Then, 100 g of the homogenised sample was put in the NIRS device. The CIE-L*a*b* colour determinants were measured by three repeated measurements on 2.5 cm meat slices with reflection spectroscopy (Miniscan® EZ 4500 L, Hunterlab, Reston (VA), USA) after 30 min of blooming at 9 °C. From the same meat slices, marbling (intramuscular fat content) was visually evaluated by a 7-point marbling level scale by two trained observers (National Pork Producers Council, 1999). Two other 100 g slices from the control and enriched group were laboratory analysed (Ecca, Merelbeke,

Belgium) to determine the level of vitamin E and Se in the muscles of the pigs using the LC-MS/MS method (vitamin E) and ICP-MS method (Se).

Validation and quality assurance

To ensure the study's reliability, a validated heating protocol was used for inducing the artificial heat waves (De Prekel et al., 2024b). Climate control HOBO sensors (HOBO MX2301A, Onset®, Bourne, MA, USA) were calibrated before start of the trial, and two sensors were positioned per compartment to monitor logging deviations. Rectal thermometers were validated every 3 weeks using a calibrated thermometer. Furthermore, an acclimatisation period for the measurement of physiological parameters was incorporated. pH meter calibration occurred initially in the slaughterhouse, followed by a 22-h interval. First, the pH meter was calibrated with a standardised buffer of pH 7; then, a buffer with pH 4 was used. The pH electrodes include a built-in temperature sensor in the tip of the electrode for fast and accurate temperature-compensated readings. After the pH measurements, the electrode was cleaned with H_2O and stored in NaCl. The reflection spectroscopy device (Miniscan® EZ 4500 L, Hunterlab, Reston, VA, USA) was standardised using a black and white panel.

Statistical analysis of data

Statistics were calculated in R® software (version 4.1.1). QQ-plots and histograms of the physiological parameters, blood (gas) parameters, performances, and meat and carcass quality parameters were evaluated to check the normality of the residuals of the models. No deviations from normality were observed. The pen was the experimental unit. The observational unit was the pen for ADFI and FCR; and the individual animal for ADG, physiological and blood parameters, and carcass and meat quality traits.

To evaluate the effects of dietary treatment in reaction on the heat load on different parameters, the following models were used. For the physiological parameters, a linear mixed model was used to determine the effect of week, dietary treatment and its interactions. A random effect for individual identification number of the animal nested within pen was added to correct for the repeated measurements within each animal:

$$Y_{physiological} = W \times \beta_W + DT \times \beta_{DT} + W \times DT \times \beta_{W \times DT} + A \times \beta_A + Z \times \mu + \varepsilon$$

where $Y_{physiological}$ = dependent variables (RR, T_{rectal}), W = week as independent variable (weeks 19–24), DT = dietary treatment as independent variable (control, enriched, E-dense), A = age as independent variable, β = vector of the fixed effects, Z = design matrix of random effects (weaning batch and individual identification number of the animal within pen), μ = vector of the random effects and ε = vector of random errors.

For the blood (gas) parameters, a linear mixed model was used to determine the effect of week, dietary treatment and its interactions. A random effect for pen was added to correct for the repeated measurements within pen:

$$Y_{blood(gas)} = W \times \beta_W + DT \times \beta_{DT} + W \times DT \times \beta_{W \times DT} + S \times \beta_S + Z \times \mu + \varepsilon$$

where $Y_{blood(gas)}$ = dependent variables (pH, ionised calcium, Na, K, glucose, haematocrit, haemoglobin, pO_2, pCO_2, HCO_3^-, total carbon dioxide, oxygen saturation and base excess), W = week as independent variable (week 19–24), DT = dietary treatment as independent variable (control, enriched, E-dense), S = sex as independent variable (barrow and gilt), β = vector of the fixed effects, Z = design matrix of random effects (pen), μ = vector of the random effects and ε = vector of random errors. Weaning batch was excluded as a random variable since the variance was 0 in the model. For the analysis of vitamin E and Se, no interaction term of week was implemented in the model.

For the performance parameters, a linear mixed model was used to determine the effect of week, dietary treatment and its interactions. A random effect for pen and compartment was added to correct for the repeated measurements within each pen:

$$Y_{performance} = W \times \beta_W + DT \times \beta_{DT} + W \times DT \times \beta_{W \times DT} + Z \times \mu + \varepsilon$$

where $Y_{perfomance}$ = dependent variables (ADFI, ADG and FCR), W = week as independent variable (weeks 19–24), DT = dietary treatment as independent variable (control, enriched, E-dense), β = vector of the fixed effects, Z = design matrix of random effects (compartment and pen), μ = vector of the random effects and ε = vector of random errors. Start weight and weaning batch were excluded as an independent and random variable since the variance was 0 in the week model.

For meat and carcass quality, a linear mixed model was used to determine the effect of dietary treatment:

$$Y_{meat-carcass} = DT \times \beta_{DT} + S \times \beta_S + WCW \times \beta_{WCW} + Z \times \mu + \varepsilon$$

where $Y_{meat\text{-}carcass}$ = dependent variables (initial and ultimate pH, drip loss, cooking loss, total fluid loss, shear force, intramuscular fat, L^*, a^*, b, marbling, vitamin E, Se, warm carcass weight, fat and muscle thickness, carcass lean meat content, ham fat thickness and ham meat content), DT = dietary treatment as independent variable (control, enriched, E-dense), S = sex as independent variable (barrow and gilt), WCW = warm carcass weight as independent variable, β = vector of the fixed effects, Z = design matrix of random effects (slaughter date and pen), μ = vector of the random effects and ε = vector of random errors. Weaning batch and pen (only for carcass traits) were excluded as random variable since the variance was near 0 in the model. Warm carcass weight was excluded as independent variable in the analysis of warm carcass weight itself.

Differences were considered significant if $P \leq 0.05$. Posthoc tests according to the Kenward-Roger df approximation on heat period within the dietary group and dietary group in the overall period were performed to evaluate the effect of heat period. In addition, out of the same linear mixed model, ΔRR, ΔT_{rectal}, ΔADFI, ΔADG, ΔFCR and delta of all blood gas parameters were calculated by the differences between the average of the parameters in thermoneutral and heat weeks per dietary group. This analysis of contrasts did not assume that the responses between the two heat waves were the same, acknowledging potential effects, such as acclimation, age, weight, and experimental conditions. Afterwards, a posthoc test according to the Kenward-Roger df approximation was performed on these least square means to compare the reaction on a higher heat load between the different dietary groups. The data of non-significant posthoc tests are not given.

Results

Four pigs were removed during the trial due to sickness/lameness: three pigs from the enriched group (before the acclimatisation period) and one pig from the control group (in week 24). Data of this pig were excluded from the dataset as it was lame, did not eat and lost weight. However, this did not alter the number of pens measured per week for performance parameters. During slaughter, data of two pigs (from the control and enriched group) got lost. Data from these pigs were excluded from the dataset.

Physiological parameters

The response in RR due to heat load did not significantly differ between the dietary treatment groups (interaction term, $P = 0.728$). All dietary groups showed a significant increase in their RR for both heat waves compared to the thermoneutral weeks (Table 2). In line with the response in RR, the response in T_{rectal} also did not significantly differ between the dietary treatment groups due to heat load (interaction term, $P = 0.386$). However, according to the post-hoc test, T_{rectal} increased within the enriched and E-dense group during the second heat wave but not during the first heat wave in comparison with the preceding thermoneutral weeks. Within the control and E-dense group, T_{rectal} was significantly higher during the first heat wave (± 39.51 °C) compared to the second (± 39.33 °C). The average increase in T_{rectal} during heat load (ΔT_{rectal}) was significantly higher for E-dense compared with the control group (0.38 vs 0.28 °C, $P = 0.018$) (Fig. 2). Note that the mean baseline of T_{rectal} of the E-dense group was numerically lower during the thermoneutral weeks than the control group (39.05 vs 39.12 °C).

Blood (gas) parameters

The average change in blood pH during heat load (ΔpH) was significantly different between the enriched and E-dense groups (Fig. 3). Average blood pH decreased during the heat load in the E-dense group while it increased in the enriched group (−0.011 vs 0.034, $P = 0.035$) (Fig. 3A). Furthermore, ΔpCO_2 was lower for the E-dense than for the enriched group (−0.31 vs −1.14, $P = 0.032$) (Fig. 3B); however, both dietary treatments were not significantly different from the control group. The detailed values of the other blood gas parameters can be found in Supplementary Table S1. The enriched group had a significantly higher total vitamin E level in the serum compared with the control group (3.78 vs 3.22 mg/L, $P < 0.001$). Selenium serum levels were also higher in the enriched group than in the control group (189 vs 172 µg/L, $P = 0.004$).

Performance parameters

Response in ADFI (interaction term, $P = 0.461$), ADG ($P = 0.437$) and FCR ($P = 0.448$) did not differ significantly among dietary treatment groups during heat load (Table 3). Nevertheless, all dietary groups significantly increased their ADFI after each of the two heat waves. All dietary groups showed a significant decrease in ADG during the heat waves in comparison to the prior thermoneutral weeks. Posthoc results also indicated a lower ADG for the E-dense group in the second heat wave compared to the first one ($P = 0.021$). Moreover, FCR increased during the second heat wave in comparison to the first heat wave in the control group. Over the whole experimental period, the average FCR was significantly higher for the E-dense group compared to the control group (2.95 vs 2.67, $P = 0.006$). Overall ADG of the enriched group was numerically higher than the other dietary treatments ($P = 0.090$).

Carcass traits and meat quality

The fat thickness of the E-dense group was 0.9 mm higher than the control group ($P = 0.035$), and ham fat thickness was higher in comparison to the other dietary groups ($P = 0.018$) (Table 4). In accordance with the above results, carcass lean meat content ($P = 0.002$) and ham meat thickness ($P = 0.002$) of the E-dense group was ± 1.1% and ± 1.0 mm lower in comparison to the control and enriched groups. For meat quality, drip loss did not differ significantly, but the total fluid loss of the E-dense group was 1.1% lower than the enriched group ($P = 0.050$) mainly due to a numerically lower cooking loss ($P = 0.097$). Furthermore, IMF content of the E-dense group tended to be 0.3% higher compared to the control group ($P = 0.066$). There was no significant difference between dietary groups in pH, shear force, meat colour and marbling ($P > 0.05$). Vitamin E and Se content in the meat of the enriched group was 11% ($P < 0.001$) and 43% ($P < 0.001$) higher than the control group, respectively.

Discussion

Physiological parameters

No large differences were observed in the physiological parameters of the pigs fed the diet enriched with betaine, vitamin E and C and Se in comparison to the control group. Research that focused solely on betaine within normal concentration ranges (0.1–0.125%) and in its natural form often demonstrates positive effects on RR or T_{rectal} during short or long cyclic heat stress conditions (Attia et al., 2009; Gabler et al., 2013; Le et al., 2020; Shakeri et al., 2020). Conversely, studies that investigate the supplementation of antioxidants such as vitamin E and Se within recommended levels often fail to show significant improvements in these parameters during (long cyclic) heat loads (Liu et al., 2018a; Shakeri et al., 2019). However, provision of feed additives above recommended or maximum levels permitted by EU legislation supplementation can lead to improvements in rectal temperature and/or respiration rate during heat stress (minimum 7 days of cyclic heat periods) in various animal species (Chauhan et al., 2014; Liu et al., 2018b;

Table 2
Physiological parameters (estimated means) of fattening pigs according to the effect of dietary treatment (control, enriched and E-dense) and the week (control and heat weeks).

Parameter	Week	N	Diet			SEM	*P*-value		
			Control	Enriched	E-dense		Treatment	Week	Treatment × Week
Respiration rate [breaths/min]	19 (thermoneutral)	288	48[a]	45[a]	49[ab]	0.816			
	20 (heat 1)	288	72[b]	68[b]	70[c]	1.737			
	21 (thermoneutral)	288	45[a]	44[a]	45[ab]	0.889			
	22 (heat 2)	288	74[b]	71[b]	77[c]	1.735	0.554	<0.001	0.728
	23 (thermoneutral)	288	40[a]	40[a]	39[a]	0.939			
	24 (thermoneutral)	288	46[a]	46[a]	50[b]	1.022			
Rectal temperature [°C]	19 (thermoneutral)	283	39.47[de]	39.45[d]	39.42[de]	0.012			
	20 (heat 1)	284	39.50[e]	39.49[d]	39.52[e]	0.020			
	21 (thermoneutral)	288	39.22[c]	39.16[c]	39.12[c]	0.013	0.621	<0.001	0.386
	22 (heat 2)	288	39.32[cd]	39.35[d]	39.35[d]	0.026			
	23 (thermoneutral)	288	38.99[b]	38.93[b]	38.90[b]	0.018			
	24 (thermoneutral)	288	38.80[a]	38.80[a]	38.76[a]	0.016			

n = number of observed fattening pigs.
[a-e] Values within a column (respiration rate or rectal temperature) with different superscripts differ significantly at $P < 0.05$ for week within a dietary group.

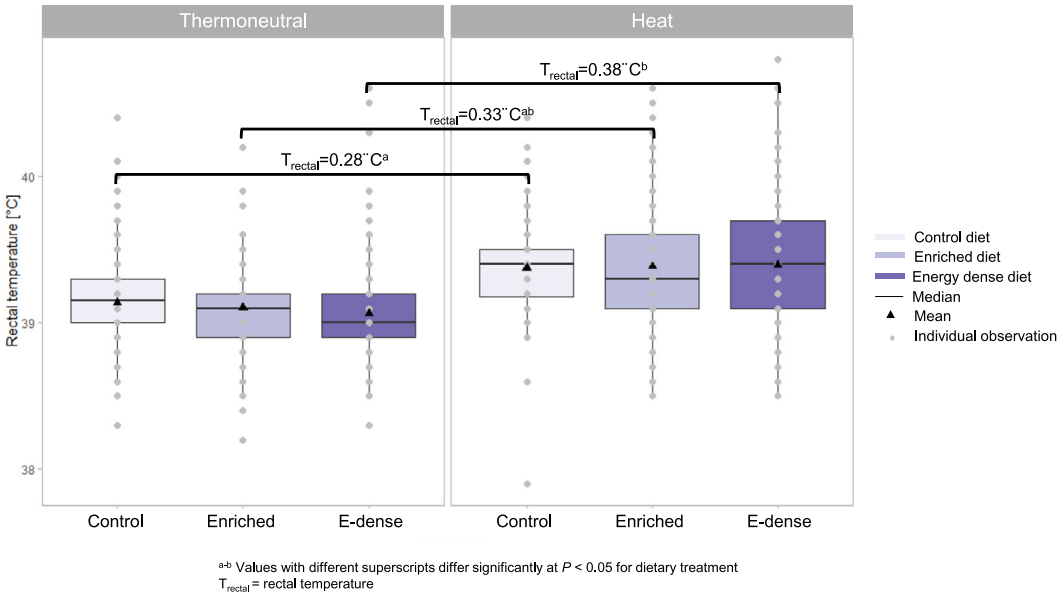

Fig. 2. Mean rectal temperature (T_{rectal}) of the three dietary treatment groups of fattening pigs (control, enriched and E-dense) during all thermoneutral weeks and the two heat waves, and differences in T_{rectal} between these means, representing ΔT_{rectal}.

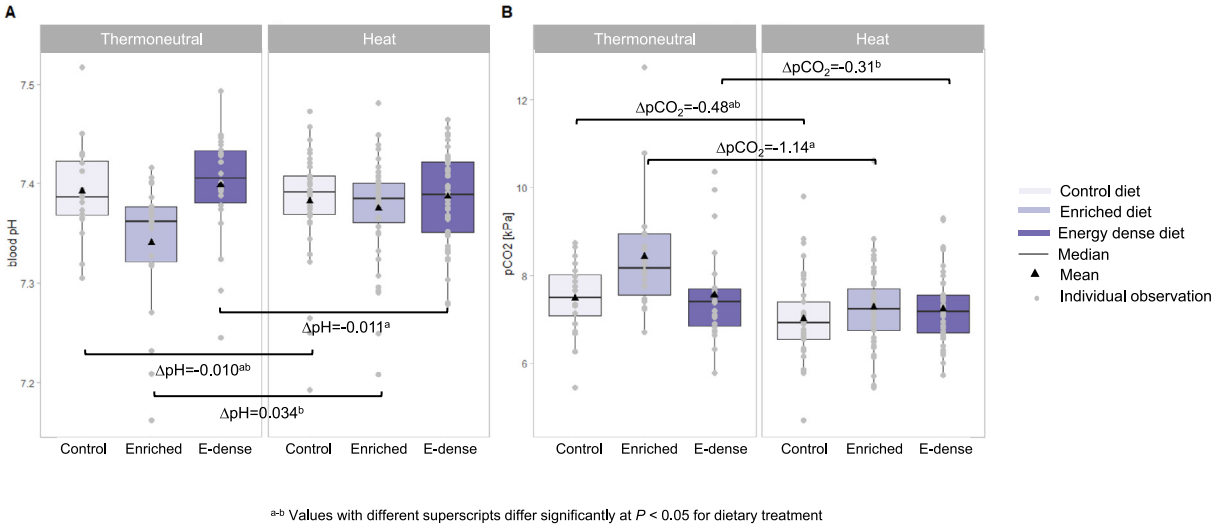

Fig. 3. Mean blood pH (A) and carbon dioxide partial pressure (pCO_2) (B) of the three dietary treatment groups of fattening pigs (control, enriched and E-dense) during the first thermoneutral week (week 19) and the two heat waves, and differences of these parameters between the means, representing ΔpH and ΔpCO_2.

Shakeri et al., 2018). In accordance with our findings, previous studies on feed additives in heat-stressed pigs that use almost the same quantity and types of additives within maximum permitted levels have shown no improvement in physiological parameters (Liu et al., 2017; De Prekel et al., 2024a). This suggests that the supplemented amount of antioxidants was insufficient and that synthetic betaine-HCL supplementation has less pronounced effects on physiological parameters compared to its natural form, as also indicated by Awad et al. (2022). To our knowledge, the combination of vitamin E, vitamin C, selenomethionine and betaine in feed as a measure to combat heat stress in growing-finishing pigs has not been frequently tested (De Prekel et al., 2024a). Knowledge is therefore lacking on possible synergistic, neutralising or antagonistic effects of feed additives given simultaneously. Despite this possibility, we did not anticipate any of these effects among the different feed additives. For example, simultaneous supplementation of vitamin E and Se does not hinder each other's positive effects, as these additives have a synergistic action (Cottrell et al., 2015). Watts (1994) did not observe any antagonistic effects between Se and betaine.

For the E-dense group, the increase in ΔT_{rectal} during the heat waves was greater in comparison to the control group. It should be noted that T_{rectal} was numerically lower for this group during thermoneutral weeks, while it was similar among groups during the heat waves. Decreasing CP levels (16 vs 12%) can contribute to reduced thermal effects (Kerr et al., 2003), due to the lower heat increment, which is consistent with our findings across all thermoneutral weeks. However, this effect was not sustained during heat waves. During long cyclic periods of high heat load (30.1–35.4 °C), a substantial reduction in CP content (21.6 vs 10.8%) can lead to improved body temperature (Morales et al., 2019). Pathak et al. (2018) also noted better physiological parameters (lower RR and

Table 3
Performance parameters (estimated means) of fattening pigs according to the effect of dietary treatment (control, enriched and E-dense) and the week (control and heat week) and the overall trial period.

Parameter	Week	n	Diet			SEM	*P*-value		
			Control	Enriched	E-dense		Treatment	Week	Treatment × Week
Start weight [kg]	19	32	81.2	78.0	81.5	0.72	0.08	NA	NA
End weight [kg]	24	32	114.0	112.0	115.0	0.91	0.34	NA	NA
Daily feed intake [g/day]	19 (thermoneutral)	32	2 627^{c}	2 504^{b}	2 667^{bc}	44			
	20 (heat 1)	32	2 115^{a}	2 134^{a}	2 183^{a}	35			
	21 (thermoneutral)	32	2 506^{bc}	2 566^{b}	2 743^{bc}	41	0.204	<0.001	0.461
	22 (heat 2)	32	2 030^{a}	2 053^{a}	2 099^{a}	39			
	23 (thermoneutral)	32	2 399^{b}	2 457^{b}	2 572^{b}	43			
	24 (thermoneutral)	32	2 634^{c}	2 603^{b}	2 807^{c}	53			
	Total	32	2 376	2 380	2 505	34	0.123	NA	NA
Daily gain [g/day]	19 (thermoneutral)	32	1 081^{d}	1 043^{c}	984^{cd}	33			
	20 (heat 1)	32	681^{ab}	735^{ab}	746^{b}	37			
	21 (thermoneutral)	32	823^{bc}	966^{bc}	965^{bcd}	28	0.504	<0.001	0.437
	22 (heat 2)	32	458^{a}	532^{a}	486^{a}	35			
	23 (thermoneutral)	32	955^{cd}	970^{bc}	1 050^{d}	31			
	24 (thermoneutral)	32	884^{bcd}	807^{b}	750^{bc}	37			
	Total	32	891	925	851	14	0.090	NA	NA
Feed conversion ratio [g/g]	19 (thermoneutral)	32	2.46^{a}	2.43^{a}	2.91^{ab}	0.11			
	20 (heat 1)	32	3.23^{a}	2.96^{ab}	3.62^{abc}	0.22			
	21 (thermoneutral)	32	3.14 ^{a}	2.72^{ab}	2.87^{ab}	0.09			
	22 (heat 2)	31	5.06^{b}	3.81^{b}	4.62^{c}	0.29	0.432	<0.001	0.448
	23 (thermoneutral)	32	2.66^{a}	2.63^{ab}	2.49^{a}	0.11			
	24 (thermoneutral)	32	3.29 ^{a}	3.35^{ab}	3.89^{bc}	0.17			
	Total	32	2.67^{x}	2.60^{x}	2.95^{y}	0.05	0.006	NA	NA

n = number of observed pens, NA = not applicable.
$^{a-d}$ Values within a column with different superscripts differ significantly at $P < 0.05$ for week within a dietary group.
$^{x-y}$ Values within a row with different superscripts differ significantly at $P < 0.05$ for dietary group in the overall trial period.

Table 4
Carcass traits and meat quality parameters (estimated means) of fattening pigs according to the effect of dietary treatment (control, enriched and E-dense).

Parameter	N	Diet			SEM	*P*-value
		Control	Enriched	E-dense		
Carcass traits						
Warm carcass weight [kg]	186	93.9	92.2	93.2	0.57	0.420
Fat thickness [mm]	186	12.2^{x}	12.4^{xy}	13.1^{y}	0.18	0.035
Muscle thickness [mm]	186	71.9	71.4	71.0	0.31	0.467
Carcass lean meat content [%]	186	64.7^{y}	64.5^{y}	63.5^{x}	0.19	0.002
Ham fat thickness [mm]	186	14.7^{x}	14.7^{x}	16.6^{y}	0.38	0.018
Ham meat content [%]	186	78.5^{y}	78.3^{y}	77.4^{x}	0.17	0.002
Meat quality						
Initial pH	96	6.46	6.45	6.38	0.02	0.400
Ultimate pH	96	5.58	5.57	5.52	0.01	0.129
Drip loss [%]	96	6.2	6.0	6.1	0.17	0.872
Cooking loss [%]	96	33.6	34.1	33.2	0.15	0.097
Total fluid loss [%]	96	44.5^{xy}	44.7^{y}	43.6^{x}	0.18	0.050
Shear force [N]	96	30.8	31.9	32.2	0.50	0.646
Intramuscular fat [%]	96	1.83	1.90	2.05	0.03	0.066
Lightness L*	96	57.2	58.2	57.9	0.21	0.302
Redness a*	96	7.6	7.1	7.5	0.09	0.121
Yellowness b*	96	16.3	16.2	16.3	0.06	0.807
Marbling[1]	96	1.2	1.1	1.3	0.04	0.298
Vitamin E [μg/g meat]	42	3.9^{x}	4.4^{y}	NA	0.07	<0.001
Selenium [μg/kg meat]	42	140^{x}	247^{y}	NA	8.61	<0.001

n = number of observed carcasses/meat loin
[1] Visual score of intramuscular fat on a seven–point marbling scale (1–7).
$^{x-y}$ Values within a row with different superscripts differ significantly at $P < 0.05$ for dietary group.

T_{rectal}) in pigs on a high-energy diet (15 mJ/kg) compared to pigs fed a low-energy diet (12.6 mJ/kg) during summer, although they tested generally higher levels of net energy than those used in our study, including the difference between the two levels. In our study, the differences in CP and net energy levels were only 1.4% (15 vs 13.6%) and 0.6 mJ/kg (9.1 vs 9.7 mJ/kg), respectively, suggesting that this alteration was insufficient to reduce physiological parameters during periods of high heat load.

Blood (gas) parameters

The control group did not show indications of respiratory alkalosis, as there was no elevation in blood pH during periods of high heat load. Although the evolution in blood pH (ΔpH) of the enriched and E-dense groups differed significantly from each other, the results for these groups did not differ significantly from the control group, suggesting the absence of respiratory alkalosis in all groups.

Additionally, blood pH levels for all groups remained within normal values during the weeks of heat stress (7.38–7.43) (Liu et al., 2016; Liu et al., 2018a; Liu et al., 2018b). An increase in blood pH and a decrease in pCO_2 were expected during the heat weeks due to elevated respiration rates. In this study, we observed higher respiratory rates, but no signs of respiratory alkalosis. The utilisation of venous blood instead of arterial blood for blood pH measurements does not affect the results (Augustinsson and Forslid, 1989). Concerning pCO_2, the use of venous blood may result in a different acid-base profile (Patience et al., 2005), potentially resulting in less pronounced differences in pCO_2 levels (Augustinsson and Forslid, 1989). This may be reflected in our study.

The E-dense group showed fewer variations during heat or thermoneutral weeks compared to the enriched group. This group had no increase in blood pH and showed the smallest decrease in ΔpCO_2. Normally, blood pH is influenced by the electrolyte balance in the diet (Kellum, 2000). As the E-dense diet had a higher calculated electrolyte balance (220 meq/kg) than the other diets (185.2 meq/kg), an increase in blood pH was expected (Haydon et al., 1990). However, no significant differences were found, probably because all dietary groups had electrolyte balances around the optimal level of 250 mEq/kg (NRC, 2012).

Performance parameters

Pigs fed the enriched diet showed slight numerical improvements in feed efficiency. Although not significant, the FCR values observed during both heat waves were lower than the other dietary treatments. Moreover, a numerically better growth throughout the entire trial period was found. These non-significant, however relevant outcomes, may be attributable to the role of Se, as it has been demonstrated to enhance ADG and feed efficiency under conditions of high heat load, particularly when derived from organic sources (Quisirumbay-Gaibor et al., 2020). In our previous study, wherein a similar combination of additives was supplemented during mild heat loads, performance parameters were numerically impaired (De Prekel et al., 2024a). This could be explained by the lower concentration of organic selenium used (0.1 mg/kg instead of 0.2 mg/kg selenomethionine). The impact of betaine on the performance of heat-stressed pigs remains unclear across various studies (Ratriyanto et al., 2009; Sales, 2011). In a study with a design similar to ours where different quantities of natural betaine were tested during a 7-week cyclic heat load with the range of 26–32 °C, no improved performances were observed in 90 kg pigs (Mendoza et al., 2017). In addition, betaine-HCL is thought to have less potential than natural betaine for enhancing ADG and FCR, as it may affect gut physiology, gut microbiota, amino acid digestibility and intestinal pH due to its HCL component (Awad et al., 2011; Awad et al., 2022). We therefore suggest that the betaine-HCL used in the present trial provided limited added value in terms of performance in pigs during periods of high heat load in comparison with organic selenium.

Low-protein diets, which reduce internal heat production during digestion, may be generally advantageous during periods of high heat stress (Dunshea et al., 2007; Patience et al., 2015). Based on this, it was expected that diets with lower protein content (Dunshea et al., 2007), a higher fat content (Spencer et al., 2005) and supplemented crystalline amino acids (Kerr et al., 2003) could mitigate the adverse effects of heat stress without compromising growth or body composition (Cottrell et al., 2015). However, pigs fed the E-dense diet had a numerically higher ADFI but grew slower, significantly less efficiently, and had significantly increased fat depositions. All these results suggest a potential issue with the diet formulation, likely due to an imbalance in the ratio of certain essential amino acids to lysine, since the digestible lysine:net energy ratio is constant. This effect might have been exacerbated by the numerically higher net energy intake in the E-dense pigs. Normally, a low-protein diet formulated with a constant digestible lysine:net energy (Lebret, 2008) and a digestible essential amino acids:lysine that respects the ideal protein should not impair performance. However, our diets were based on the ideal amino acid profile for fatting pigs (gilts and barrows) from 80 to 120 kg following CVB (2023) recommendations. Also, no major differences in the essential digestible and total amino acids:lysine ratio of the formulated and analysed diet were observed that could explain the variations in FCR and lipid deposition. Another possible explanation for the worse results of the E-dense group could be the asynchrony in the availability of energy and amino acids. Fat was mainly used as an energy source in the E-dense feed. This possibly leads to slower absorption and energy metabolisation than rapidly available carbohydrates (Wang et al., 2018). In turn, the supplementation with crystalline amino acids may be absorbed faster than amino acids bound in protein (Chung and Baker, 1992). van den Borne et al. (2007) showed that performance may decrease when the availability of amino acids is not in synchrony with the availability of energy. Hence, it is conceivable that asynchrony as described by van den Borne et al. (2007) could have occurred.

Carcass traits and meat quality

The enriched diet did not alter any carcass traits in comparison to the control group, in accordance with the results of a similar study during mild summer conditions (De Prekel et al., 2024a). Other studies have reported better carcass traits: Eklund et al. (2005) and Ratriyanto et al. (2009) reported that betaine supplementation increased lean meat content and Zhu et al. (2022) also showed that a combination of organic Se and vitamin E supplementation reduced backfat thickness during thermoneutral conditions. In terms of meat quality, the enriched group had the lowest water-holding capacity, but this was not significantly different from the control group. Water-holding capacity may be improved by supplementing with vitamin E, vitamin C and Se (Dugan et al., 2004; Lahučký et al., 2005; Zhu et al., 2022) and the same is found for ultimate pH and colour, depending on the experimental setting (Dugan et al., 2004; Lahučký et al., 2005; Ngapo and Gariépy, 2008; Zhu et al., 2022). The unchanged parameters may be attributed to the controlled use of feed additives within permitted limits or to the specific form utilised. The enriched group exhibited higher Se levels in their loins compared to the control group. This can be attributed to the greater bioavailability of the organic Se source in the enriched group as opposed to the inorganic variant in the control group (Zhan et al., 2007), or the slightly higher analysed Se content in the feed. The extra levels of Se in pig meat can be interesting for the consumer, as Se is important for the prevention of different diseases and general health (Fairweather-Tait et al., 2011; Huang et al., 2023). The recommended dose of Se for adults is between 60 and 70 μg/day (Fairweather-Tait et al., 2011; Kipp et al., 2015). However, the daily intake of Se in Belgium is ± 40 μg/day, which is typical for most European countries (Fairweather-Tait et al., 2011). In the context of this study, a 100 g serving of enriched meat can provide an additional 10 μg of Se which may have a positive impact on the consumer's health. The extra vitamin E in the loins due to the enriched dietary treatment may also improve human health as 73% of the population does not reach the desirable serum vitamin E concentration of ≥ 30 μmol/L (Péter et al., 2019).

The E-dense diet showed some significant yet less favourable changes in carcass quality. Pigs fed the E-dense diet had lower lean meat and ham meat content and higher fat and ham fat thickness in comparison to the control group. This is in accordance with performance results and the hypothesis that the availability of amino acids was not synchronised with the availability of energy (van den

Borne et al., 2007). In addition, this effect is probably enhanced by the numerically higher feed and net energy intake. The numerically higher IMF content of pigs fed E-dense vs control diet can be linked to the lower carcass lean meat content and higher back fat thickness (Burkett et al., 2009; Pietruszka et al., 2015).

Conclusions

Under the present heat load conditions, the antioxidant and osmolyte–enriched diet did not improve physiological and performance parameters, carcass and meat quality nor respiratory alkalosis compared to the control diet. The meat from pigs fed the enriched diet contained significantly more Se and vitamin E. The E-dense diet as presented in this study resulted in an increase in rectal temperature and a less favourable feed conversion ratio and carcass quality compared to pigs fed the control diet during heat load. Overall, the nutritional strategies were not able to significantly decrease or prevent important physiological heat stress parameters in fattening pigs compared to the control diet, but the supplementation of antioxidants and osmolytes numerically improved daily gain over the entire trial. Further research is warranted to explore other nutritional strategies in pigs to alleviate the negative effects of heat stress.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101514) can be found at the foot of the online page, in the Appendix section.

Ethics approval

The Ethics Committee of Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) approved all experimental procedures (number 2023/434).

Data and model availability statement

The data/models were not deposited in an official repository. The analysed datasets from the current study are available from the corresponding author upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors used ChatGPT and Grammarly for grammar and vocabulary checks during the writing process. Critical review and modifications of grammar and vocabulary were undertaken by the authors themselves. Miriam Levenson (ILVO) provided English-language editing. The authors take full responsibility for the final version of the paper. No content information from AI-assisted technologies was utilised.

Author ORCIDs

LDP: https://orcid.org/0000-0001-6796-6596.
DM: https://orcid.org/0000-0003-1149-9348.
AVDB: https://orcid.org/0000-0002-1353-8112.
MA: https://orcid.org/0000-0002-4007-7497.

CRediT authorship contribution statement

L. De Prekel: Writing – review & editing, Writing – original draft, Visualisation, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualisation. **D. Maes:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualisation. **A. Van den Broeke:** Writing – review & editing, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualisation. **M. Aluwé:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualisation.

Declaration of interest

The authors declare no personal or financial conflicts of interest.

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References

Attia, Y.A., Hassan, R.A., Qota, E.M.A., 2009. Recovery from adverse effects of heat stress on slow-growing chicks in the tropics: effect of ascorbic acid and different levels of betaine. Tropical Animal Health and Production 41, 807–818. https://doi.org/10.1007/s11250-008-9256-9.

Augustinsson, O., Forslid, A., 1989. Aldosterone secretion during acute respiratory acidosis and NH_4Cl-induced metabolic acidosis in the goat. Acta Physiologica Scandinavica 136, 339–345. https://doi.org/10.1111/j.1748-1716.1989.tb08674.x.

Awad, W.A., Hess, M., Twarużek, M., Grajewski, J., Kosicki, R., Böhm, J., Zentek, J., 2011. The impact of the fusarium mycotoxin deoxynivalenol on the health and performance of broiler chickens. International Journal of Molecular Sciences 12, 7996–8012. https://doi.org/10.3390/ijms12117996.

Awad, W.A., Ruhnau, D., Gavrău, A., Dublecz, K., Hess, M., 2022. Comparing effects of natural betaine and betaine hydrochloride on gut physiology in broiler chickens. Poultry Science 101, 102173. https://doi.org/10.1016/j.psj.2022.102173.

Boccard, R., Buchter, L., Casteels, E., Cosentino, E., Dransfield, E., Hood, D.E., Joseph, R.L., MacDougall, D.B., Rhodes, D.N., Schön, I., Tinbergen, B.J., Touraille, C., 1981. Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the commission of the European communities' (CEC) beef production research programme. Livestock Production Science 8, 385–397. https://doi.org/10.1016/0301-6226(81)90061-0.

Burg, M.B., 1995. Molecular basis of osmotic regulation. American Journal of Physiology-Renal Physiology 268, 983–996. https://doi.org/10.1152/ajprenal.1995.268.6.F983.

Burkett, J.L., Baas, T.J., Rothschild, M., Stalder, K., Prusa, K., Bailey, T., 2009. The effect of selection for intramuscular fat on fatty acid composition in Duroc pigs. PhD, Iowa State University, Ames, IA, USA.

Chauhan, S.S., Celi, P., Fahri, F.T., Celi, P., Dunshea, F.R., 2014. Dietary antioxidants at supranutritional doses modulate skeletal muscle heat shock protein and

inflammatory gene expression in sheep exposed to heat stress. Journal of Animal Science 92, 4897–4908. https://doi.org/10.2527/jas.2014-8047.
Chung, T.K., Baker, D.H., 1992. Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. Journal of Animal Science 70, 3781–3790. https://doi.org/10.2527/1992.70123781x.
Collin, A., Lebreton, Y., Fillaut, M., Vincent, A., Thomas, F., Herpin, P., 2001. Effects of exposure to high temperature and feeding level on regional blood flow and oxidative capacity of tissues in piglets. Experimental Physiology 86, 83–91. https://doi.org/10.1113/eph8602102.
Cottrell, J.J., Liu, F., Hung, A.T., DiGiacomo, K., Chauhan, S.S., Leury, B.J., Furness, J.B., Celi, P., Dunshea, F.R., 2015. Nutritional strategies to alleviate heat stress in pigs. Animal Production Science 55, 1391–1402. https://doi.org/10.1071/AN15255.
Csonka, L.N., 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiological Reviews 53, 121–147. https://doi.org/10.1128/mr.53.1.121-147.1989.
CVB, 2023. Tabellenboek Voeding Varkens 2023. Stichting CVB, Wageningen Livestock Research (WLR) & Instituut voor Landbouw-, Visserij- en Voedingsonderzoek (ILVO). Stichting CVB, Venray, The Netherlands.
De Prekel, L., Maes, D., Van den Broeke, A., Ampe, B., Aluwe, M., 2024a. Effect of simultaneous dietary supplementation of betaine, selenomethionine, and vitamins E and C under summer conditions in growing-finishing pigs. Veterinary Sciences 11, 110. https://doi.org/10.3390/vetsci11030110.
De Prekel, L., Maes, D., Van den Broeke, A., Ampe, B., Aluwé, M., 2024b. Evaluation of a heating protocol and stocking density impact on heatstressed fattening pigs. Animal 18, 101172. https://doi.org/10.1016/j.animal.2024.101172.
Dugan, M., Aalhus, J., Uttaro, B., 2004. Nutritional manipulation of pork quality: current opportunities. Advances in Pork Production 15, 237–243.
Dunshea, F.R., Ostrowska, E., Ferrari, J.M., Gill, H.S., 2007. Dairy proteins and the regulation of satiety and obesity. Australian Journal of Experimental Agriculture 47, 1051. https://doi.org/10.1071/EA06263.
Eklund, M., Bauer, E., Wamatu, J., Mosenthin, R., 2005. Potential nutritional and physiological functions of betaine in livestock. Nutrition Research Reviews 18, 31–48. https://doi.org/10.1079/nrr200493.
Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J.E., Hurst, R., 2011. Selenium in human health and disease. Antioxidants & Redox Signaling 14, 1337–1383. https://doi.org/10.1089/ars.2010.3275.
Gabler, N.K., Frouel, S., Awati, A., Owusu-Asiedu, A., Amerah, A.M., Patridge, G.G., Dunshea, F.R., 2013. Betaine mitigates intestinal permeability in growing pigs induced by heat stress. Proceedings of the Manipulating Pig Production XIV, 24-27 November 2013, Melbourne, Australia, p. 24-27.
Gan, F., Chen, X., Liao, S.F., Lv, C., Ren, F., Ye, G., Pan, C., Huang, D., Shi, J., Shi, X., Zhou, H., Huang, K., 2014. Selenium-enriched probiotics improve antioxidant status, immune function, and selenoprotein gene expression of piglets raised under high ambient temperature. Journal of Agricultural and Food Chemistry 62, 4502–4508. https://doi.org/10.1021/jf501065d.
Hanson J, S.W.F., Olley, 1963. Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Biochemical Journal 89, 101–102.
Haydon, K.D., West, J.W., McCarter, M.N., 1990. Effect of dietary electrolyte balance on performance and blood parameters of growing-finishing swine fed in high ambient temperatures. Journal of Animal Science 68, 2400. https://doi.org/10.2527/1990.6882400x.
Honikel, K.O., 1987. How to measure the water-holding capacity of meat? Recommendation of standardized methods. In: Tarrant, P.V., Eikelenboom, G., Monin, G. (Eds.), Evaluation and Control of Meat Quality in Pigs. Springer, Dordrecht, the Netherlands, pp. 129–142.
Honikel, K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Science 49, 447–457. https://doi.org/10.1016/S0309-1740(98)00034-5.
Huang, J.Q., Wang, Z.H., Sun, L.H., Wang, L.L., Yin, Y.L., 2023. Selenium in modern agriculture. Modern Agriculture 1, 34–42. https://doi.org/10.1002/moda.2.
Huynh, T.T.T., Aarnink, A.J.A., Verstegen, M.W.A., Gerrits, W.J.J., Heetkamp, M.J.W., Kemp, B., Canh, T.T., 2005. Effects of increasing temperatures on physiological changes in pigs at different relative humidities. Journal of Animal Science 83, 1385–1396. https://doi.org/10.2527/2005.8361385x.
Kellum, J.A., 2000. Determinants of blood pH in health and disease. Critical Care 4, 6–14. https://doi.org/10.1186/cc644.
Kerr, B.J., Yen, J.T., Nienaber, J.A., Easter, R.A., 2003. Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs. Journal of Animal Science 81, 1998–2007. https://doi.org/10.2527/2003.8181998x.
Kettunen, H., Peuranen, S., Tiihonen, K., 2001. Betaine aids in the osmoregulation of duodenal epithelium of broiler chicks, and affects the movement of water across the small intestinal epithelium in vitro. Comparative Biochemistry and Physiology Part a: Molecular & Integrative Physiology 129, 595–603. https://doi.org/10.1016/S1095-6433(01)00298-7.
Kipp, A.P., Strohm, D., Brigelius-Flohé, R., Schomburg, L., Bechthold, A., Leschik-Bonnet, E., Heseker, H., 2015. Revised reference values for selenium intake. Journal of Trace Elements in Medicine and Biology 32, 195–199. https://doi.org/10.1016/j.jtemb.2015.07.005.
Kowalski, E., Vossen, E., Millet, S., Ampe, B., Callens, B., Van Royen, G., De Smet, S., Aluwé, M., 2020. Performance and carcass, loin and ham quality in crossbreds from three terminal sire lines. Meat Science 167, 108–158. https://doi.org/10.1016/j.meatsci.2020.108158.
Lahučký, R., Bahelka, I., Novotná, K., Vašíčková, K., 2005. Effects of dietary vitamin E and vitamin C supplementation on the level of α-tocopherol and L-ascorbic acid in muscle and on the antioxidative status and meat quality of pigs. Czech Journal of Animal Science 50, 175–184. https://doi.org/10.17221/4012-CJAS.
Le Bellego, L., Van Milgen, J., Noblet, J., 2002. Effect of high ambient temperature on protein and lipid deposition and energy utilization in growing pigs. Animal Science 75, 85–96. https://doi.org/10.1017/s1357729800052863.
Le, H.H., Shakeri, M., Suleria, H.A.R., Zhao, W., McQuade, R.M., Phillips, D.J., Vidacs, E., Furness, J.B., Dunshea, F.R., Artuso-Ponte, V., Cottrell, J.J., 2020. Betaine and isoquinoline alkaloids protect against heat stress and colonic permeability in growing pigs. Antioxidants 9, 1–15. https://doi.org/10.3390/antiox9101024.
Lebret, B., 2008. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. Animal 2, 1548–1558. https://doi.org/10.1017/S1751731108002796.
Liu, F., Cottrell, J.J., Furness, J.B., Rivera, L.R., Kelly, F.W., Wijesiriwardana, U., Pustovit, R.V., Fothergill, L.J., Bravo, D.M., Celi, P., Leury, B.J., Gabler, N.K., Dunshea, F.R., 2016. Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. Experimental Physiology 101, 801–810. https://doi.org/10.1113/EP085746.
Liu, F., Cottrell, J.J., Collins, C.L., Henman, D.J., O'Halloran, K.S.B., Dunshea, F.R., 2017. Supplementation of selenium, vitamin E, chromium and betaine above recommended levels improves lactating performance of sows over summer. Tropical Animal Health and Production 49, 1461–1469. https://doi.org/10.1007/S11250-017-1348-Y/TABLES/5.
Liu, F., Celi, P., Chauhan, S.S., Cottrell, J.J., Leury, B.J., Dunshea, F.R., 2018a. A short-term supranutritional Vitamin E supplementation alleviated respiratory alkalosis but did not reduce oxidative stress in heat stressed pigs. Asian-Australasian Journal of Animal Sciences 31, 263–269. https://doi.org/10.5713/ajas.17.0256.
Liu, F., Celi, P., Cottrell, J.J., Chauhan, S.S., Leury, B.J., Dunshea, F.R., 2018b. Effects of a short-term supranutritional selenium supplementation on redox balance, physiology and insulin-related metabolism in heat-stressed pigs. Journal of Animal Physiology and Animal Nutrition 102, 276–285. https://doi.org/10.1111/jpn.12689.
Lv, C.H., Wang, T., Regmi, N., Chen, X., Huang, K., Liao, S.F., 2015. Effects of dietary supplementation of selenium-enriched probiotics on production performance and intestinal microbiota of weanling piglets raised under high ambient temperature. Journal of Animal Physiology and Animal Nutrition 99, 1161–1171. https://doi.org/10.1111/jpn.12326.
Mendoza, S.M., Boyd, R.D., Zier-Rush, C.E., Ferket, P.R., Haydon, K.D., van Heugten, E., 2017. Effect of natural betaine and ractopamine HCl on whole-body and carcass growth in pigs housed under high ambient temperatures. Journal of Animal Science 95, 3047–3056. https://doi.org/10.2527/jas.2017.1622.
Morales, A., Ibarra, N., Chávez, M., Gómez, T., Suárez, A., Valle, J.A., Camacho, R.L., Cervantes, M., 2018. Effect of feed intake level and dietary protein content on the body temperature of pigs housed under thermo neutral conditions. Journal of Animal Physiology and Animal Nutrition 102, 718–725. https://doi.org/10.1111/jpn.12824.
Morales, A., Valle, J.A., Castillo, G., Antoine, D., Avelar, E., Camacho, R.L., Buenabad, L., Cervantes, M., 2019. The dietary protein content slightly affects the body temperature of growing pigs exposed to heat stress. Translational Animal Science 3, 1326–1334. https://doi.org/10.1093/tas/txz143.
Musharaf, N.A., Latshaw, J.D., 1999. Heat increment as affected by protein and amino acid nutrition. World's Poultry Science Journal 55, 233–240. https://doi.org/10.1079/WPS19990017.
National Pork Producers Council, 1999. Official color and marbling standards. National Pork Producers Council, Des Moines, IA, USA.
Ngapo, T.M., Gariépy, C., 2008. Factors affecting the eating quality of pork. Critical Reviews in Food Science and Nutrition 48, 599–633. https://doi.org/10.1080/10408390701558126.
NRC, 2012. Nutrient Requirements of Swine. National Academies Press, Washington, DC, USA. https://doi.org/10.17226/13298.
NWSCR, 1976. Livestock hot weather stress. Regional operations manual letter. National Weather Service Central Region Kansas. City, MO, USA.
Pathak, P.K., Roychoudhury, R., Saharia, J., Borah, M.C., Dutta, D.J., Bhuyan, R., Kalita, D., 2018. Impact of seasonal thermal stress on physiological and blood biochemical parameters in pigs under different dietary energy levels. Tropical Animal Health and Production 50, 1025–1032. https://doi.org/10.1007/s11250-018-1526-6.
Patience, J.F., Umboh, J.F., Chaplin, R.K., Nyachoti, C.M., 2005. Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. Livestock Production Science 96, 205–214. https://doi.org/10.1016/j.livprodsci.2005.01.012.
Patience, J.F., Rossoni-Serão, M.C., Gutiérrez, N.A., 2015. A review of feed efficiency in swine: biology and application. Journal of Animal Science and Biotechnology 6, 1–9. https://doi.org/10.1186/S40104-015-0031-2/TABLES/4.
Pearce, S.C., Gabler, N.K., Ross, J.W., Escobar, J., Patience, J.F., Rhoads, R.P., Baumgard, L.H., 2013a. The effects of heat stress and plane of nutrition on metabolism in growing pigs. Journal of Animal Science 91, 2108–2118. https://doi.org/10.2527/jas.2012-5738.
Pearce, S.C., Mani, V., Boddicker, R.L., Johnson, J.S., Weber, T.E., Ross, J.W., Rhoads, R.P., Baumgard, L.H., Gabler, N.K., 2013b. Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. PLoS One1 8, 70215. https://doi.org/10.1371/journal.pone.0070215.

Péter, S., Eggersdorfer, M., Weber, P., 2019. Vitamin E intake and serum levels in the general population: a global perspective. In: Weber, P., Birringer, M., Blumberg, J.B., Eggersdorfer, M., Frank, J. (Eds.), Vitamin E in Human Health. Humana Press, Cham, Switzerland, pp. 175–188. https://doi.org/10.1007/978-3-030-05315-4_13.

Pietruszka, A., Jacyno, E., Kawęcka, M., Biel, W., 2015. The relation between intramuscular fat level in the longissimus muscle and the quality of pig carcasses and meat. Annals of Animal Science 15, 1031–1041. https://doi.org/10.1515/AOAS-2015-0046.

Quisirumbay-Gaibor, J., Patiño-Patroni, D.M., Perales, C.V., 2020. Effect of selenium supplementation on productive performance in pigs: meta-analysis. Revista De Investigaciones Veterinarias Del Peru 31, 17551. https://doi.org/10.15381/rivep.v31i1.17751.

Ratriyanto, A., Mosenthin, R., Bauer, E., Eklund, M., 2009. Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. Asian-Australasian Journal of Animal Sciences 22, 1461–1476. https://doi.org/10.5713/ajas.2009.80659.

Renaudeau, D., Gourdine, J.L., St-Pierre, N.R., 2011. Meta-analysis of the effects of high ambient temperature on growth performance of growing-finishing pigs. Journal of Animal Science 89, 2220–2230. https://doi.org/10.2527/jas.2010-3329.

Sales, J., 2011. A meta-analysis of the effects of dietary betaine supplementation on finishing performance and carcass characteristics of pigs. Animal Feed Science and Technology 165, 68–78. https://doi.org/10.1016/J.ANIFEEDSCI.2011.02.008.

Schauberger, G., Mikovits, C., Zollitsch, W., Hörtenhuber, S.J., Baumgartner, J., Niebuhr, K., Piringer, M., Knauder, W., Anders, I., Andre, K., Hennig-Pauka, I., Schönhart, M., 2019. Global warming impact on confined livestock in buildings: efficacy of adaptation measures to reduce heat stress for growing-fattening pigs. Climatic Change 156, 567–587. https://doi.org/10.1007/S10584-019-02525-3/FIGURES/6.

Shakeri, M., Cottrell, J.J., Wilkinson, S., Ringuet, M., Furness, J.B., Dunshea, F.R., 2018. Betaine and antioxidants improve growth performance, breast muscle development and ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. Animals 8, 162. https://doi.org/10.3390/ani8100162.

Shakeri, M., Cottrell, J.J., Wilkinson, S., Le, H.H., Suleria, H.A.R., Warner, R.D., Dunshea, F.R., 2019. Growth performance and characterization of meat quality of broiler chickens supplemented with betaine and antioxidants under cyclic heat stress. Antioxidants 8, 336. https://doi.org/10.3390/antiox8090336.

Shakeri, M., Cottrell, J.J., Wilkinson, S., Le, H.H., Suleria, H.A.R., Warner, R.D., Dunshea, F.R., 2020. Dietary betaine reduces the negative effects of cyclic heat exposure on growth performance, blood gas status and meat quality in broiler chickens. Agriculture 10, 176. https://doi.org/10.3390/agriculture10050176.

Siljander-Rasi, H., Peuranen, S., Tiihonen, K., Virtanen, E., Kettunen, H., Alaviuhkola, T., Simmins, P.H., 2003. Effect of equi-molar dietary betaine and choline addition on performance, carcass quality and physiological parameters of pigs. Animal Science 76, 55–62. https://doi.org/10.1017/S1357729800053315.

Spencer, J.D., Gaines, A.M., Berg, E.P., Allee, G.L., 2005. Diet modifications to improve finishing pig growth performance and pork quality attributes during periods of heat stress. Journal of Animal Science 83, 243–254. https://doi.org/10.2527/2005.831243x.

Stewart, K.R., Cabezon, F.A., Boyd, R.D., 2015. Betaine for boars and sows during heat stress. Book of abstracts of the 15th Annual Midwest Swine Nutrition Conference Proceedings, 10 September 2015, Indianapolis, Indiana, USA, p. 45-48.

St-Pierre, N.R., Cobanov, B., Schnitkey, G., 2003. Economic losses from heat stress by US livestock industries. Journal of Dairy Science 86, E52–E77. https://doi.org/10.3168/jds.S0022-0302(03)74040-5.

Tang, J., Cao, L., Jia, G., Liu, G., Chen, X., Tian, G., Cai, J., Shang, H., Zhao, H., 2019. The protective effect of selenium from heat stress-induced porcine small intestinal epithelial cell line (IPEC-J2) injury is associated with regulation expression of selenoproteins. British Journal of Nutrition 122, 1081–1090. https://doi.org/10.1017/S0007114519001910.

van den Borne, J.J.G.C., Schrama, J.W., Heetkamp, M.J.W., Verstegen, M.W.A., Gerrits, W.J.J., 2007. Synchronising the availability of amino acids and glucose increases protein retention in pigs. Animal 1, 666–674. https://doi.org/10.1017/S1751731107736741.

Wang, Y., Zhou, J., Wang, G., Cai, S., Zeng, X., Qiao, S., 2018. Advances in low-protein diets for swine. Journal of Animal Science and Biotechnology 9, 60. https://doi.org/10.1186/s40104-018-0276-7.

Watts, D.L., 1994. The nutritional relationships of selenium. Journal of Orthomolecular Medicine 9, 111–117.

Zhan, X., Wang, M., Zhao, R., Li, W., Xu, Z., 2007. Effects of different selenium source on selenium distribution, loin quality and antioxidant status in finishing pigs. Animal Feed Science and Technology 132, 202–211. https://doi.org/10.1016/j.anifeedsci.2006.03.020.

Zhu, C., Yang, J., Nie, X., Wu, Q., Wang, L., Jiang, Z., 2022. Influences of dietary vitamin E, selenium-enriched yeast, and soy isoflavone supplementation on growth performance, antioxidant capacity, carcass traits, meat quality and gut microbiota in finishing pigs. Antioxidants 11, 1510. https://doi.org/10.3390/ANTIOX11081510.