

Evaluation of a heating protocol and stocking density impact on heatstressed fattening pigs

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ABSTRACT

As climate change intensifies, heat stress mitigation for pigs becomes more important. Trials involving induced heat waves are useful to test several measures (e.g. reduced stocking density) at a faster rate, but only when accurately evaluated and validated. In the present study, we investigated the suitability of an artificial heating protocol at different pig weights (experiment 1). The impact of different stocking densities on fattening pigs during an artificial heat wave (experiment 2) was also investigated. Experiment 1: Forty 20-week-old pigs weighing 96.5 ± 7.3 kg (W_{100}) and forty 17-week-old pigs weighing 72.7 ± 9.9 kg (W_{70}) were housed in two compartments. An artificial heat wave (heat load) was induced for 3 days. During 3-day periods before, during and after the heat load, physiological parameters (respiration rate (RR), rectal temperature (T_{rectal}), skin temperature (T_{skin}) and behavior) were measured and average daily feed intake was observed. Ambient temperature, relative humidity and temperature-humidity index (THI) were monitored. Experiment 2: A total of 150 fattening pigs were randomly divided into three treatment groups: $SD_{1.3}$ (1.3 m²/pig), $SD_{1.0}$ (1.0 m²/pig) and $SD_{0.8}$ (0.8 m²/pig). All pens had a total pen surface of 4.88 m², corresponding with 4, 5 and 6 fattening pigs in the $SD_{1.3}$, $SD_{1.0}$ and $SD_{0.8}$ groups, respectively. The heat load was induced for 7 days on week 21. Respiration rate and T_{rectal} were observed as in experiment 1. Average daily gain and average daily feed intake were also noted. During the heat load, THI reached ≥ 75 (78.4 (experiment 1) and 78.6 (experiment 2)), even when relative humidity decreased to $\pm 45\%$. Every physiological parameter showed significant increases during the heat load. The prolonged heating protocol in experiment 2 also provoked significant decreases in average daily feed intake (15%) and average daily gain (19%) for all groups. Weight within the studied range of 70–100 kg did not have a significant impact on any of the parameters. However, T_{skin} was affected by both weight and heat load ($P < 0.05$), where T_{skin} from W_{100} was always lower in comparison to W_{70} . In addition, we found that 0.8 m²/pig doubled the increase of T_{rectal} during the heat load, namely $SD_{0.8}$ (0.22 °C) compared to $SD_{1.0}$ (0.12 °C) ($P = 0.033$) and $SD_{1.3}$ (0.13 °C) ($P = 0.053$). This suggests that pigs housed at higher densities are less able to regulate their internal heat production. However, RR and performances were not significantly affected by heat load in this experimental set-up. A stocking density of 1.0 m²/animal may be sufficient to mitigate some negative effects of heat stress.

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Implications

This research shows the value of reducing stocking density for mitigating the negative effects of a high heat load in fattening pigs. Management strategies such as reducing stocking density can be applied in the short term at a relatively low cost compared to housing and climate adaptations. Increasing space for pigs has positive effects during thermoneutral conditions; thus, it might also have an impact on physiological and performance parameters during

heatwave periods. The results of the study following a successfully validated artificial heating protocol show some mild advantages (smaller increase in rectal temperature) of lower stocking density during a heatwave.

Introduction

Pigs are more sensitive to higher ambient temperatures than most other farm animals due to their poor ability to sweat and pant (Mount, 1979b). Pigs have only a few functional sweat glands, and their subcutaneous adipose tissue layer further inhibits heat loss (Collier and Gebremedhin, 2015). The effects of climate change

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are already being felt and are expected to increase (KMI, 2020). These weather extremes will exert major pressure on animal health and welfare. Measures to effectively mitigate heat stress are therefore urgently needed. Performing induced heat wave trials is a useful way to test various measures in a controlled and efficient manner. However, it is crucial that the artificial heat load closely resembles the actual climate conditions experienced during natural heat waves. This ensures the reliability and relevance of a study.

Accurate evaluation and validation of artificial heat waves are crucial for research. Some studies have used artificial heating protocols without testing physiological reactions related to animal welfare such as respiration rate (RR) or rectal temperature (T_{rectal}) in animals subjected to high temperatures (Collin et al., 2001; Cui et al., 2019; Guo et al., 2020). Further, an artificial heating protocol should also consider the age of the animals, as older and thus heavier pigs are more sensitive to heat stress: the upper critical temperature drops according to age or BW (Quiniou et al., 2000).

Various measures are known to reduce heat stress in pigs, such as environmental modifications (Mayorga et al., 2019; Schaubberger et al., 2020), dietary changes (Cottrell et al., 2015), improved drinking water characteristics (Jeon and Kim, 2014) and the use of different genetic lines (Silió, 2000; Gaughan and Cawdell-Smith, 2015; Rauw et al., 2017; Cross et al., 2018; Mayorga et al., 2019). Another commonly proposed, simple and low-cost method is to lower the stocking density, which increases the space available for pigs. The minimum floor surface for housing fattening pigs between 85 and 110 kg is 0.65 m²/animal in Belgium. From 110 kg live weight and up, a floor surface of at least 1 m²/animal is mandatory (Federale overheidsdienst volksgezondheid, 2003). Many pig farmers keep pigs in a space of 0.65–0.85 m²/pig, without adjusting the allowed space according to live weight during the fattening period. However, most of them split-market pigs at slaughter weight, which means that the surface area remains the same but the floor area per pig increases as the heavier pigs per pen are removed for slaughter. Numerous studies have shown that reducing stocking density can have positive effects on pig performance and welfare under normal climate conditions (Dewulf et al., 2007). Concerns regarding heat stress in pig farming are increasing. An investigation of the effect of stocking density on heat stress in pigs may therefore offer valuable insights. More space per pig means that the animals have less direct contact with their pen mates, can choose to move away from their pen mates and have better access to the cooler floor. All of these can increase heat radiation and encourage convective heat losses (Mount, 1979a; Huynh, 2005; Dewulf et al., 2007). Kerr et al. (2005) and White et al. (2008) have already demonstrated the positive effects of extra space on productivity during periods of high heat load. In addition, model calculations by Schaubberger et al. (2019) have shown that reducing the stocking density by 20–40% could decrease the negative effects of heat stress on pig growth by 5–9%, respectively. However, it is important to examine the reduction of stocking density not only in terms of performance parameters, as high productivity does not necessarily equate to high animal welfare in all situations (Fraser, 2008; Jensen et al., 2012). To the best of our knowledge, no studies have been published on the effect of additional space on physiological parameters (which can be partly linked to the animal's well-being) during higher heat loads. One study reported that heavier pigs with low space allowance and increased ambient temperatures had elevated cortisol levels, indicating a stress reaction (Hilmann et al., 2004). This may be associated with a certain level of heat stress as acute heat exposure stimulates the hypothalamic–pituitary–adrenal axis and associated cortisol release (Campos et al., 2017).

In the present study, we investigated the suitability and validation of an artificial heating protocol on pigs of different weights.

We further assessed the impact of different stocking densities on fattening pigs during periods with a high heat load induced by an artificial heat wave. The possible impact of a lower stocking density on heat stress was determined by evaluating physiological and performance parameters of the animals. Preliminary results have already been published in abstract form at the European Federation of Animal Science 2023 conference (De Prekel et al., 2023).

Material and methods

The Ethics Committee of Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) approved all experimental procedures (number 2021/393 and 2022/405).

Experiment 1: evaluation and validation of an artificial heating protocol for two weight groups

Experimental setup and animals

The trial was conducted with 80 barrows (Topigs TN70 × Belgian Piétrain) in two compartments of the Pig Campus, the experimental pig housing from Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Ghent University (UGent) and University College Ghent (HOGENT) in Melle, Belgium. Each compartment was occupied by a different weight group and consisted of four pens with ten pigs each. An artificial heat wave (heat load) was induced for 3 days. During 3 days before (preheat), during (heat) and after the heat load (postheat 1), the physiological parameters of the animals were observed. Fattening pigs of two consecutive weaning batches (3-week interval) were exposed to the heat load at the same time, thus enabling assessment of the effect of pig weight. At the start of the trial, the first weight group (W_{100}) consisted of forty 20-week-old fattening pigs of 96.5 ± 7.3 kg (at the end of the finisher phase but still in need of efficient growth) while the second weight group (W_{70}) consisted of forty 17-week-old fattening pigs weighing 72.7 ± 9.9 kg (at the beginning of the finisher phase) (Fig. 1). Three animals were removed from W_{70} (one from pen 2 and two from pen 4) during the trial due to tail biting. The pen ($n = 4$) was considered as the experimental unit.

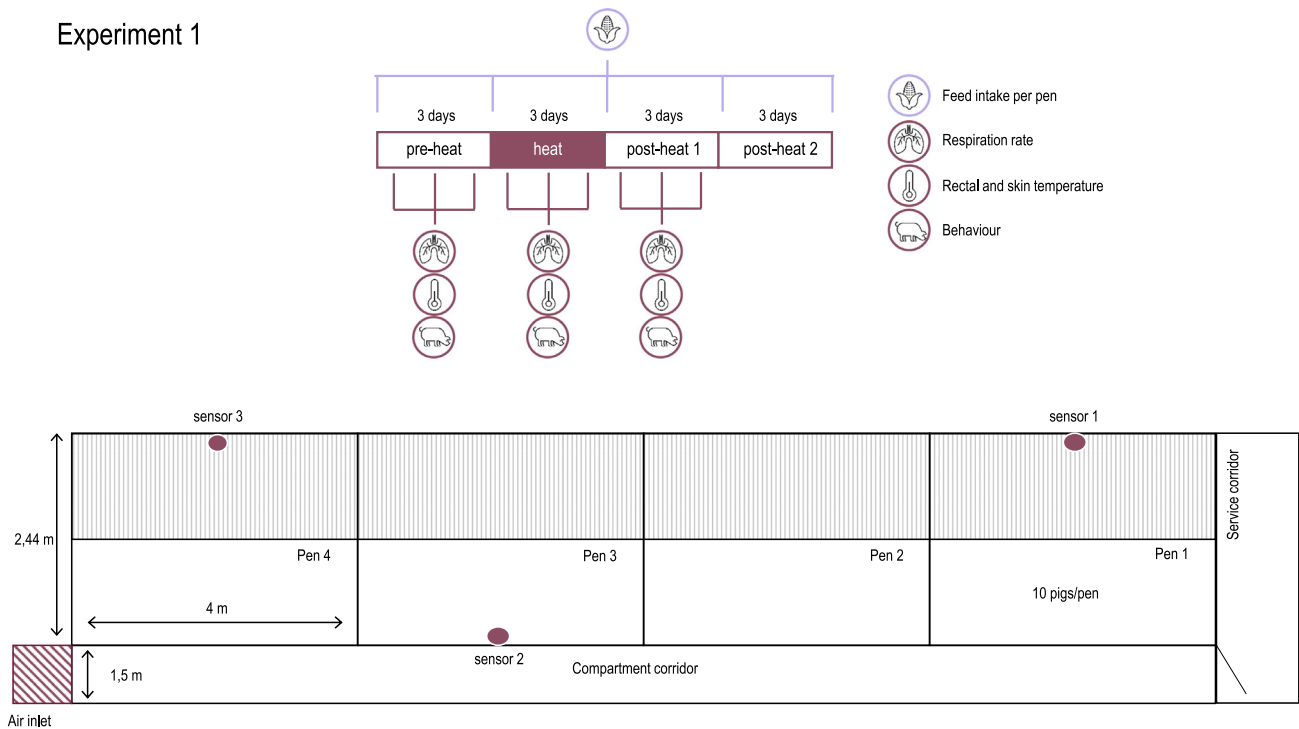
Housing and management

All pens had a partially slatted floor with a total pen surface of 9.76 m². The pigs were fed a standard two-phase pig diet with a phase change when the average compartment weight reached 65 kg. Feeders were located in the right and left front corners of each pen with two drinking nipples at the center back of the pen. Feed and water were provided *ad libitum* to all groups. The compartment was artificially lit from 07:30 to 15:30 h with additional natural light emanating from one window (2.07 m²) on the north side of compartment W_{70} and on the south side of compartment W_{100} .

Climate control

The compartment was mechanically ventilated by channel ventilation, meaning that the incoming air entered the compartment via the slats of the corridor. In thermoneutral conditions (before and after the artificial heat wave), the indoor climate was automatically controlled by a climate computer (Hotraco Agri©, Hotraco Group, Hegelsom, The Netherlands). During the artificial heat wave, the ventilation rate was fixed between 30 and 40% to achieve a compromise between good air quality and the preservation of heat in the compartment. The heating devices (Thermobile ITA-45 Robust™, Thermobile, Breda, The Netherlands) used to create the artificial heat wave had a heating power of 45.1 kW and an air displacement of 3 000 m³ via a heating duct (6 m, Ø 0.4 m).

Experiment 1



Experiment 2

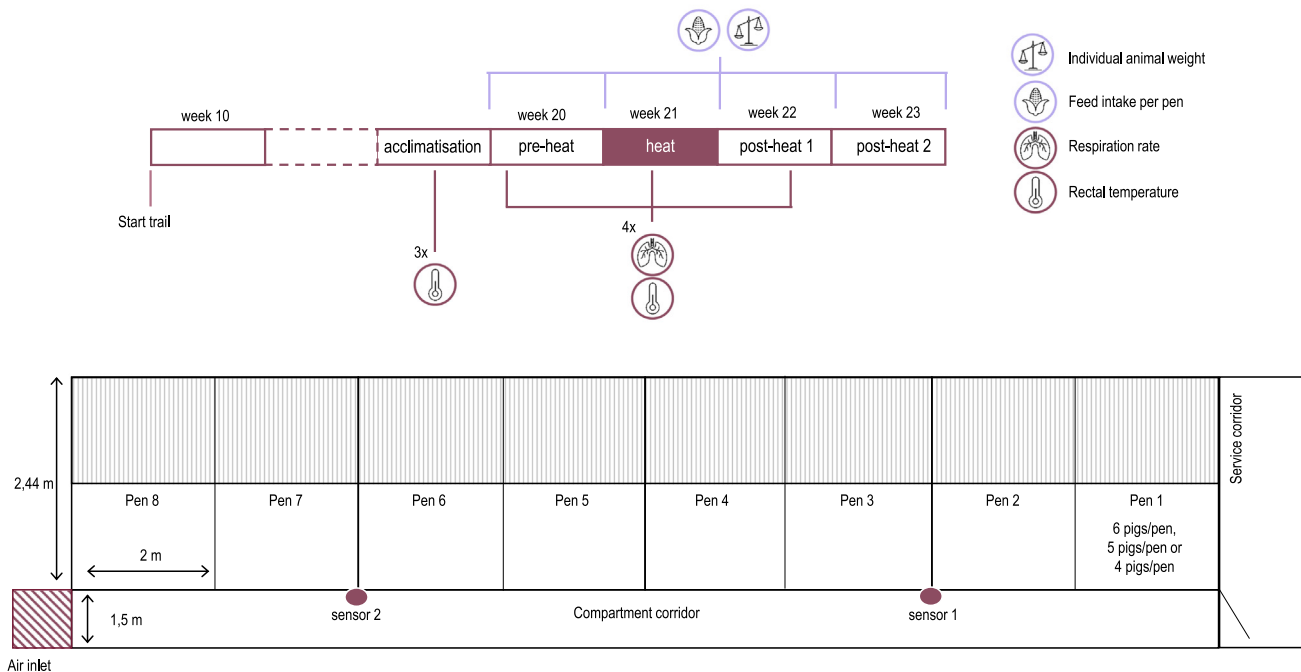


Fig. 1. Schematic overview of the experimental design (timeline and follow-up of the different parameters of the fattening pigs) and the compartments of experiments 1 and 2.

Throughout the artificial heat wave, the temperature remained consistent at around 31 °C day and night. Two climate sensors (FarmConnect™, Stienen, Nederweert, The Netherlands) were placed in the center back of the first and last pen, and one sensor was placed in the corridor at the level of the third pen in every

compartment. These sensors logged the relative humidity, ambient temperature, CO₂ and NH₃ concentration every 10 min for the entire trial period. CO₂ and NH₃ values were validated before starting with a portable sensor (Dräger X-am 700, Drägerwerk AG & Co. KGaA, Lübeck, Germany). The data from the climate sensors were

used to calculate the Temperature-Humidity Index (THI). This value combines ambient temperature and relative humidity according to the following formula (1):

$$THI = 0.72 * T_{DB} + 0.72 * T_{WB} + 40.6 \tag{1}$$

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

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 based on the behavior of pigs during handling and transport, i.e. 75 ≥ THI > 79: warning for heat stress, 79 ≥ THI > 84: danger for heat stress, THI ≥ 84: great danger for heat stress (National Weather Service Central Region, 1976).

Physiological parameters and behavior

Four reference animals per pen were randomly selected to monitor the physiological parameters individually throughout the study: RR, T_{rectal} and skin temperature (T_{skin}). The same reference animals were evaluated throughout the trial. The parameters were measured daily before (preheat), during (heat) and after (postheat) the artificial heat wave. The observations started at 12:00 h (until 17:00 h) and were performed by the same observers. Respiration rate (breaths/min) was scored visually based on the number of flank movements per 30 s multiplied by two. Respiration rate was only evaluated when a pig was lying in a resting position. Rectal temperature (°C) was measured using a digital thermometer (Veterinär-thermometer SC12, Scala electronics GmbH, Stahnsdorf, Germany) for approximately 15 s. Observations of pigs with fever due to infected tails or wounds were excluded from the dataset. Skin temperature (°C) was measured using an IR camera (Testo 875-i2™, Testo, Almere, The Netherlands). The emission coefficient was set to ε = 0.98, which is the value for biological tissues (Brown-Brandl et al., 2013; Petry et al., 2017). The camera was placed on a tripod and set at a height just above the pen fence. The camera was focused on the entire flank of the pig. The images were analyzed using Testo software (Testo, Almere, The Netherlands) which calculates the average (T_{skin,A}), the coldest (T_{skin,C}) and hottest (T_{skin,H}) point of the T_{skin} from a manually indicated oval area of the pig's flank as described by Brown-Brandl et al. (2013). Behavior was observed by scan sampling modified by Ekkel et al. (2003). Observations started with the first pen where each animal was categorized according to the type of activity (Table 1). The behavioral assessment then continued in the other pens. After evaluating all pens, the observer waited 3 min before starting with the first

pen again. In total, the behavior was evaluated ten times per pen per observation day. Afterward, the average of all the behavior results was calculated and converted into a percentage.

Performance parameters

Feed intake per pen was measured every 3 days before, during and after the heat load, as well as for an extra 3-day period after the postheat period (postheat 2). At the beginning of each period, the feeders were manually filled and weighed. Every extra feed addition within the same period was also monitored. At the end of each period (every 3 days), residual feed was weighed. Based on this data, average daily feed intake per animal was calculated.

Statistical analysis

The statistical analysis was conducted using R® software version 4.1.1. To ensure the normality of the distribution of the physiological (RR, T_{rectal}, T_{skin}), behavior (passive, active, and eating/drinking) and performance parameters (average daily feed intake), QQ-plots and histograms of the residuals were visually evaluated. No relevant deviations from normality were observed. The effect of the weight group (W₇₀ and W₁₀₀), heat load (preheat, heat and postheat 1 (and postheat2 for average daily feed intake)) and the interaction of heat load × weight group on the dependent variables were determined using linear mixed models:

$$Y = HL \times \beta_{HL} + WG \times \beta_{WG} + HL \times WG \times \beta_{HL \times WG} + Z \times \mu + \varepsilon$$

where Y = dependent variables (RR, T_{rectal}, T_{skin}, passive behavior, active behavior, eating/drinking behavior and average daily feed intake), HL = heat load as independent variable (preheat, heat, postheat 1 and postheat 2), WG = weight group as independent variable (W₇₀ and W₁₀₀), β = vector of the fixed effects, Z = design matrix of random effects (for the physiological parameters: observation date and the pig-ID; for behavior parameters: observation date and pen; for performance parameters: pen within the compartment and date), μ = vector of the random effects and ε = vector of random errors. Differences were considered significant if P ≤ 0.05. If the interaction term was significant for a specific variable, it indicated that the weight groups reacted differently to the heat load. Posthoc tests according to the Kenward-Roger df approximation on heat load within the weight group and weight group within heat load were performed when significant P-values were found.

Experiment 2: effect of stocking density during a period of high heat load

Experimental setup and animals

A total of 150 mixed-sex fattening pigs (Topigs TN70 × Belgian Piétrain) were randomly divided into three treatment groups: SD_{1.3} (stocking density of 1.3 m²/pig, n = 12 pens), SD_{1.0} (stocking density of 1.0 m²/pig, n = 11 pens) and SD_{0.8} (stocking density of

Table 1
Definitions of active and inactive behavior of fattening pigs.

Animal behavior ¹	Description
Active	
Standing	Body supported by three or more legs and with head raised
Moving	Walking or running, body supported by three or more legs, position change possible and head held high.
Exploring	Sniffing the floor and feeder, interacting with materials or pen mates
Sitting	One or two front legs support the body, with hindquarters touching the ground.
Feeding/drinking	Head to drinking nipple or head to feeder
Inactive	
Sternal lying	The body is not supported by any of the legs. Instead, the pig lies on its sternum with its head high or down.
(Semi-)lateral lying	The pig lies half on its torso and half on its belly or entirely on its side with all four legs extended.

¹ Revised fromEkkel et al. (2003).

0.8 m²/pig, n = 6 pens). Sex was balanced within the pen. The trial started at 10 weeks of age (23.6 ± 2.6 kg) and ended at slaughter age (114.2 ± 13.2 kg). An artificial heat wave (heat load) was induced for 7 days when the animals were 21 weeks old (88.1 ± 12.2 kg). In the week before (preheat period), during and after the artificial heat wave (postheat 1), the physiological parameters of the animals were observed. (Fig. 1). As ten pigs were removed from the trial due to illness or lameness before the preheat period, some SD_{0.8} pens (six pigs per pen) were changed into SD_{1.0} or SD_{1.3} pens (5 and 4 pigs per pen, respectively). The actual densities at the time of observation were used, resulting in uneven pen replicates compared to the original set-up. Pens from which pigs were removed during the observation period (between 20 and 23 weeks old) were excluded from the dataset due to changes in stocking density mid-observation. The pen (n = 12, 11 or 6 for SD_{1.3}, SD_{1.0} and SD_{0.8}, respectively) was considered as the experimental unit.

Housing and management

The trial was performed in four compartments of the Pig Campus (Melle, Belgium) in three batches which took place at different periods. Each compartment was comprised of eight pens with a random distribution of the three treatment groups. The pigs were fed a standard two-phase pig diet with a phase-change when the average compartment weight reached 65 kg. Feed and water were provided *ad libitum* to all 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 had a partially slatted floor with a total pen surface of 4.88 m². The compartment was artificially lit from 07:30 to 15:30 h plus natural light from one window (2.07 m²) on the south side of compartments 1, 3 and 4 and on the north side of compartment 2.

Climate control

The same heating protocol as described in experiment 1 was used for experiment 2, except for the used climate sensors: two sensors (HOBO MX2301A, Onset®, Bourne (MA), USA), which were calibrated before start, 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 every 10 min for the entire trial period. The data from the climate sensors were also used to calculate the THI as describes in experiment 1. Also, the periods before, during and after the heat load lasted 7 days instead of 3 days.

Physiological parameters

Four reference animals per pen were randomly selected to monitor the physiological parameters RR and T_{rectal} individually as described in experiment 1. The parameters were measured daily for 4 days during preheat, heat, and postheat 1. Before the start of the preheat period, T_{rectal} was measured for 3 consecutive days. This allowed the animals to become accustomed to the presence of the observers in the pen, which reduced the risk of a stress-induced increase in T_{rectal} during the first observations. Animal behavior and T_{skin} were not evaluated in this experiment.

Performance parameters

Individual animal weight and feed intake per pen were measured weekly for the preheat, heat, and postheat 1 period, as well as for an extra period during the 2nd week after the heat load (postheat 2 period). Based on the data, average daily feed intake, average daily gain and feed conversion ratio were calculated.

Statistical analysis

Statistics were calculated in R® software version 4.1.1. QQ-plots and histograms of the physiological (RR, T_{rectal}) and performance (average daily feed intake, average daily gain and feed conversion

ratio) parameters were evaluated to check the normality of the residuals of the models. No deviations from normality were observed. The effect of stocking density (SD_{1.3}, SD_{1.0} and SD_{0.8}), heat load (preheat, heat and postheat 1 (and postheat 2 for the performance parameters)) and the interaction of heat load × stocking density on the dependent variables was determined using linear mixed model:

$$Y = HL \times \beta_{HL} + SD \times \beta_{SD} + HL \times SD \times \beta_{HL \times SD} + Z \times \mu + \varepsilon$$

where Y = dependent variables (RR, T_{rectal}, average daily feed intake, average daily gain and feed conversion ratio), HL = heat load as independent variable (preheat, heat, postheat 1 and postheat 2), SD = stocking density as independent variable (SD_{1.3}, SD_{1.0} and SD_{0.8}), β = vector of the fixed effects, Z = design matrix of random effects (for the physiological parameters: observation date and pig-ID within pen and compartment; performance parameters: pen within the compartment and date), μ = vector of the random effects and ε = vector of random errors. Differences were considered significant if $P \leq 0.05$. If the interaction term was significant for a specific variable, animals housed in different stocking densities reacted differently to the heat load. Posthoc tests according to the Kenward-Roger df approximation on heat load within the stocking density group and stocking density group within heat load were performed when significant P-values were found. In addition, out of the same linear mixed model ΔRR, ΔT_{rectal}, Δaverage daily feed intake, Δaverage daily gain and Δfeed conversion ratio were calculated by the differences between the average of the parameters on control days (pre- and postheat load) and days with a high heat load for every stocking density. Afterward, 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 stocking density groups. The data of non-significant posthoc test are not given.

Results

Experiment 1: evaluation and validation of an artificial heating protocol

Climate control

During the artificial heat wave, the average temperature was 30.3 °C (min. 30.0 °C – max. 30.6 °C) and the average relative humidity was 46.7% (min. 41.4% – max. 51.3%) in both weight groups. A slight difference in the temperature and relative humidity values was noted among the three sensors (Fig. 2). The average relative humidity dropped below 50% during the heat load, while it was 58.7% (min. 50.9% – max. 63.3%) in the pre- and postheat period. The THI increased from an average of 71.5 ± 0.2 to 78.4 ± 0.1 during the heat period in both compartments of W₇₀ and W₁₀₀ (Fig. 3). The THI during the heat period of W₁₀₀ (78.8) reached slightly higher values than in W₇₀ (78.0). Despite the change in ventilation during the heat load, the CO₂ levels during the heat period remained below 3 000 ppm (Fig. 4). A single peak in CO₂ occurred at the beginning of the artificial heat wave but dropped relatively quickly. The NH₃ concentration of both compartments during the entire trial period exceeded 25 ppm (Fig. 4). However, the NH₃ levels did not show a notable increase during the artificial heat wave compared to the periods before and after the heat load when the mechanical ventilation operated under normal working conditions (no restricted ventilation rates).

Physiological parameters and behavior

For RR, the weight groups did not react differently to the heat load (interaction term, $P = 0.514$) (Table 2). Independent of weight, RR increased by ± 41 breaths/min during the heat load ($P < 0.001$).

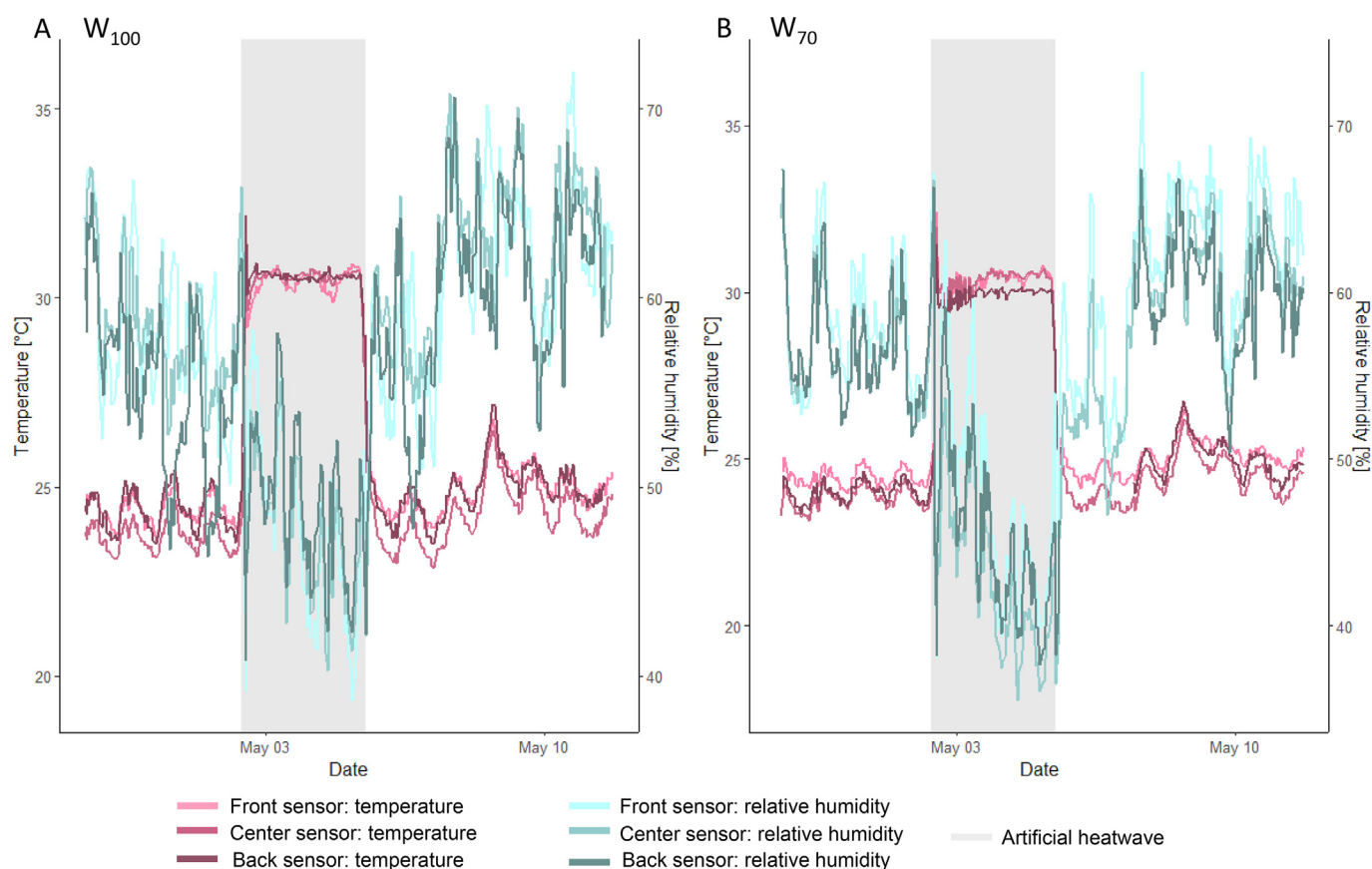


Fig. 2. Ambient temperature and relative humidity of the front, center and back sensors with a ten-minute interval during the trial in the compartment of A) W₁₀₀ (forty 20-week-old fattening pigs of 96.5 ± 7.3 kg) and B) W₇₀ (forty 17-week-old fattening pigs weighing 72.7 ± 9.9 kg) (experiment 1).

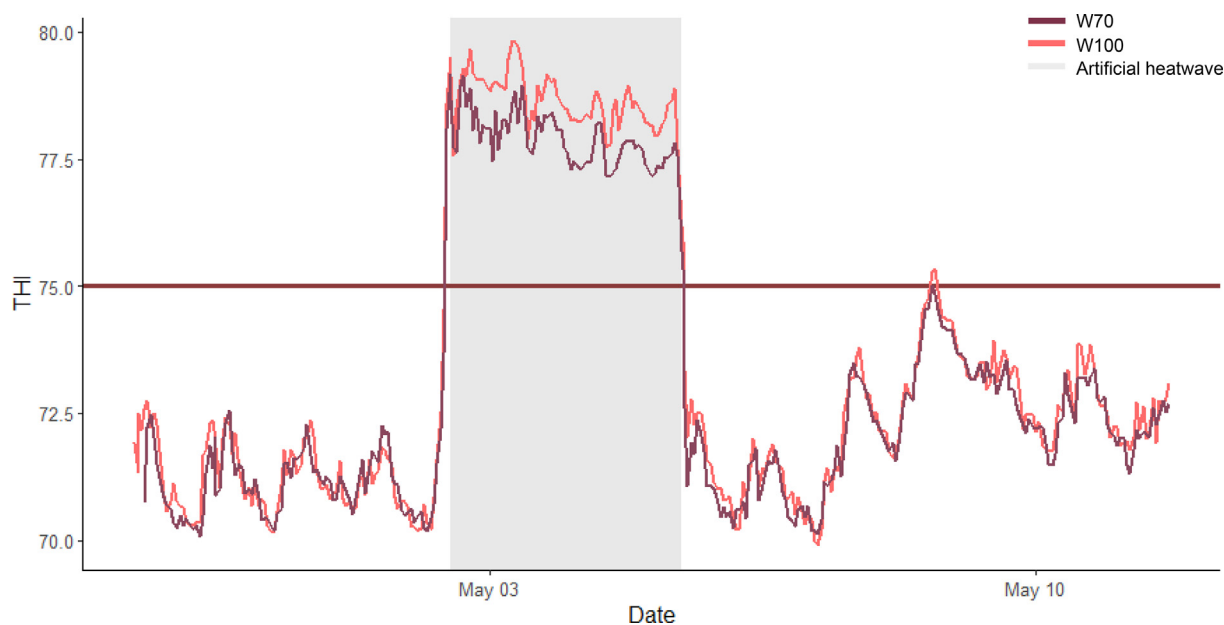


Fig. 3. Average Temperature Humidity Index (THI) of the front, center and back sensors with a ten-minute interval during the trial in the compartment of W₁₀₀ (forty 20-week-old fattening pigs of 96.5 ± 7.3 kg) and W₇₀ (forty 17-week-old fattening pigs weighing 72.7 ± 9.9 kg) (experiment 1).

For T_{rectal} , no differences between W₇₀ and W₁₀₀ were observed during the heat load (interaction term, $P = 0.403$). In both weight groups, T_{rectal} increased during the heat load by ± 0.35 °C and ± 0.25 °C for W₁₀₀ and W₇₀, respectively ($P = 0.013$), but did not differ significantly from each other. The different weight

groups had different T_{skin} during the heat load (interaction terms, $T_{\text{skin,A}} P < 0.001$, $T_{\text{skin,C}} P = 0.004$ and $T_{\text{skin,H}} P = 0.035$). All T_{skin} measurements were higher during a period of high heat load compared to the periods with low heat load and T_{skin} from W₁₀₀ was always lower compared to W₇₀, except for $T_{\text{skin,C}}$ during the heat load. Pig

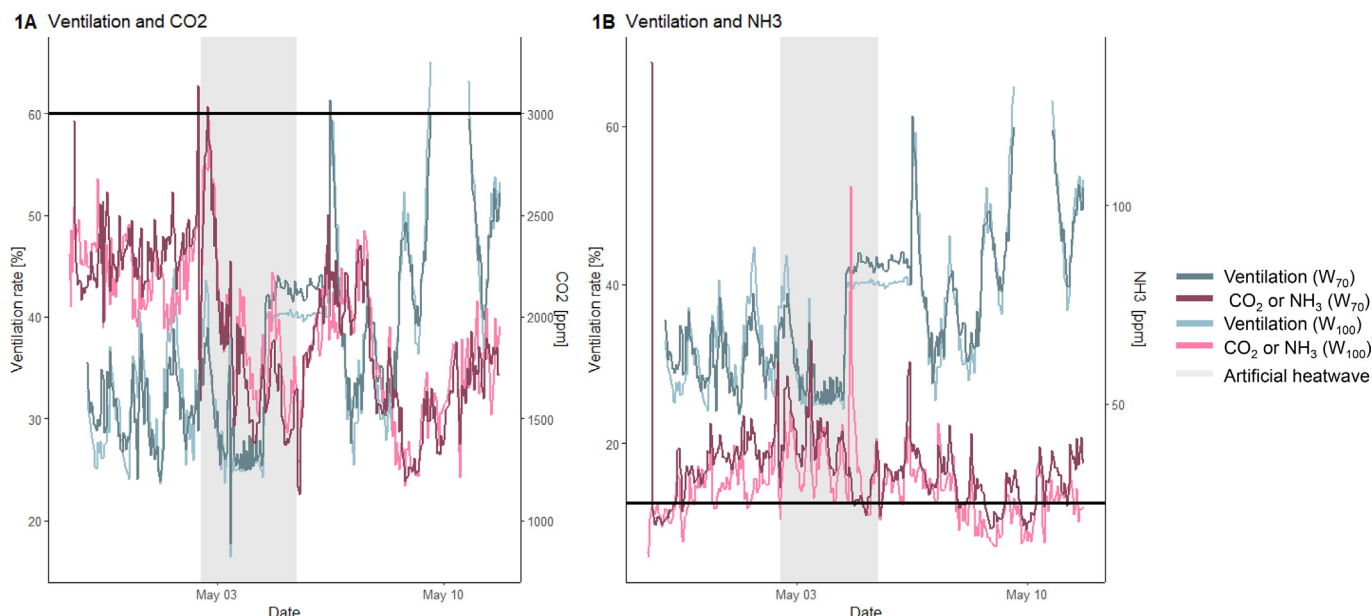


Fig. 4. Ventilation rate [%], average CO₂ [ppm] (A) and average NH₃ [ppm] (B) with a ten-minute interval during the entire trial in the compartment of W₁₀₀ (forty 20-week-old fattening pigs of 96.5 ± 7.3 kg) and W₇₀ (forty 17-week-old fattening pigs weighing 72.7 ± 9.9 kg) (experiment 1).

Table 2

The physiological parameters (respiration rate (breaths/min), rectal temperature (°C) and skin temperature (°C) of the mean, the coldest and the hottest point of the indicated flank area of the pig), behavior (passive (%), active (%) and feeding/drinking behavior (%)) and the feed intake (kg/day per animal) of fattening pigs (estimated means) according to the effect of weight group (W₇₀ and W₁₀₀) and heat load (preheat, heat or postheat period) (experiment 1, n = 4).

Parameter	Weight group								SEM	P-value		
	W ₇₀				W ₁₀₀					Weight group	Heat load	Weight × heat load
	Preheat	Heat	Postheat 1	Postheat 2	Preheat	Heat	Postheat 1	Postheat 2				
Physiological parameters												
Respiration rate [bpm]	36 ^a	74 ^b	35 ^a	NA	37 ^a	79 ^b	33 ^a	NA	1.67	0.908	<0.001	0.514
Rectal temperature [°C]	38.9 ^a	39.2 ^b	38.9 ^a	NA	39.0 ^a	39.3 ^b	38.9 ^a	NA	0.03	0.520	0.013	0.403
Skin temperature: average [°C]	32.7 ^{a,x}	35.1 ^{b,x}	32.4 ^{a,x}	NA	31.7 ^{a,y}	34.4 ^{b,y}	30.8 ^{a,y}	NA	0.11	<0.001	<0.001	<0.001
Skin temperature: cold point [°C]	29.7 ^{a,x}	32.8 ^{b,x}	29.7 ^{a,x}	NA	28.5 ^{a,y}	32.0 ^{b,x}	27.6 ^{a,y}	NA	0.14	0.003	<0.001	0.004
Skin temperature: hot point [°C]	34.2 ^{a,x}	36.3 ^{b,x}	34.0 ^{a,x}	NA	33.5 ^{a,y}	35.7 ^{b,y}	32.9 ^{a,y}	NA	0.09	0.001	<0.001	0.035
Behavior												
Passive [%]	75%	82%	75%	NA	70%	83%	75%	NA	1.31	0.234	0.625	0.511
Active [%]	14% ^x	10%	18%	NA	23% ^y	10%	18%	NA	1.08	0.016	0.452	0.082
Feeding/drinking [%]	9%	8%	7%	NA	8%	7%	7%	NA	0.40	0.348	0.776	0.924
Feed intake												
Average daily feed intake [kg/day per animal]	2.53	2.26	2.45	2.70	2.79	2.24	2.59	2.85	0.05	0.159	0.933	0.296

W₇₀ = Weight group of ± 70 kg; W₁₀₀ = Weight group of ± 100 kg, NA = not applicable.

^{x-y} Values within a row with different superscripts differ significantly at $P < 0.05$ for weight group within heat load period.

^{a-b} Values within a row with different superscripts differ significantly at $P < 0.05$ for heat load within a weight group.

behavior differed only slightly: W₇₀ tended to be less active (8%) before the heat load compared to W₁₀₀, while these differences disappeared during and after the heat load (interaction term, $P = 0.082$). For passive and eating/drinking behavior, neither weight groups reacted differently to the heat load ($P = 0.511$ and $P = 0.924$, respectively) and even regardless of weight group, passive or eating/drinking behavior did not significantly alter during the heat load.

Performance parameters

For average daily feed intake, neither weight groups showed changes in the evolution of feed intake during the heat load (interaction term, $P = 0.296$). For both weight groups, no reduction

of feed intake was observed during the heat load ($P = 0.933$) (Table 2).

Experiment 2: effect of stocking density during a period with high heat load

Climate control

In compartments 1, 2, 3 and 4, the average maximum THI per day during the heat load was 77.4, 80.8, 78.5 and 77.8, respectively. Temperature and relative humidity of the highest THI per day during the observation time (14:00–17:00 h) are given for every compartment, which took place during different periods (Fig. 5). All compartments had an average THI above 75. The THI of compartment 2 even surpassed a THI of 79 with a THI of 80.8.

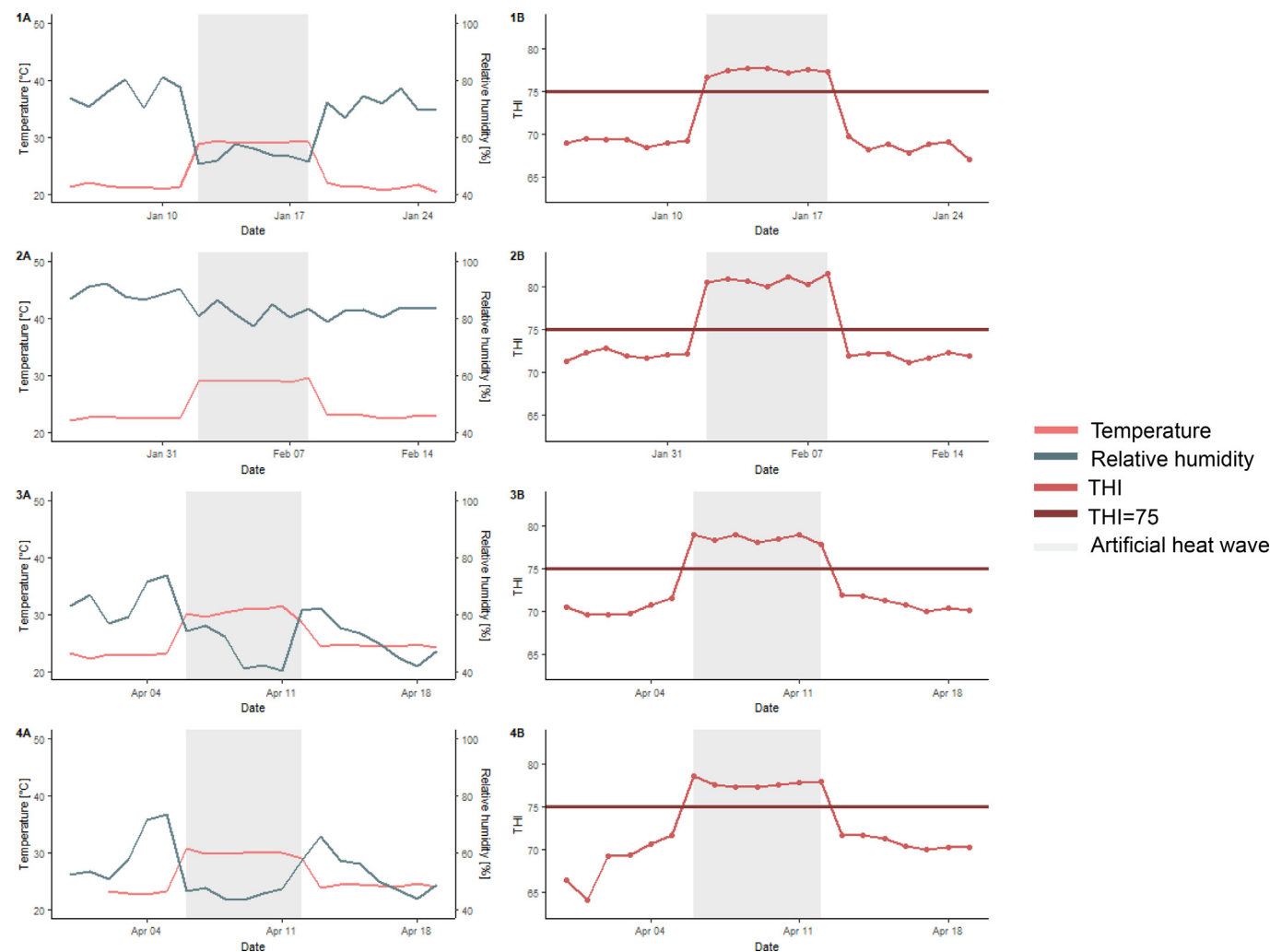


Fig. 5. Ambient temperature and relative humidity (A) of the highest Temperature Humidity Index (THI) (B) during the observation time from 14:00–17:00 h in every compartment of the housed fattening pigs (1, 2, 3 and 4) (experiment 2).

Table 3
Respiration rate (breaths/min) and rectal temperature (°C) of fattening pigs (estimated means), according to the effect of stocking density (SD_{1.3}, SD_{1.0}, SD_{0.8}) and heat load (preheat, heat or postheat period) (experiment 2).

Parameter	Heat load	Stocking density			SEM	P-value		
		SD _{1.3} (n = 12)	SD _{1.0} (n = 11)	SD _{0.8} (n = 6)		Heat load	Stocking density	Stocking density × heat load
Respiration rate [breaths/min]								
	Preheat	48 ^a	50 ^a	50 ^a	0.649	0.001	0.544	0.532
	Heat	69 ^b	70 ^b	68 ^b	1.022			
	Postheat	50 ^a	53 ^{ab}	51 ^{ab}	0.670			
Rectal temperature [°C]								
	Preheat	39.3 ^{ab}	39.3	39.3 ^a	0.012	0.004	0.521	0.126
	Heat	39.4 ^b	39.4	39.5 ^b	0.016			
	Postheat	39.3 ^a	39.3	39.3 ^a	0.011			

SD_{1.3} = stocking density at 1.3 m²/pig; SD_{1.0} = stocking density at 1.0 m²/pig; SD_{0.8} = stocking density at 0.8 m²/pig.
^{a-b}Values within a column (respiration rate and rectal temperature) with different superscripts differ significantly at *P* < 0.05 for heat load within a stocking density.

Physiological parameters

For RR, the stocking density groups did not react differently to the heat load (interaction term, *P* = 0.532) (Table 3), but independent of the different stocking densities, RR increased by ± 18 breaths/min during the heat load in comparison to the preheat period (*P* = 0.001). For *T*_{rectal}, the stocking density groups showed no differences in their reaction to the heat load (interaction term,

P = 0.126). Comparisons of Δ*T*_{rectal}, however, revealed notable differences for the three stocking density groups during the heat load: SD_{0.8} had a higher Δ*T*_{rectal} than SD_{1.0} (*P* = 0.033) and tended to be higher than SD_{1.3} (*P* = 0.053) (Fig. 6). This means that *T*_{rectal} in SD_{0.8} increased by 0.22 °C between the control period and the heat load period, while the increase was limited to 0.13 °C and 0.12 °C for SD_{1.3} and SD_{1.0}, respectively.

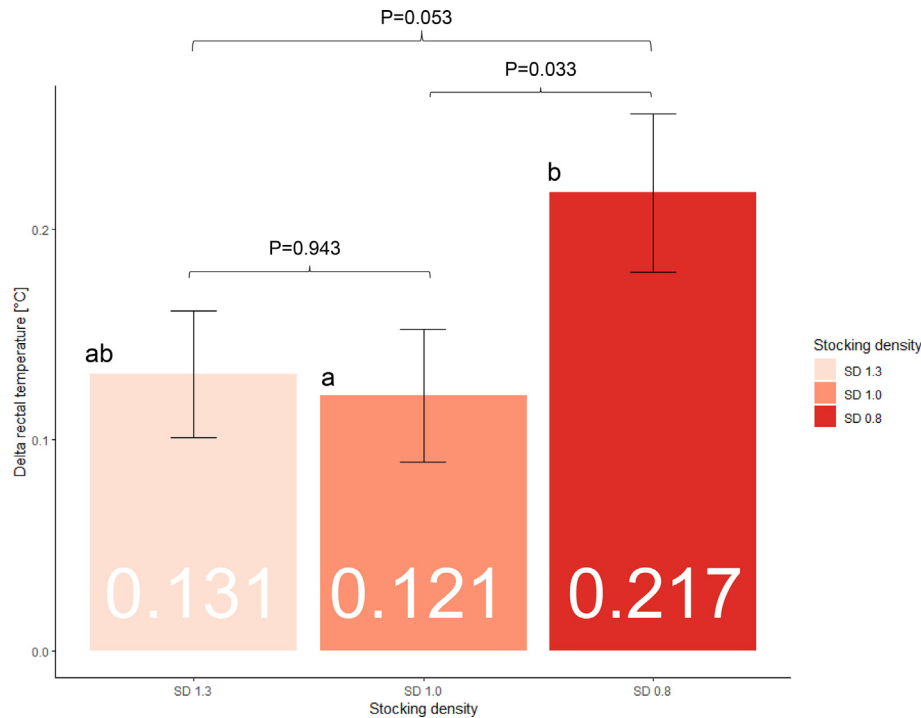


Fig. 6. Effect of stocking density (SD_{1.3} = stocking density at 1.3 m²/pig; SD_{1.0} = stocking density 1.0 m²/pig; SD_{0.8} = stocking density at 0.8 m²/pig) on ΔT_{rectal} (delta rectal temperature) of fattening pigs between control (pre- and postheat period) and the heat load period (experiment 2).

Table 4

Start and end weight (kg), average daily feed intake (g/day), average daily gain (g/day) and feed conversion ratio (g/g) of fattening pigs (estimated means), according to the effect of stocking density (SD_{1.3}, SD_{1.0}, SD_{0.8}) and heat load (preheat, heat or postheat period) (experiment 2).

Parameter	Heat load	Stocking density			SEM	P-value		
		SD _{1.3} (n = 12)	SD _{1.0} (n = 11)	SD _{0.8} (n = 6)		Heat load	Stocking density	Stocking density × heat load
Start weight [kg]		74.1	72.9	73.0	0.84	NA	0.580	NA
End weight [kg]		108	104	105	1.17			
Average daily feed intake [g/day]								
	Preheat	2 697 ^b	2 592 ^b	2 647 ^b	57	<0.001	0.653	0.258
	Heat	2 321 ^a	2 259 ^a	2 208 ^a	40			
	Postheat 1	2 637 ^b	2 654 ^b	2 713 ^b	61			
	Postheat 2	3 017 ^c	2 784 ^b	3 002 ^b	69			
Average daily gain [g/day]								
	Preheat	1 019 ^b	937	973 ^{ab}	26	0.001	0.428	0.758
	Heat	820 ^a	784	764 ^a	26			
	Postheat 1	1 024 ^b	950	1 016 ^b	24			
	Postheat 2	1 041 ^b	912	866 ^{ab}	37			
Feed conversion ratio [g/g]								
	Preheat	2.67	2.77	2.73 ^a	0.04	0.236	0.904	0.209
	Heat	2.95	2.94	2.94 ^a	0.11			
	Postheat 1	2.59	2.83	2.71 ^a	0.08			
	Postheat 2	2.93 ^x	3.14 ^x	3.81 ^{by}	0.15			

SD_{1.3} = stocking density at 1.3 m²/pig; SD_{1.0} = stocking density at 1.0 m²/pig; SD_{0.8} = stocking density at 0.8 m²/pig.

^{a-c} Values within a column (average daily feed intake, average daily gain and feed conversion ratio) with different superscripts differ significantly at $P < 0.05$ for heat load within a stocking density group.

^{x-y} Values within a row (average daily feed intake, average daily gain and feed conversion ratio) with different superscripts differ significantly at $P < 0.05$ for stocking density within a heat load period.

Performance parameters

For average daily feed intake, none of the stocking density groups showed a different reaction to the heat load when compared to each other (interaction term, $P = 0.258$) (Table 4), but the heat load did influence the feed intake within the stocking density groups ($P < 0.001$). The average daily feed intake decreased significantly during the heat load, after which all groups recovered by increasing their average daily feed intake. Notably, the SD_{1.3} group

showed significant increases in average daily feed intake after the heat load and continued to increase it significantly by ± 350 g/day, measured 2 weeks after the heat period (postheat 2).

For average daily gain, the stocking density groups all reacted similarly to the heat load (interaction term, $P = 0.758$), with the heat load causing a lower average daily gain ($P = 0.001$). However, average daily gain of SD_{1.3} was numerically always higher than SD_{1.0} and SD_{0.8}. In addition, when examined across all 4 weeks of

the trial, average daily gain of SD_{1.3} tended to be higher (977 g/day) than average daily gain of SD_{1.0} (893 g/day) and SD_{0.8} (905 g/day) ($P = 0.073$) (data not given in the table). For the feed conversion ratio, no significant effects were found.

Discussion

Evaluation and validation of the artificial heating protocol

Evaluation using climate parameters

According to the climate data, the protocol for conducting an artificial heat wave was successfully generated and could be replicated effectively, even under varying environmental conditions.

In experiment 1, a 3-day artificial heat wave caused an increase in THI above the cut-off value of 75 (78.4), which corresponds to a warning of risks associated with heat stress (NWSCR, 1976). The variation in ambient temperature during the heat wave was small in both compartments (30.0–30.6 °C). During the heat load, a decrease in relative humidity was seen, which is expected when heating with hot air. This occurs because higher indoor temperatures allow the air to hold more water vapor while the absolute humidity remains constant (Elovitz, 1999). Despite the lower relative humidity values, the THI remained stable between 75 and 79 during the heat wave. In experiment 2, the same heating protocol was used, but with a longer (7-day) heat wave to be able to better observe possible effects on performance parameters. Again, an average THI > 75 was achieved for all compartments. For both experiments, the main factor of variance in THI was related to relative humidity. For experiment 1, slightly higher THI values observed in W₁₀₀ may have been due to higher relative humidity values in that specific compartment. The outside relative humidity of both weight groups (both compartments) was the same as they were exposed to heat on the same date. Therefore, it is recommended to test different management strategies within a compartment with sufficient compartment replicates. For experiment 2, one compartment had a higher THI (80.8 in compartment 2) compared to the others (± 77.9), despite the application of the same heating device and thermostat setting. This difference can be explained by the high outside relative humidity during the artificial heating period in compartment 2 (which took place during a different period than compartments 1, 3 and 4). Outside relative humidity was between ± 75 –100% due to rainy and misty conditions, which affected the interior relative humidity and heat load. We can thus conclude that with the protocol used, we succeed in generating a controlled heat load with a THI of at least 75, but when setting up experiments, obviously the impact of varying outdoor relative humidity and possible differences between compartments should be taken into account.

Safety of the indoor climate was tested based on CO₂ and NH₃ values. CO₂ values remained within acceptable limits (Van Gansbeke et al., 2010; Klimaatplatform, 2021). The heating devices were placed outside the stable. This allowed the waste gases generated by combustion to be released into the outside air, and only warm air was directed into the compartment. NH₃ values were higher than the cut-off value of 20–25 ppm (Van Gansbeke et al., 2010). Similar concentrations were measured before, during and after the heat load, indicating that heating according to our protocol did not have a major impact on NH₃ levels, while normally a higher temperature accelerates the ammonia production (Van Gansbeke and Van den Bogaert, 2020). This may be due to the hot air movement generated by the heating device, which causes the air to rise rapidly to the upper air layers (KMI, 2023), and not mix with the air present in the compartment. Alternatively, the unaltered ammonia levels can be due to the lowered ventilation rate during the heat load, as the combination of a higher ventila-

tion rate at higher ambient temperature can increase ammonia emissions (Van Gansbeke and Van den Bogaert, 2020). Regardless, it is important to note that high NH₃ levels can negatively affect the health of animals and humans. For humans, 25 ppm NH₃ is the maximum permissible concentration in the workplace. For pigs, this concentration causes higher susceptibility to respiratory diseases. NH₃ concentrations above 50 ppm reduce the pig's general health status and zootechnical performance (Van Overbeke et al., 2010). When high NH₃ levels are measured, measures should be taken to mitigate pen fouling or limit ammonia emissions (Klimaatplatform, 2021).

Validation through assessment of changes in physiological, behavior and performance parameters

The artificial heat wave protocol can also be confirmed to induce a heat stress response in the animals, as evident by the significant changes in RR, T_{rectal} and also in feed intake when applying a 7-day heat load.

All physiological parameters, regardless of weight and stocking density, increased during the heat load. Respiration rate, which is the first physiological adaption to heat stress (Huynh et al., 2005b), doubled in experiment 1. Rectal temperature, the most relevant parameter for determining the level of homeothermy during heat stress (Renaudeau et al., 2010), also significantly increased by approximately 0.15 °C (experiment 2)–0.25 °C (experiment 1) during the heat load. At lower relative humidity values such as those measured during the heat load, the inflection point of RR and T_{rectal} will typically occur at a higher temperature (Huynh et al., 2005b). Even under the trial conditions of low relative humidity and a moderately elevated temperature of ± 30 °C, RR and T_{rectal} showed significant increases. This allowed us to conclude that the heat load effectively induced heat stress as measured by these two parameters. Skin temperature was included in the validation study as a non-invasive indicator for body temperature. Given that the utilization of IR technology for assessing body temperature and health status is still in its early stages of development, further research is essential to validate its efficacy and reliability (Zhang et al., 2019; da Fonseca et al., 2020). During the heat load, T_{skin} did increase, but T_{skin} may not be the most reliable physiological parameter. It was found to be correlated with the surrounding temperature ($r = 0.48$, $P < 0.001$), in contrast to T_{rectal} ($r = 0.12$, $P = 0.004$) (De Prekel et al., 2024) and reflects the balance between the heat generated by the animal and the energy dissipated. This has led to concerns about its usefulness (Mayorga et al., 2019). Further, the measurement of T_{skin} using an IR camera can be influenced by various factors, such as how the camera is handled, the software used to process the images, the animal's recent behavior and position, the chosen emission coefficient and the degree of soiling on the skin (Petry et al., 2017). For these reasons and due to the time-consuming nature of these measurements, T_{skin} was not included in the second experiment. In the first experiment, the occurrence of passive (lying) behavior increased to a non-significant degree in both weight groups by $\pm 10\%$ during the heat load, mainly at the expense of active behaviors. Other studies also showed a significant increase in lying behavior (3–5%) when fattening pigs were exposed to high ambient temperatures (Hilman et al., 2004; Huynh et al., 2005a). As the effect of heat stress on these main behavioral classes was relatively limited when using the applied scan sampling method, it was excluded from the second experiment.

An important adaptation during a period of heat stress is reduced feed intake, which effectively lowers the animal's internal heat production (Kemp and Versteegen, 1987; Quiniou et al., 2000; Collin et al., 2001; Huynh et al., 2005b). In the first experiment, a numerical but non-significant decrease in feed intake (10% for W₇₀ and 17% for W₁₀₀) was observed. Collin et al. (2001) did find

a significant reduction of 30% in the average daily feed intake of young pigs (± 21 kg) during a relatively short (4 days) incremental rise in temperature (from 25 to 33 °C) at a relative humidity of 60% compared to a thermoneutral group (from 25 to 23 °C). This conforms to a THI of 83.6, which is much higher than the THI of 78.4 in our trial. Other studies found also significant decreases in average daily feed intake when pigs were exposed to a longer heat load period (Nienaber et al., 1996; Quiniou et al., 2000; Huynh et al., 2005b). In experiment 2, when the heat load was extended from 3 to 7 days, we did observe a significant reduction in average daily feed intake during the heat load (15%). This validated our prolonged heating protocol for the measurement of average daily feed intake.

The effect of pig weight during artificial heat load

In order to determine the effect of pig weight on the effects of heat stress during artificial heating conditions, two weight groups (70 and 100 kg) were studied. The results showed no significant differences in physiological and performance measures, except for skin temperature and active behavior. As noted above, skin temperature can be considered as a less reliable parameter. Results indicate that heat stress trials can be conducted conveniently with both weight groups, as no relevant differences were found.

During the heat load, the weight groups showed no differences in RR and T_{rectal} . Various studies have suggested that heavier pigs are more susceptible to the effects of heat stress (Quiniou et al., 2000; Li et al., 2018). The current study therefore suggests that this weight effect is not, or is at least less relevant within the weight range of 70–100 kg in terms of these physiological parameters. Further, the T_{skin} of the 100 kg fattening pigs was almost always lower than the pigs weighing ± 70 kg. This is likely due to the fact that older and heavier pigs have a more extensive layer of subcutaneous lipid tissue, which functions as insulation (Schmidt et al., 2013) and impedes sensible heat loss (Mayorga et al., 2019). Heavier pigs have also a lower volume ratio, which limits the heat flow from the core of the body to the skin and the surrounding environment (Soerensen and Pedersen, 2015). This would mean that heavier fattening pigs cannot lose radiative heat through their skin as easily as their lighter counterparts, making heavier pigs more susceptible to the effects of heat stress. Alternatively, a lower T_{skin} can indicate a lower internal heat production that leads to lower blood flow to the skin to increase heat loss (Simmons et al., 2011; Brown-Brandl et al., 2013). However, the latter hypothesis is unlikely, as the T_{rectal} of W_{100} numerically increased more during the heat load than W_{70} , indicating that W_{100} had a higher core temperature.

During the heat load, W_{100} showed a relatively stronger decrease in feed intake compared to W_{70} , although not significant ($P > 0.05$). We expected to see a significant difference in average daily feed intake between the different weight groups, as feed intake is influenced by age, BW (Kanis and Koops, 1990; Quiniou et al., 2001) and increasing ambient temperature (Close, 1989; Massabie et al., 1991). Important to note is that feed intake was only measured over a period of 3 days, which may be too brief to show significant results since we indeed found significant differences when applying a 7-day heat load.

The effect of stocking density on pigs during high heat load

Reducing the number of animals per m^2 did not decrease the impact on RR or significantly improve average daily feed intake, average daily gain or feed conversion ratio during higher heat loads. However, ΔT_{rectal} increased more in the highest stocking density, suggesting that the effects of heat stress may be reduced to a slight degree by lowering the stocking density. Further, pigs at lower stocking density showed a better recovery in average daily feed intake and average daily gain after the heat load.

Pigs housed at a stocking density of 0.8 m^2/animal showed nearly twice the increase in ΔT_{rectal} compared to those housed at 1.0 m^2/animal or more space allowance. This may be explained by the radiant heat emitted from pen mates, as the heat loss of pigs depends on the emissivity of radiating surfaces (Fialho et al., 2004). Further, pigs stocked at a higher density have less access to the floor to achieve sensible heat loss via conduction, resulting in a reduced ability to maintain their internal metabolic temperature. Pigs housed at 1.0 m^2/animal or more space allowance already had a lower ΔT_{rectal} compared to those housed at 0.8 m^2/animal , suggesting that stocking density of ≥ 1.0 m^2 per pig may have already allowed the pigs to cope with the effects of heat stress created within the settings of this trial.

Lowering the stocking density for fattening pigs did not significantly affect the average daily feed intake during the heat load. In contrast to our study, White et al. (2008) found a significant reduction in the average daily feed intake of pigs housed under 0.66 m^2/pig compared to 0.93 m^2/pig during the heat load. Another study found that pigs housed individually at 2.0 m^2/pig had a higher feed intake in the week after the heat load than those housed at 1.0 m^2/pig (Kerr et al., 2005). This suggests that regardless of the presence of pen mates, additional available floor space can minimize the decrease in average daily feed intake during the heat load by compensation for feed intake after the heat load. We also observed that the group with the lowest stocking density (and the most space allowance) had a noticeable increase in their feed intake in the week following the heat load and continued to significantly increase in the 2nd week after the heat load. This might suggest that pigs with more space allowance recover faster from a heat wave.

The average daily gain of pigs was not affected by stocking density during the heat load. However, the pigs housed with the lowest stocking density had a numerically higher average daily gain compared to those housed with higher stocking density, even during high heat loads, and tended to show a higher average daily gain across the entire trial period. White et al. (2008) found that higher stocking density and increased temperature independently reduced average daily gain and suggested that providing pigs with a 28% increase in space allowance (from 0.66 m^2/pig to 0.93 m^2/pig) could mitigate 50% of the negative growth results due to increased heat. In addition, Kerr et al. (2005) note that pigs with extra available floor area had a higher average daily gain 1 week after being exposed to heat. They suggest that the increased space allowed the pigs to compensate for their weight loss during the heat wave by increasing their feed intake. Although not significant, we observed a similar trend in the present trial, where pigs given more space ($SD_{1.3}$) had a slightly higher average daily gain in the 2 weeks following heat exposure compared to those at higher stocking densities. Typically, an increase in average daily gain can be attributed to increased average daily feed intake, for example, due to less feeding competition at a lower number of pigs per feeder (Hyun and Ellis, 2001) as can be applied in the study by White et al. (2008). However, several studies (Pearce and Paterson, 1993; Kerr et al., 2005; Jensen et al., 2012; Edilson et al., 2020) have not revealed any significant difference in average daily feed intake, while the average daily gain tended to change (in the present study across the entire trial period) or significantly changed. Observed lower values of average daily gain in higher stocking densities, in the present study, might be due to stress induced by higher stocking densities, which is known to lead to increased cortisol levels, which in turn may affect gluconeogenesis and protein deposition (Barnett et al., 1983; Hemsworth et al., 1986). Alternatively, the change in average daily gain may be due to more efficient feed utilization (Kerr et al., 2005).

Conclusion

A successful artificial heating protocol was established, as the THI remained above the threshold of 75 even when relative humidity decreased. Every physiological parameter was altered during the period of heat load. The altered heating protocol of 7 days led to a significant drop in average daily feed intake and gain. The present study showed that, aside from skin temperature and active behavior, weight within the studied range of 70–100 kg had no significant impact on the evaluated parameters. This suggests that fattening pigs with an average weight of ± 70 kg are equally affected by higher heat loads in terms of respiration rate, rectal temperature, and passive behavior compared to pigs that are 3 weeks older and 20 kg heavier. In addition, increasing the stocking density did result in a doubling of the increase of rectal temperature during the heat load of pigs housed at 0.8 m²/animal compared to a stocking density of 1.0 m²/animal and 1.3 m²/animal. This suggests that pigs housed at higher stocking densities (0.8 m²/animal) are less able to regulate their internal metabolic heat production and that their core temperature remained high. This may potentially lead to a larger impact of heat stress. Our recommendations for pig welfare under conditions of high head loads, based on measurements of the increase in rectal temperature during increased heat load within the current trial design, are to increase stocking densities to 1.0 m²/animal as this appears to be sufficient to mitigate some negative effects of heat stress.

Ethics approval

The Ethics Committee of Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) approved all experimental procedures (number 2021/393 and 2022/405).

Data and model availability statement

None of the data were deposited in an official repository. The analyzed 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

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare no personal or financial conflicts of interest.

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