MITIGATING HEAT STRESS IN FATTENING PIGS

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LIST OF ABBREVIATIONS

List of abbreviations

AA	Amino acid
ADFI	Average daily feed intake
ADG	Average daily gain
ADWI	Average daily water intake
AIC	Akaike information criterion
BP	Breakpoint
CF	Crude fat
E	Energy
EB	Electrolyte balance
FCR	Feed conversion ratio
FHP	Fasting heat production
HPA	Hypothalamic-pituitary-adrenal
HSP	Heat shock protein
HSS	Heat stress score
ID	Identification number of the animal
IMF	Intramuscular fat
IR	Infrared
LCT	Lower critical temperature
LYS	Lysine
NA	Not applicable
NDT	Number of days when THI ≥ 77 within a week
NE	Net energy
NEFA	Non-esterified fatty acids
pCO2	Partial pressure of CO2
pH_i	Initial pH
pH_u	Ultimate pH
R^2_c	Pseudo conditional R^2
R^2_m	Pseudo marginal R^2
RCP	Representative concentration pathways
RFID	Radio frequency identification
RH	Relative humidity
ROS	Reactive oxygen species
RR	Respiration rate
$S_{carcass}$	Progeny of TN70 sows x a terminal sire line selected for optimal carcass quality
SD	Stocking density
Se	Selenium
S_{growth}	Progeny of TN70 sows x a terminal sire line selected for optimal growth rate
SID	Standardized ileal digestible
TCI	Thermal circulation index

T_{DB}	Dry-bulb temperature
THI	Temperature-humidity index
THP	Total heat production
T_{rectal}	Rectal temperature
T_{skin}	Skin temperature
T_{wb}	Wet-bulb temperature
UCT	Upper critical temperature
VAS	Visual analogue scale
W_{100}	20-week-old fattening pigs of 96.5 ± 7.3 kg
W_{70}	17-week-old fattening pigs weighing 72.7 ± 9.9 kg

CHAPTER 1 | BACKGROUND & SCIENTIFIC OUTLINES

1 Climate change as an emerging stress factor in the livestock industry

The emission of greenhouse gasses, caused by human activities, is the primary driver of global warming and climate change. This leads to rapid and severe changes in the atmosphere, oceans, and biosphere on a global scale, with detrimental effects for both ecosystems and people (IPCC, 2023a). Over the past century, the global surface temperature has increased by 1.5°C above pre-industrial levels and will increase even further (IPCC, 2024). Furthermore, global relative humidity (RH) levels are expected to become more extreme under worst-case climate scenarios. This implies a trend in which already humid regions are predicted to experience increased humidity, while drier regions are expected to become even more arid (IPCC, 2023b). In Belgium, there is an increasing trend in annual temperature compared to the reference period of 1961-1990 (Figure 1). This trend will continue with projected increases ranging from 0.7°C to 5°C by the end of the century, depending on different greenhouse gas emission scenarios represented by the Representative Concentration Pathways (RCPs) (Figure 1). The 3 RCPs present outcomes based on different emission quantities models: RCP2.6 (optimistic scenario with a strong reduction of emissions), RCP4.5 (intermediate reduction of emissions), and RCP8.5 (pessimistic scenario with a strong increase of emissions) (KMI, 2020). However, climate prediction models frequently fail to predict accurate scenarios, as it is shown that temperatures are rising at a much faster rate than initially predicted (van Oldenborgh et al., 2009; Vautard et al., 2023). This highlights the urgency of 1) taking action to address climate change and reduce its impacts through human behaviour (climate mitigation), and 2) managing the considerable challenges caused by the current climate situation (climate adaptation). One of these challenges is the impact of heat stress on livestock, as climate change will directly affect animal welfare, health, and performance (Campos et al., 2017).

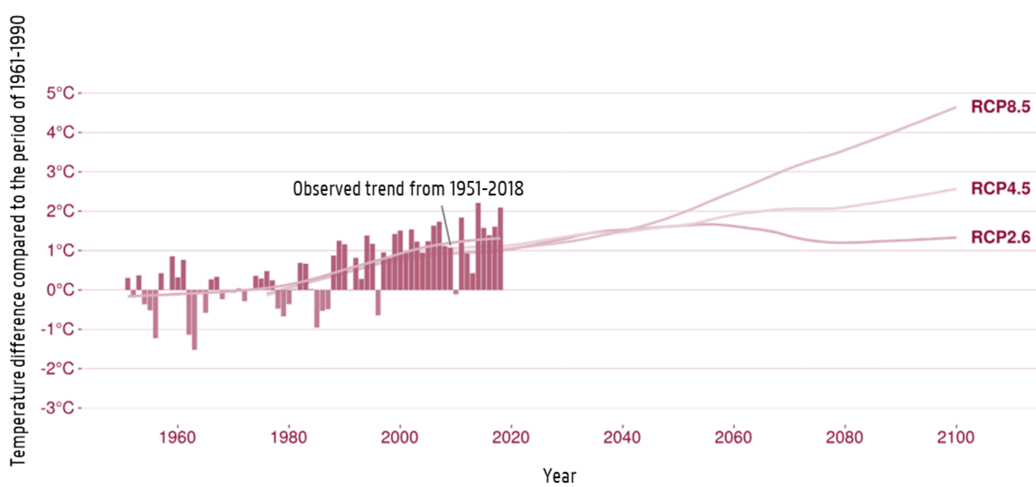

Figure 1: Evolution of the average annual temperature (temperature difference [°C] compared to the reference period of 1961-1990) in Belgium predicted by the Representative Concentration Pathways (RCPs). RCP2.6 = optimistic scenario with a strong reduction of emissions, RCP4.5 = intermediate reduction of emissions and RCP8.5 = pessimistic scenario with a strong emission increase. Adapted from KMI (2020)

2 High-performance breeds in temperate environments

To enhance production in pig farming, there has been an increased emphasis on selecting high-performance breeds in the last decades. These breeds developed to be raised in temperate environments such as Western Europe, have a lower tolerance to heat stress compared to breeds in tropical areas. Pigs selected for high efficiency of lean growth have a greater effect of increasing temperature on average daily gain (ADG) and feed conversion ratio (FCR) (Renaudeau et al., 2011). In contrast, tropical breeds like Creole (Gourdine et al., 2007; Gourdine et al., 2021; Renaudeau et al., 2007) and Iberian pigs (Rauw et al., 2020; Silió, 2000) show greater heat stress tolerance due to their slower growth and higher fat content. However, these breeds may be less economically viable due to their higher production costs (Gourdine et al., 2021; Rose et al., 2017).

The lower production efficiency makes tropical breeds less attractive in temperate climates. Therefore, it is important to develop solutions for widely used breeds, including adjustments in management, feeding practices, and climate-control technologies. Additionally, modern breeds can be refined to achieve a balance between high performance and enhanced heat tolerance, ensuring they remain viable across diverse environmental conditions.

3 Objectives and scientific outline

The primary objective of this thesis is to evaluate the impact of heat stress on fattening pigs and the influencing factors, as well as to investigate management strategies to mitigate the effects of heat loads in temperate environments, like Belgium (Figure 2). The specific objectives are:

a) To assess the impact of heat load on fattening pigs and investigate the variations in pig and farm-related characteristics;
b) To develop and validate a heating protocol that enables rapid investigation of heat stress mitigation measures;
c) To investigate the effects of different management strategies on the physiological and performance parameters of fattening pigs under heat stress

Chapter 2 provides a literature review of thermoregulation mechanisms in pigs and the key factors influencing them. The physiological and performance-related consequences of heat stress are outlined and discussed. To assess whether these effects are comparable under controlled artificial heat waves, frequently used in subsequent experiments, a standardized heating protocol was developed and validated, as detailed in Chapter 3. The general introduction also explores various heat stress mitigation strategies, with a particular focus on management and microclimate modifications. This focus on management-based interventions is further examined in Chapters 4 to 6.

Chapter 4 investigates feed adaptations as a strategy to alleviate heat stress. Chapter 4A evaluates the potential of feed additives, such as antioxidants and osmolytes, under high heat load conditions, while Chapter 4B also examines the effects of different feed compositions. These studies were conducted during mild summer conditions as well as two consecutive artificial heat waves. Chapter 5 explores the impact of space allowance on pigs during an artificial heat wave, and Chapter 6 examines whether two modern terminal sire lines exhibit different physiological and performance responses to natural summer conditions. In Chapter 7, data from all experimental studies are compiled into a single dataset to assess thermoregulation models in fattening pigs and identify key thermoregulatory characteristics influencing these models. Finally, Chapter 8 discusses the overall findings of this thesis and addresses efficacy and challenges of implementing strategies to mitigate the impact of heat stress in fattening pigs.

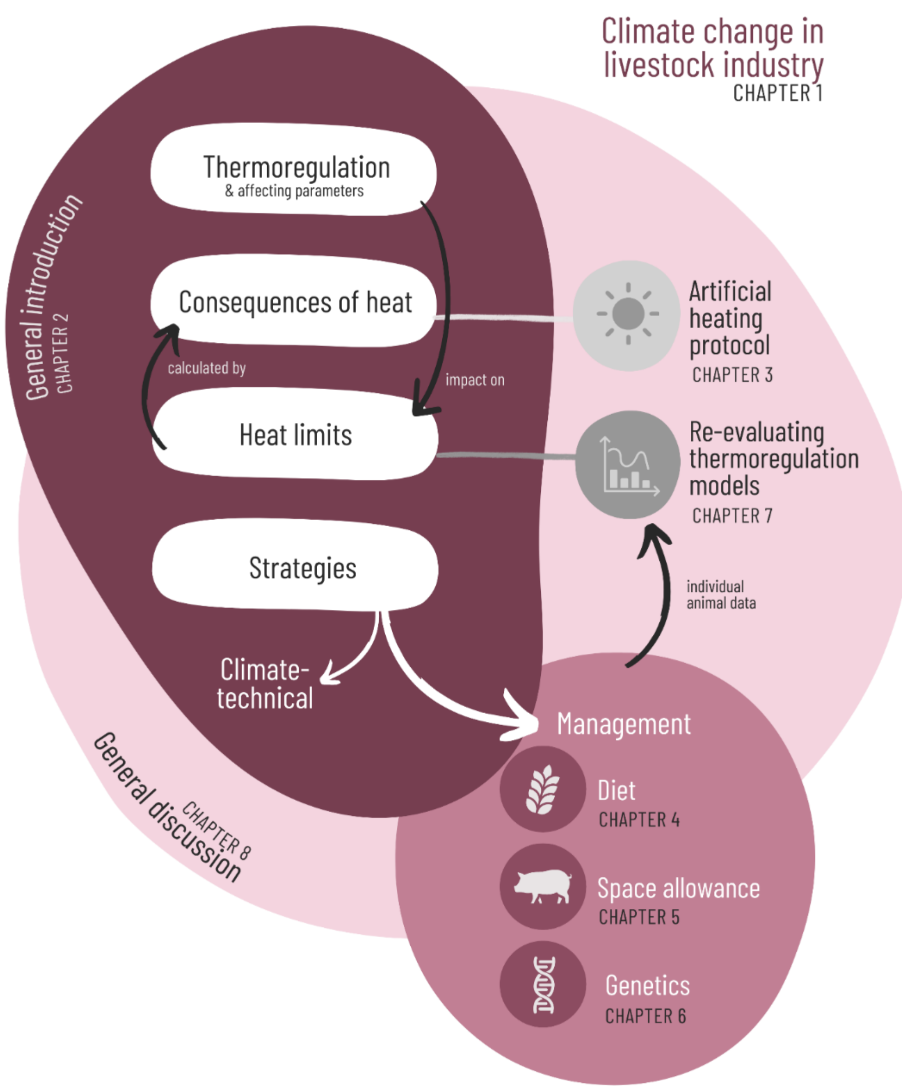

Figure 2: Outline of the thesis

CHAPTER 2 | GENERAL INTRODUCTION

1 Thermoregulation of the pig

1.1 Heat production

A crucial aspect of understanding thermoregulation in pigs, or any other animal, is heat production. Heat is produced as a result of the animal's metabolic activity, which is largely determined by the rate of oxygen consumption (Mount, 1979a). Heat production is closely linked to the animal's energy intake, and the two terms are often used interchangeably. To clarify this relationship, a brief overview of energy intake and its connection to heat production is provided in Figure 1.

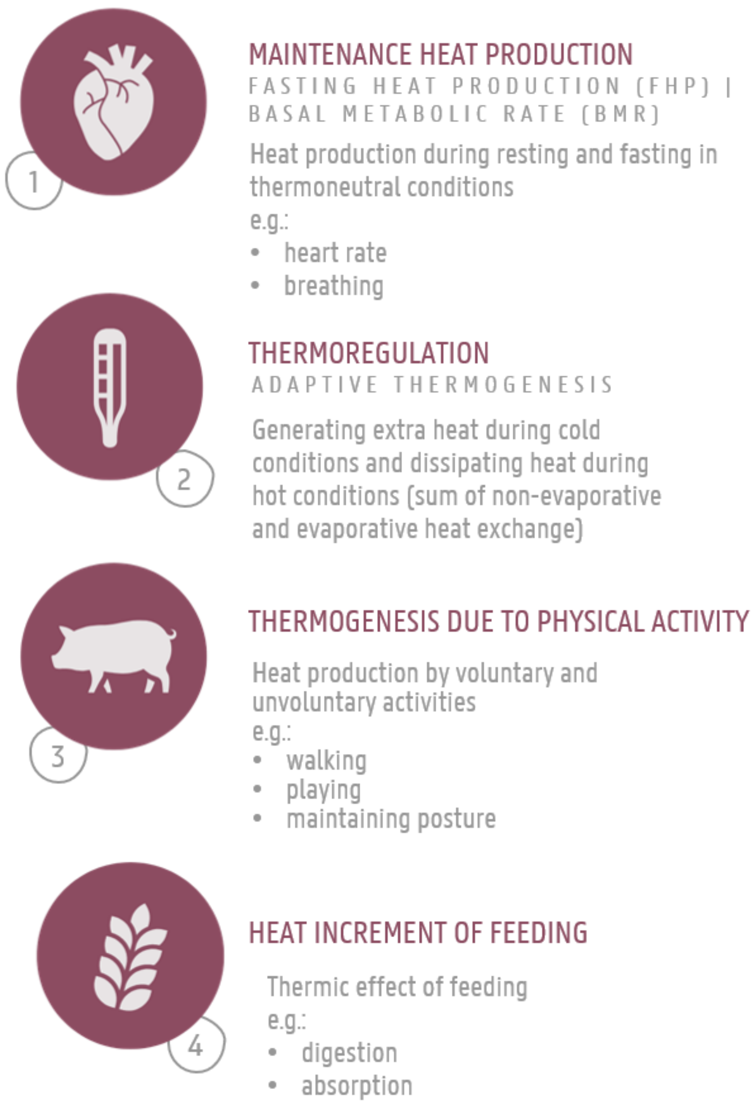

Figure 1A: An overview of an animal's heat production. Based on the information of National Research Council (2012)

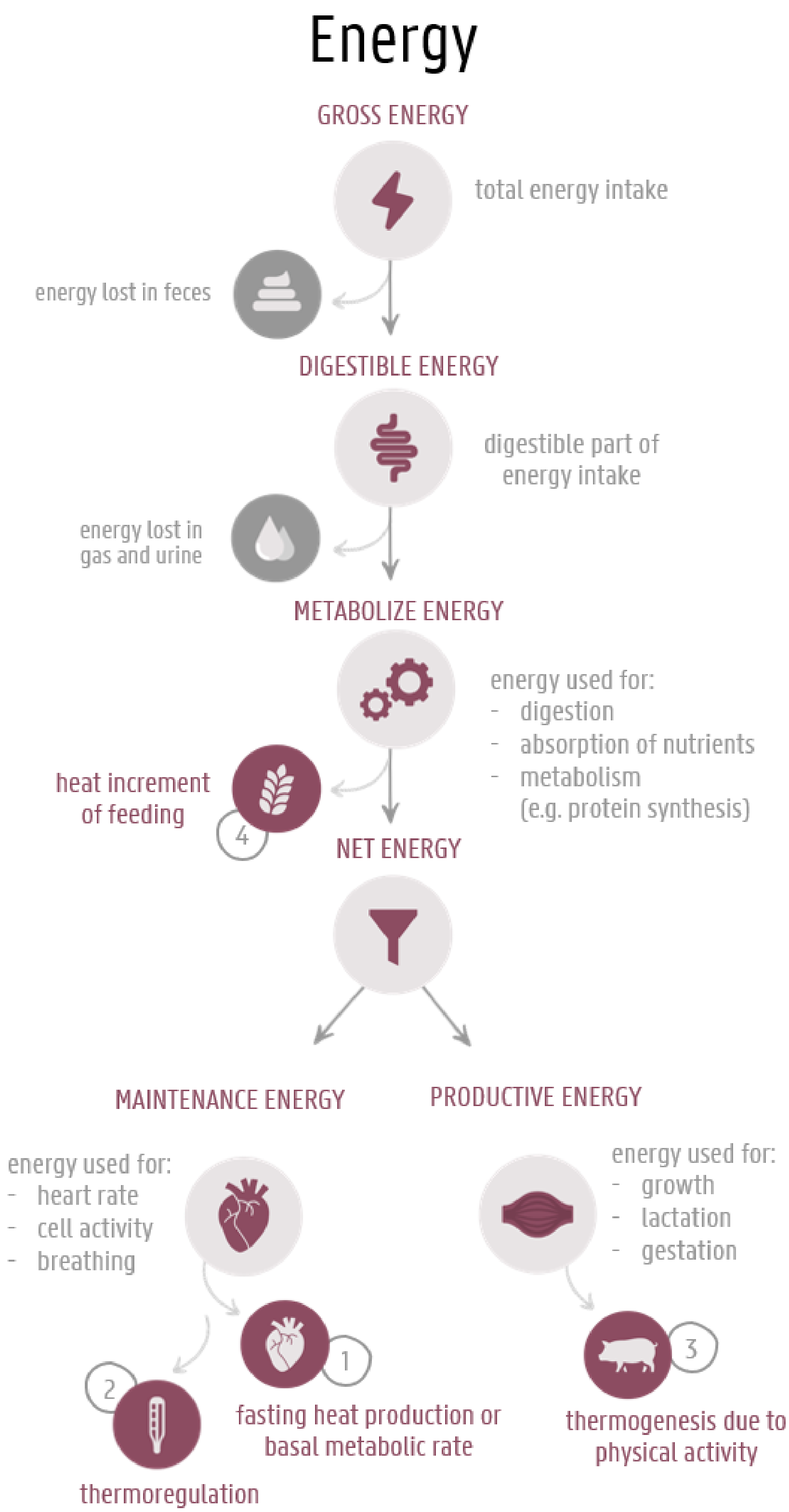

Figure 1B: A schematic overview of energy intake of an animal. Grey circles represent the energy used for a certain purpose, while red circles represent heat exchange. The numbers can be linked to Figure 1A. Based on the information of National Research Council (2012)

1.2 Normal functioning

Homeothermic animals, such as pigs, typically maintain a constant deep body (core) temperature despite varying environmental conditions and metabolic heat production rate (Mount, 1979c). In their thermoneutral zone, the energy demands for maintaining homeothermy are minimal, allowing for optimal production. Pigs regulate body temperature through a balance of heat production at lower ambient temperatures and heat loss at higher ambient temperatures (Huynh, 2005; Mount, 1979c).

Thermoregulation (adaptive thermogenesis, Figure 1) in pigs is maintained through sensible (non-evaporative) and latent (evaporative) heat exchange mechanisms (Figure 2). Non-evaporative heat exchange occurs via radiation (heat transfer by radiation, e.g. cool environment), conduction (heat transfer by direct contact, e.g. cool floor), or convection (heat transfer by movement of fluids, e.g. high air velocity), while evaporative heat exchange occurs through evaporation (heat transfer by the transition of liquid water into gas, e.g. sweating). These mechanisms provide a heat transfer between the body surface and the environment (Mount, 1979c). Non-evaporative heat exchange requires a temperature gradient between the animal and its surroundings (Collier & Gebremedhin, 2015; Mount, 1979c). This type of heat transfer is prevalent in cooler conditions or within the thermoneutral zone, where heat losses or gains occur. For instance, an animal may gain heat through convection and radiation from a hotter environment while losing heat conductively to a cooler floor (Mount, 1979c). As ambient temperature rises and approaches the animal's body temperature, the temperature gradient diminishes, reducing heat loss and potentially resulting in heat gain from the environment. At this point, heat transfer relies more on evaporative heat exchange. When the ambient temperature exceeds the upper critical temperature (UCT), evaporation becomes the only way for heat loss (Collier & Gebremedhin, 2015). Evaporative heat exchange is driven by a water vapor pressure gradient between the pig's outer surface and the environment and therefore becomes more important in hotter (and dryer) conditions (Mayorga et al., 2019; Mount, 1979c). Pigs lose heat by evaporation through a wet skin surface or respiration. Skin evaporation is limited in current housing condition of commercial pigs and consequently, pigs primarily depend on respiratory evaporation for heat loss (Huynh, 2005).

The thermoregulation of pigs can be illustrated by different temperature zones (Figure 2), assuming a certain feed intake (resulting in a constant heat increment of feeding, Figure 1) and a resting position (resulting in a constant thermogenesis due to physical activity, Figure 1). Within the thermoregulatory range A-D, pigs can keep their deep body temperature constant through adaptive thermogenesis, the combination of non-evaporative and evaporative heat exchange. Outside this zone, body temperature will fall when the ambient temperature is below point A, or rise when the ambient temperature is above point D. In zone A-B, the pig can maintain its body temperature constant due to heat production, for example shivering, which will require extra energy. In zone B-D, the pig's body temperature is kept constant by heat loss regulations, for example lying on a cold floor. This zone is also called the thermoneutral zone or zone of minimal metabolism. It is bounded by two critical points, between which optimal production can be achieved with the most efficient energy use for maintaining body temperature (the adaptive thermogenesis remains constant). The lower critical temperature (LCT, point B); and the upper critical temperature (UCT, point D), mark these boundaries. Within the thermoneutral zone lies the comfort zone (zone B-C) (Mount, 1979a), where pigs manage heat exchange without additional thermoregulatory efforts (Curtis, 1985). However, from point C onward, at the evaporative critical temperature (ECT), evaporative heat loss mechanisms, such as panting (Huynh, 2005), will start to increase. The exact temperature thresholds and slopes of these zones in Figure 2 vary depending on several characteristics, including species, age, nutrition, adaptation history, and environmental conditions, which are discussed in detail in Section 1.3

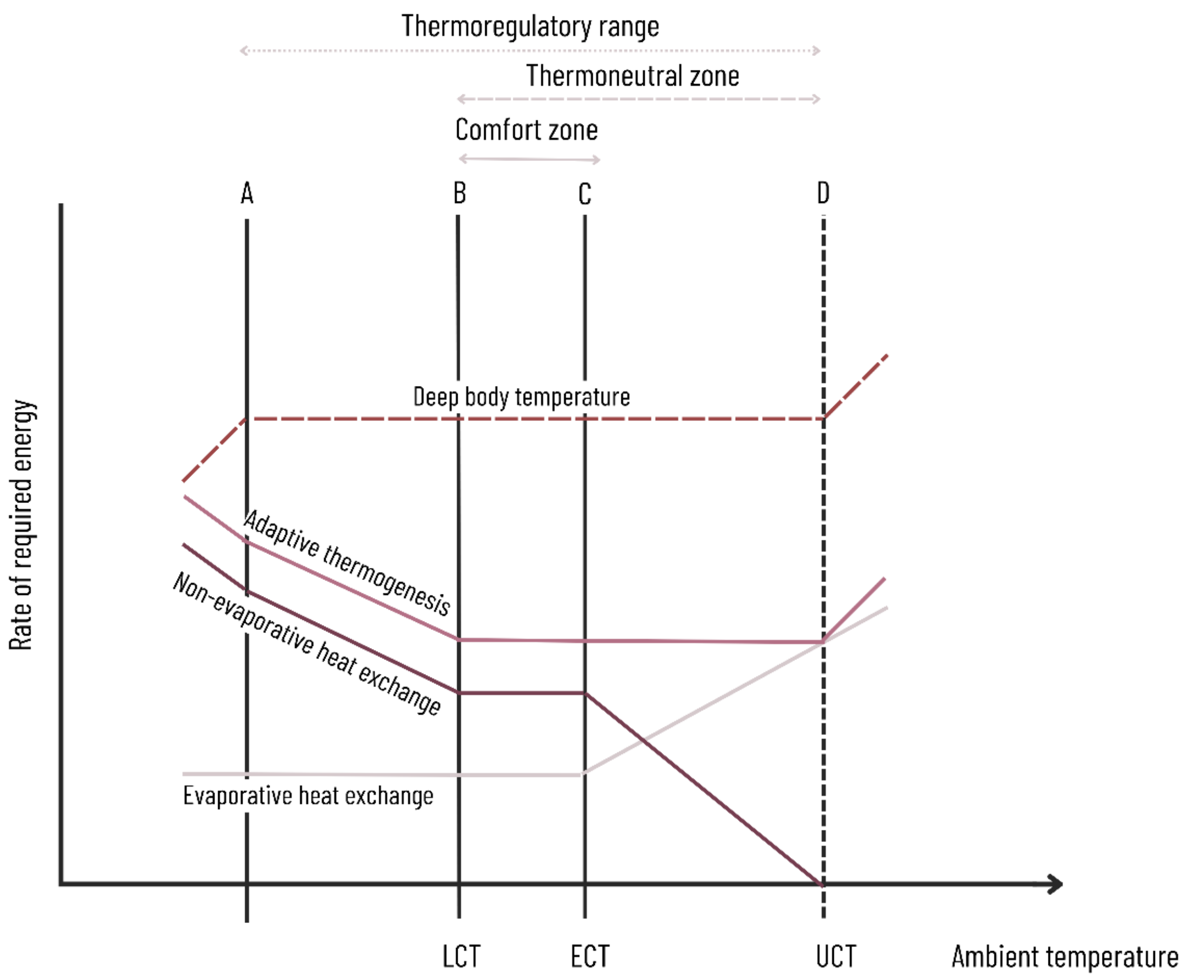

Figure 2: Schematic representation of the thermoregulation of pigs. B = lower critical temperature (LCT), C = evaporative critical temperature (ECT), D = upper critical temperature (UCT), zone A-D = zone of thermoregulatory range, zone B-D = thermoneutral zone, B-C = comfort zone. Zone -∞-A = cold stress, zone D- +∞ = heat stress. Adapted from Mount (1979a)

As ambient temperature continues to rise, the energy required for maintaining a stable adaptive thermogenesis is no longer constant. Beyond the UCT, energy required for maintaining homeothermy increases along with the deep body temperature, despite all efforts to dissipate heat (Mount, 1979c). When heat loss efforts become insufficient, animals will initiate various strategies to cope with the increasing heat load, including behavioural, physiological, and anatomical changes (Mayorga et al., 2019; Mount, 1979a), which will be described further in the thesis.

1.3 Characteristics affecting thermoregulation

There is an important distinction, often misunderstood, between characteristics that influence thermoregulation (section 1.3) and parameters that are affected by high heat loads, in other words, the consequences of high heat load (section 2).

Animal- or climate-related characteristics that affect thermoregulation are specific to the individual animal or the environmental situation, such as genetic background or air movement. These parameters influence the lower and upper critical temperature, causing these thresholds to shift to either lower or higher temperatures. In this sense, these characteristics determine how sensitive an animal is to heat. Adjusting these influencing factors, may alter the animal's sensitivity to heat stress.

On the other hand, the consequences of a high heat load, are the outcomes of the animal's heat stress sensitivity, such as increased rectal temperature (T_{rectal}) or decreased daily gain. In other words, the effect of heat load can lead to changes in these parameters, which will impact the animal's overall condition, influencing the well-being and performance. Concerning this, characteristics that can affect thermoregulation can influence the consequences of a high heat load, resulting in a favourable or unfavourable outcome, while this is not the case the other way around.

1.3.1 Climate- and environment-related characteristics

1.3.1.1 Ambient temperature

The most critical climate-related factor influencing thermoregulation is ambient temperature. The ambient temperature affects the rate at which various physiological and performance parameters adjust during increasing heat loads. Moreover, the duration of the changing parameters is also influenced by the ambient temperature (Renaudeau et al., 2010).

Under prolonged and continued heat load, pigs lack adequate recovery during brief intervals of lower temperatures. When pigs experience chronic heat stress, they adjust their thermoregulation through a process called heat acclimation, characterized by changes in physiological and metabolic traits (Kingsolver & Huey, 1998). This heat acclimation is divided into a biphasic profile (Horowitz, 2001), as can be seen in Figure 3. The first phase, also called short-term heat acclimation, occurs during the first 24h-48h after heat exposure. It is characterized by quick physiological adaptions (Figure 3; v_1) like increased T_{rectal}, T_{skin} and respiration rate (RR), and reduced average daily feed intake (ADFI). During short-term acclimation, pigs predominantly rely on evaporative heat loss rather than non-evaporative heat exchange, as indicated by a reduced Thermal Circulation Index (TCI, discussed further) observed within the first hours of heat exposure (Renaudeau et al., 2010). The changes during the short-term heat acclimation depend on the ambient temperature. For example, RR or T_{rectal} will increase much faster during higher ambient temperatures (36°C) whereby the slope of the graph (Figure 3; v_1) will be much steeper, compared to lower temperatures (28 °C) (Renaudeau et al., 2010). Interestingly, the endpoint of the short-term acclimation phase (Figure 3; td_1) appears to be independent of the ambient temperature, whether 28°C, 32°C, or 36°C, for the physiological parameters measured. This suggests that the duration of this phase remains consistent at approximately 1.10 days, as indicated by T_{rectal} and RR. However, when considering ADFI, the short-term acclimation period was notably longer at 28°C, lasting approximately 2.30 days, compared to 0.29 days at both 32°C and 36°C (Renaudeau et al., 2010). The second phase, or long-term heat acclimation, is defined by an incremental decrease/increase and stabilization (Figure 3; v_2 and v_3) of the parameters mentioned above (Horowitz, 2001; Renaudeau et al., 2010; Renaudeau et al., 2007). For some parameters, like RR and T_{rectal}, Renaudeau et al. (2010) found a curvilinear response, meaning there are two sub-phases within the long-term heat acclimation (Figure 3; v_2 and v_3). The first sub-phase v_2 can be described as a strong decline for RR, T_{rectal} and T_{skin}, or an increase in terms of ADFI and is followed by the second sub-phase v_3, which shows a slight but significant change in the parameter's profile. Notably, the duration of the first sub-phase is temperature-dependent: as ambient temperature rises, the length of this period increases (Figure 3; td_2) (Renaudeau et al., 2010). It is important to note that this model of heat acclimation was developed using pigs from 12 weeks of age that were exposed to a constant heat load (28 °C, 32 °C, or 36 °C with 80% relative humidity (RH)) for 20 days (Renaudeau et al., 2010). However, the model does not account for the influence of weight. Heavier fattening pigs are more susceptible to heat stress than lighter ones, which is discussed further. This could steepen the slope of the short-term heat acclimation, as heavier pigs may exacerbate this effect under high temperatures. Similarly, RH can further increase heat load, thereby influencing the slope of the curve. Nonetheless, the model proposed by Renaudeau et al. (2010) remains a solid model.

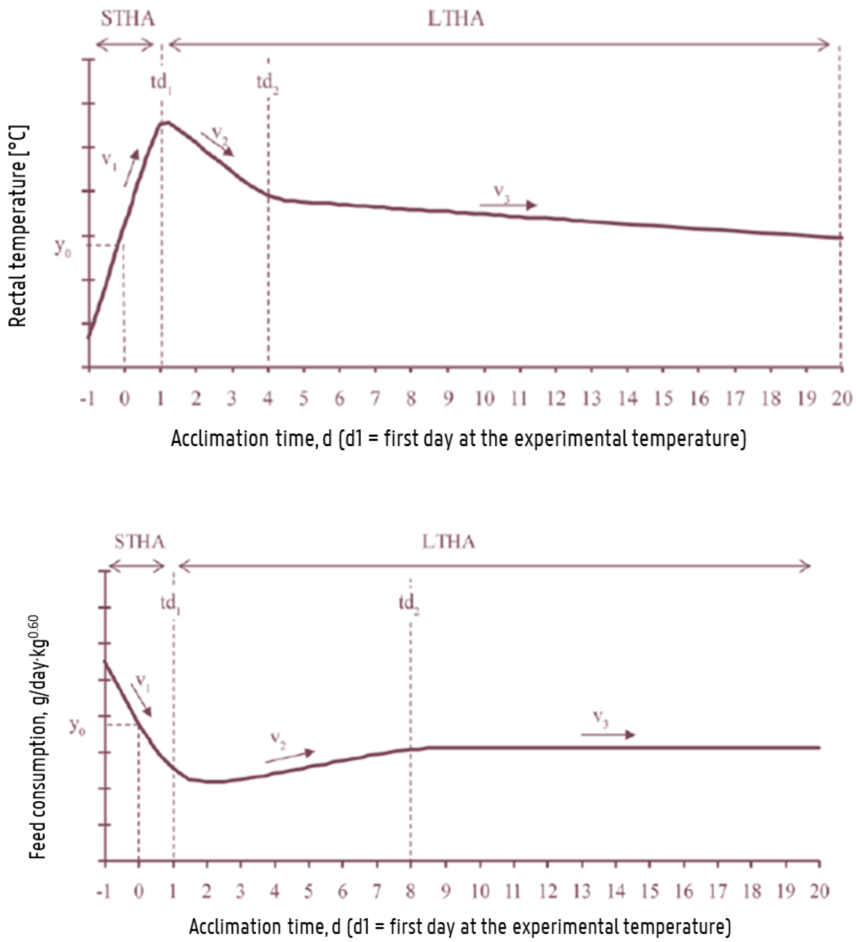

Figure 3: Theoretical biphasic model of the rectal temperature [°C] and feed consumption [g/day · $kg^{0.6}$] during thermal acclimation of fattening pigs. The model of respiration rate can be represented as the model of rectal temperature. STHA = short-term heat acclimation; LTHA = long-term heat acclimation. y_0 is the value of Y at d = 0, td_1 and td_2 (day of exposure) are the thresh hold days, and v_1, v_2, and v_3 are the linear variations of Y before and after td_1 and after td_2, respectively (Renaudeau et al., 2010)

In general, pigs will adapt to a certain degree of chronic heat stress by a period of acclimation. This adaptation is primarily driven by an increase in heat loss effectors and a reduction in heat production (Brown-Brandl et al., 2001; Renaudeau et al., 2010). The reduction in heat production is linked to a decline in oxygen consumption (Giles & Black, 1991). Additionally, as RR decreases gradually during the long-term acclimatation period, heat production related to physical activity, an important contributor to heat production during panting, also reduces (Collin et al., 2001b; Quiniou et al., 2001). This decrease in RR may be attributed to a reduced need for body cooling as acclimation progresses (Bianca, 2009) or to a more efficient evaporative heat loss per breath. Additionally, higher heat loads activate mechanisms primarily aimed at enhancing heat loss, such as increased pulmonary ventilation (the movement of air in and out of the lungs) and elevated heart rate. These responses, however, also elevate metabolic rate due to their energy demands, which can be counterproductive during the heat acclimation process (Saxton, 1981). Overall, it seems that the reduction in heat production during long-term acclimation plays a more critical role than the increase in heat loss effectors (Renaudeau et al., 2010). When the heat load becomes too severe and thermoregulatory adaptations fail to sufficiently lower body temperature, total heat production (THP) increases. This occurs because rising body temperature accelerates the rate of physiological reactions, such as muscle activity during panting (Brown-Brandl et al., 2001). When this rise continues, the animal will die due to hyperthermia (Mount, 1979c).

A constant ambient temperature will affect the pig's productivity more than an ambient temperature that is fluctuating diurnally (Nienaber et al., 1989). During periods of fluctuating temperatures, pigs can compensate for reduced feed intake during the hotter periods of the day by increasing their intake when the temperatures are lower, such as at night (Huynh et al., 2005a). However, this compensation is effective or sufficient only when diurnal temperature variations are small. When the diurnal temperature variation exceeds ±1.5°C, the increased nocturnal feed intake does not fully compensate the reduced intake during the day. Consequently, this results in an overall decline in performance (Quiniou et al., 2010).

The frequency of heat waves also influences the intensity of heat stress effects. For example, a study investigating multiple four-day heat waves at approximately 32°C, combined with a thermoneutral week in between, revealed that the first heat wave caused a more substantial reduction in ADFI and ADG compared to subsequent heat waves. Not only was the decline in performance parameters less severe during later heat waves, but the ADG during the second and third heat waves also exceeded that of the first, with age being corrected in the statistical analysis (Rauw et al., 2017). This pattern suggests a possible adaptive response in thermoregulation to recurring cycles of heat exposure.

1.3.1.2 Relative humidity

A higher RH increases the heat load experienced by pigs, as also reflected in the Temperature-humidity index (THI), discussed below. When RH is elevated, the vapor pressure gradient between the pig's body and the surrounding environment diminishes, reducing the effectiveness of evaporative cooling. Consequently, physiological parameters such as RR and T_{rectal} begin to increase at a lower temperature, referred to as the inflection point. For instance, the inflection point of the physiological parameters is approximately 2°C lower when the RH is 80% compared to 50%. High RH also exacerbates the reduction in daily gain during a high heat load (Huynh et al., 2005a). Generally, from an ambient temperature of 30°C, an 18% increase in RH is equivalent to a 1°C rise in temperature (Curtis, 1983; Huynh et al., 2005a). Above 80% RH, heat stress effects intensify, as the rate of evaporative heat loss decreases (Granier et al., 1998).

1.3.1.3 Temperature-humidity index

The THI is a metric that combines ambient temperature and RH (measured through dry-bulb (T_{DB}) and wet-bulb temperatures (T_{WB})) to assess the severity of heat stress. Originally developed for humans, it was first referred to as the "discomfort index" (Thom, 1959). Its primary goal was to provide a simple, effective measure of human discomfort caused by environmental conditions. While the concept of "effective temperature" remains widely accepted, it is not easily derived from standard meteorological data. However, because dry- and wet-bulb temperatures closely correlate with effective temperature, a formula was created based on a large dataset that included these parameters along with effective temperature values. Using a linear model, the first THI for discomfort was formulated:

$$THI_{Thom} = 0.4\ T_{DB} + 0.4\ T_{WB} + 15$$

Thresholds were then established to categorize comfort levels:

- THI > 70: Discomfort
- THI > 75: Moderate discomfort
- THI > 79: Most individuals feel significant discomfort
- THI > 80: Serious discomfort

This formula was the foundation of many other indexes, including THI for livestock. The first THI specifically designed for pigs (young pigs weighing between 20-30 kg) was introduced by Ingram (1965). Ingram (1965) examined various physiological responses to changes in dry- and wet-bulb temperatures. Ultimately, the change in core body temperature was chosen as the key parameter for determining the formula's coefficients:

$$THI_{Ingram} = 0.65\ T_{DB} + 0.35\ T_{WB}$$

Subsequent coefficients were refined using data from different physiological and behavioural responses, such as core body temperature, RR, and performance traits (Fehr et al., 1983; NWSCR, 1976; Roller & Goldman, 1969). These developments were later summarized in reviews by Vitt et al. (2017) and Cao et al. (2021). As result, many THI formulas and thresholds values were developed over the years, which vary depending on species, animal category, and measurement method.

In the next chapters, the THI formula first mentioned by Maust et al. (1972) for lactating cows, and later applied by Lucas et al. (2000) for pigs with the threshold values from NWSCR (1976), will be used. This formula represents an adaptation of the original THI formula, with coefficients based on the work of the Livestock Conservation Institute where animal behaviour was evaluated:

$$THI_{Lucas} = 0.72\ T_{DB} + 0.72\ T_{WB} + 40.2$$

With different thresholds, indicating risks of confined livestock, especially pigs (Lucas et al., 2000; NWSCR, 1976) (Figure 4):

- $THI \geq 75$: alert
- $79 \leq THI < 83$: dangerous
- $THI > 84$: very dangerous

In this formula, the T_{WB} contributes an equal proportion to the overall heat load as the T_{DB}. However, when calculating the T_{WB}, RH does not carry the same weight as the temperature:

$$T_{WB} = T_{DB} \cdot \tan^{-1}(0.151977 \cdot \sqrt{(RH + 8.313659)}) + \tan^{-1}(T_{DB} + RH) - \tan^{-1}(RH - 1.676331) + 0.00391838 \cdot RH^{3/2} \cdot \tan^{-1}(0.023101 \times RH) - 4.686035$$

Temperature [°C] \ Relative humidity [%]	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
23	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	
24	68	68	69	70	70	71	71	72	72	73	73	74	74	75	75	
25	69	70	70	71	72	72	73	73	74	74	75	75	76	76	77	Alert
26	70	71	72	72	73	73	74	75	75	76	76	77	77	78	78	
27	72	72	73	74	74	75	75	76	76	77	77	78	79	79	80	
28	73	74	74	75	76	76	77	77	78	78	79	79	80	80	81	Dangerous
29	74	75	76	76	77	78	78	79	79	80	80	81	81	82	82	
30	75	76	77	78	78	79	79	80	81	81	82	82	83	83	84	
31	77	77	78	79	80	80	81	81	82	83	83	84	84	85	85	
32	78	79	80	80	81	82	82	83	83	84	85	85	86	86	87	Very dangerous
33	79	80	81	82	82	83	84	84	85	85	86	87	87	88	88	
34	80	81	82	83	84	84	85	86	86	87	87	88	89	89	90	

Figure 4: Temperature-humidity index diagram based on the formula of Lucas et al. (2000). The thresholds are THI ≥ 75: alert, 79 ≤ THI < 83: dangerous and THI > 84: very dangerous.

The use of the THI has several advantages:

- It is relatively easy to calculate, making it a convenient tool for assessing thermal stress.
- By incorporating RH, THI may provide a more accurate measure of heat load than using temperature alone.

However, THI has also its limitations:

- A major drawback is the lack of universality; the wide variety of formulas and thresholds hinders its application across different contexts.
- The THI fails to account for other critical factors that influence thermoregulation, such as air movement, ventilation rates, and housing conditions. Many of the commonly used formulas are also based on outdated data, which may not fully reflect current understanding of environmental conditions.
- The thresholds used to define thermal stress are sometimes based on vague or unsubstantiated physiological or performance changes, reducing their reliability.

In addition to the commonly used THI, several alternative indices have been developed for assessing heat stress in e.g. dairy cattle. These indices aim to provide a more comprehensive representation of the thermal environment, particularly for animals housed outdoors, where additional climatic factors play a significant role. Notably, indices such as the Adjusted THI (THI_{adj}), the Black Globe Humidity Index (BGHI), the Comprehensive Climate Index (CCI), and the Equivalent Temperature Index (ETI) incorporate variables beyond temperature and humidity, such as wind speed and solar radiation flux (Choi et al., 2024; Yan et al., 2021). These components are especially relevant for evaluating the heat load experienced by animals in open or pasture-based systems, where exposure to direct sunlight and fluctuating airflow influence thermal stress responses.

1.3.1.4 Air movement

In general, higher air speeds enhance heat loss in pigs (a combination of non-evaporative and evaporative heat loss) across different temperature levels (10, 20, and 30 °C). However, in pigs, the improvement in heat loss due to increased air movement is more pronounced for non-evaporative heat loss compared to evaporative heat loss (Close et al., 2010).

Convective heat transfer refers to heat loss caused by the movement of fluids, including air. Therefore, a faster air movement significantly impacts pigs' convective heat dissipation. It is influenced by several factors, including surface area, temperature gradient between the skin and the environment, and convective heat transfer coefficient. This coefficient reflects the efficiency of heat transfer between the body and the surrounding air, and is affected by factors such as animal geometry, size, and airflow patterns around the pigs (Li et al., 2016). The increased convective heat transfer coefficient with increasing airspeed was observed in group-housed fattening pigs of varying body weights (30, 50, and 80 kg), where body weight had no significant effect on the convective heat transfer coefficient (Li et al., 2018). Under normal conditions, the convective heat transfer coefficient of an individual pig decreases with increasing body weight (Li et al., 2016) due to several factors discussed in Section 1.3.2.1. However, in group-housed pigs, airflow patterns become more complex, (Li et al., 2018), and heat production from neighbouring pigs affects the results.

As mentioned earlier, the evaporation rate also increases with air velocity (Ramsay, 1935). Evaporation has two aspects: energy and diffusion. The energy aspect is driven by temperature differences, while the diffusion aspect refers to the transport of water vapor into the laminar boundary layer, which in pigs is the boundary between the skin and the environment. Increased airspeed accelerates this vapor transport, enhancing evaporation (Barkman & Stoutjesdijk, 1986). However, since pigs rely mainly on their respiration as evaporation, instead of transpiration via the skin, the impact of air movement will be lower than on convection, except when they are given water to wallow in or when they wallow in their own feces.

The impact of increased airspeed on heat loss is also reflected in changes to the pig's LCT, the temperature at which heat production efforts are minimal. Higher airspeeds increase the LCT. For instance, the LCT at an airspeed of 3 cm/s is approximately 19.4 °C, while at 56 cm/s, it rises to 29.5 °C (Close et al., 2010). This effect is beneficial only when ambient temperatures exceed the LCT. For example, at an ambient temperature of 15 °C with an airspeed of 56 cm/s, pigs may experience cold stress and huddle together, as the LCT of 29.5 °C means they would need to produce heat to bridge a temperature gap of 14.5 °C before reaching the thermoneutral zone.

The impact of airspeed on pigs can be quantified using the Kata-value, which represents the amount of heat (in kcal) radiated per cm² per second from a warm surface at approximately 38°C, similar to the pig's body temperature. This value is influenced by both air movement and ambient temperature, providing an indication of the cooling effect of the environment on the animal (Tielen, 1974).

1.3.2 Animal-related characteristics

1.3.2.1 Body weight

Heavier fattening pigs experience a greater effect of high heat load than lighter ones. To explain this, different physiological aspects should be considered, that result in a decreased UCT with increasing body weight (Quiniou et al., 2000; Renaudeau et al., 2011).

First, heavier pigs have a smaller surface area-to-volume ratio, which directly impacts their ability to dissipate heat (Bruce & Clark, 2010). This means that animals with a larger surface area-to-volume ratio can lose heat more efficiently because they have more skin surface relative to their volume. This is also the case in pigs. Heavier fattening pigs must dissipate the heat through a smaller surface area compared to lighter ones. Consequently, their ability to lose heat is reduced, making them more sensitive to heat. In addition, heavier pigs have more subcutaneous fat tissue (Mount, 1979b). This acts like an insulation coat (Bruce & Clark, 2010), and impedes non-evaporative heat loss (Mayorga et al., 2019), in particular the convective heat loss (Li et al., 2018).

Furthermore, heavier pigs have higher maintenance energy requirements than lighter ones, which can be estimated using fasting heat production (FHP) as a predictor (van Milgen et al., 1998). The relationship between FHP (or maintenance energy requirements) and total body mass is described by the formula:

$$FHP = a \cdot BW^b$$

In which BW = pig's metabolic body weight, a = coefficient that varies depending on animal category (sow, piglet, fattening pig, etc.) and study, and b = exponent that varies between 0.54-0.80 (National Research Council, 2012).

However, FHP per kg of body weight decreases as body mass increases. For example, a 25 kg Piétrain pig has an FHP of 563 kJ per kg, while a 60 kg Piétrain pig has an FHP of 490 kJ per kg. The total FHP for a 25 kg pig, assuming an exponent b of 0.6, is 3,697 kJ ($536 \cdot 25^{0.6}$), whereas for a 60 kg pig, it is 5,715 kJ ($490 \cdot 60^{0.6}$). This suggests that using a fixed coefficient *a* in the represented formula for growing-finishing pigs can lead to underestimation of FHP in lighter pigs and overestimation in heavier pigs. Nonetheless, maintenance energy requirements and corresponding FHP increase with body weight due to the energy demands of additional muscle mass (van Milgen et al., 1998).

Lastly, behavioural characteristics play a role. As body weight increases, the energy cost of physical activities also rises. For instance, a 25 kg pig will use less energy to stand up compared to a heavier pig. However, heavier pigs have lower activity levels, which can reduce overall energy expenditure (van Milgen et al., 1998). This decrease in activity may partially counterbalance the higher energy costs associated with movements of heavier pigs.

1.3.2.2 Genetics

Over the last few decades, there has been a notable shift in pig genetics, focusing on increasing (re)production traits such as lean tissue accretion, milk yield, and litter size. However, this genetic progress has come with an unintended consequence, namely an increase in metabolic heat production under thermoneutral conditions (Baumgard & Rhoads, 2013; Renaudeau et al., 2012a). Between 1936 and 2002, pig FHP increased by 18.1% (Brown-Brandl et al., 2004), with a further 4.7% rise observed between 2002 and 2020 (Ramirez et al., 2022). This increase is noticeable in fattening pigs, where the selection for high muscle gain correlates directly with higher FHP due to the metabolic demands of lean tissue (van Milgen et al., 1998). For example, lean breeds like Piétrain and Large White have a FHP of around 962 $kJ/kg^{0.6}$, while slow-growing breeds with higher fat content, like the Meishan, show a much lower FHP of about 660 $kJ/kg^{0.6}$ at the same age (van Milgen et al., 1998). The difference in FHP of various breeds will in turn affect their feeding behaviour, particularly under high heat load conditions. According to Cross et al. (2018), Duroc and Yorkshire fattening pigs showed more feeding behaviour as THI increased, characterized by more frequent visits and longer time spent at the feeder. In contrast, Landrace pigs had a decrease in feeding activity under the same conditions. Duroc and Yorkshire pigs, being less lean than Landrace pigs, likely have lower FHP at the same age, giving them a broader thermoneutral zone. This wider zone could enable them to maintain a more stable ADFI, even during periods of elevated heat load.

In addition to muscle mass, other factors related to phenotype contribute to FHP variations, such as the energy demands of visceral organs. The energy cost per kg of tissue for visceral mass is almost four times higher than that of muscle tissue. This means that, even among breeds with similar lean meat percentages, differences in visceral mass can lead to variations in FHP. For instance, very lean pigs like the Piétrain have lower FHP compared to less lean breeds like the Large White, largely due to a smaller visceral mass (van Milgen et al., 1998). Thus, the maintenance requirements vary significantly across pig breeds due to differences in muscle as well as visceral organ composition (Noblet et al., 1991; Quinious & Noblet, 1995; van Milgen et al., 1998).

Also, other aspects can be linked to the varying maintenance requirements of different breeds, such as the activity levels. Breeds have distinct activity rates, and the energy cost of performing these activities varies between them. For instance, in a study by van Milgen et al. (1998), Meishan pigs stood up less frequently than Piétrain pigs. Moreover, the energy expenditure for this type of activity was nearly double for Piétrain pigs (22 $kJ/kg^{0.75}$ per hour) compared to Meishan pigs (11.2 $kJ/kg^{0.75}$ per hour). This difference likely reflects the higher metabolic demands associated with Piétrain pigs' greater muscle mass.

Another important question is whether selective breeding can focus on traits that enhance thermoregulation. It is assumed that thermal tolerance in pigs may be heritable by different gene expressions during high heat load. Under higher THI conditions, Cross et al. (2018) found some gene expressions at different regions of SSC14_11 and SSC7_53, with the expression of GNA13 and AMZ2 genes, which are associated with blood flow, and DNAJA4, HPS90AA1 and HSP90AB1 genes, associated with the release of heat shock proteins (HSP's) that protect cells from stressors. These gene expressions may be interesting components for thermal tolerance. Furthermore, CHRNA3 and CHRNB4 genes are regulating eating behaviour, which is also important as this can influence the heat increment of feeding (Cross et al., 2018). Besides gene expression, the selection on metabolic biomarkers may be interesting for the development of heat-tolerant breeds. For example, high cortisol levels, which have a high heritability ($h^2 = 0.69$) (Larzul et al., 2015), are associated with improved robustness and adaptability traits and therefore likely also heat tolerance. However, elevated cortisol levels are also linked to adverse effects on production performance (Mormede et al., 2011). A similar trend is observed for T_{rectal}, a physiological heat-stress biomarker. Rectal temperature is low to moderately heritable (Gourdine et al., 2021), but it is negatively correlated with production traits (Renaudeau et al., 2004). These findings suggest that traits related to thermoregulation and production are located on separate genomic loci. Despite these insights, the genomic variability of thermoregulation traits, the heritability of heat stress biomarkers, and their correlations with production parameters remain poorly understood. Additionally, knowledge of the differences in heat tolerance, including phenotypic, hormonal, and metabolic heat stress parameters, between commercial and tropical pig breeds is missing (Gourdine et al., 2021).

1.3.2.3 Sex

Physiological differences between gilts and barrows (castrated boars) are evident and become more pronounced when exposed to heat. Under thermoneutral conditions, gilts typically have a slightly lower T_{rectal} than barrows (39.2°C vs. 39.4°C). This difference persists during prolonged heat exposure, with gilts maintaining a significantly lower T_{rectal} when compared to barrows (40.0°C vs. 40.4°C) (Rudolph et al., 2024a). Moreover, the change in rectal temperature (ΔT_{rectal}) between thermoneutral and heat-stress conditions is smaller for gilts than for barrows (0.8°C vs. 1.0°C). Gilts also demonstrate better acclimation during extended heat exposure. After seven days of continuous high heat load, gilts show a significant reduction in T_{rectal}, from 40.3°C to 40.0°C, whereas barrows did not show a similar decrease (Rudolph et al., 2024a). This difference in thermoregulation may be partly attributed to the hormone oestrogen, which has been shown to promote lower body temperatures (Charkoudian & Stachenfeld, 2016; Chen & Yu, 2018). Additionally, gilts' lower feed intake relative to barrows could contribute to reduced heat increment from feeding, further aiding their thermoregulation. However, despite their lower body temperatures under both thermoneutral and heat-stress conditions, gilts experience greater oxidative damage in muscle tissue compared to barrows (Rudolph et al., 2024b).

Beside physiological changes, sex-dependent differences in performance are also important under thermoneutral and heat-stress conditions. Under thermoneutral conditions, barrows typically have higher feed intake and faster growth rates than gilts. However, during heat stress, their responses change. Within the first 4 hours of exposure to high heat loads, barrows have a significant lower feed intake, whereas gilts maintain feed intake levels similar to those under thermoneutral conditions. Over a prolonged heat exposure of 7 days, both sexes experience substantial declines in ADFI, with barrows reducing intake by 48% and gilts by 43% compared to ADFI under thermoneutral conditions. A similar pattern was observed for ADG, where gilts demonstrated a relatively better growth performance under high heat load conditions (Rudolph et al., 2024a). Notably, these observations were made in single-housed pigs, ensuring that competition for feed was not a factor. However, in commercial mixed-sex fattening systems, feeder competition may play a more significant role in influencing eating behaviour. During thermoneutral conditions, barrows spend more time at the feeder than gilts (Brown-Brandl et al., 2013b) a difference that persists and becomes more pronounced as temperatures rise (Cross et al., 2018).

The differences in feed intake and feeding behaviour between gilts and barrows may particularly influence the thermic effect of feeding, which directly impacts their overall heat production. However, no significant sex effect was found regarding energy maintenance requirements or FHP under standardized conditions (National Research Council, 2012; Noblet et al., 1999). That said, when considering lean-gain estimates, gilts and boars are likely to have greater maintenance requirements than barrows due to their higher rates of protein accretion (National Research Council, 2012). This suggests that while the thermic effect of feeding plays a role in heat production differences between gilts and barrows, variations in maintenance requirements may also arise indirectly from differences in growth composition.

1.3.2.4 Physiological status

Energy requirements, and consequently, heat production and thermoregulation, are influenced by physiological states (National Research Council, 2012):

- Growing pigs have relatively high maintenance energy requirements, ranging from 191-216 kcal/kg $BW^{0.6}$ (National Research Council, 2012) due to the energy-intensive demands of muscle mass.
- Gestating sows experience elevated maintenance needs, estimated of 100 kcal/$W^{0.75}$.
- The maintenance requirements of lactating sows are 5-10% greater than during pregnancy, around 110 kcal/$W^{0.75}$ (National Research Council, 2012; Noblet et al., 1990). Lactation increases a sow's metabolic rate (Eissen et al., 2000), resulting in higher heat production. This becomes particularly detrimental during periods of high heat load, because sows then reduce both feed intake and milk production to minimize THP and lower their UCT (Black et al., 1993).

As this doctoral thesis focusses on growing-fattening pigs, references to sows and their physiological and (re)production parameters will be discussed only briefly in the following sections.

1.3.3 Management-related characteristics

1.3.3.1 Diet composition and feed intake levels

Diet composition significantly affects an animal's THP due to the thermic effect of feeding, also known as the heat increment. This results from the energy costs during the digestion and absorption of nutrients, (National Research Council, 2012), which is subsequently converted into heat. Diets rich in fat generate a lower heat increment compared to those high in fibre or protein. Specifically, the heat increment for fat is approximately 15%, whereas it is 22% for carbohydrates and 36% for protein of the total metabolizable energy (Brown-Brandl et al., 2004). For example, Jorgensen et al. (1996) reported that heat production, expressed as a percentage of metabolizable energy, rose from 0.57 to 0.63 when dietary fibre increased from 59 to 238 g/kg of dry matter. Additionally, the heat increment and overall heat production are influenced by the amino acid (AA) profile of the protein in the diet. Matching the AA profile to the pig's growth requirements minimizes heat production, as excess AA's result in unproductive heat generation during their removal (Brown-Brandl et al., 2004). Further information on this topic can be found in Chapter 4B, where the effect of diet with a lower protein and higher fat content was investigated.

In addition to diet composition, feed intake volume plays a critical role in thermoregulation. Higher feed intake leads to a greater heat increment, as more energy is required for the digestion, absorption, and utilization of nutrients, which is then converted into heat (Brown-Brandl et al., 2004). Pearce et al. (2013a) demonstrated this relationship by comparing pigs under thermoneutral conditions. They showed that pair-fed pigs, whose intake matched that of heat-stressed pigs, exhibited a 0.6°C lower T_{rectal} compared to pigs fed *ad libitum*. This reduction in body temperature was attributed to the lower feed intake and the associated reduction in heat increment. It is also important to note that the heat increment values for fat, fibre, and protein are not constant but vary with feeding levels (Blaxter, 1989). Thus, both diet composition and feeding volume, as also their interaction, affect heat production and can therefore influence the UCT in pigs.

1.3.3.2 Housing conditions

The housing conditions of pigs influence thermoregulation achieved by behavioural changes. The LCT can be affected by the opportunity to huddle in group housing conditions (Gilbert et al., 2010). On the other hand, group housing may influence the UCT due to the space allowance. Group-housed pigs are less able to lose sensible heat due to the penmates' presence (Huynh et al., 2005a). More information on this topic is available in Chapter 5.

In addition to space allowance per animal, floor type is an important factor of housing conditions. Slatted floors are generally 3-5°C cooler than insulated solid floors (Randall et al., 1983), due to the cooling effect of the slurry pit beneath. As temperatures rise, pigs prefer slatted floors over solid floors, which were originally intended for lying and resting. This behavioural shift can lead to increased excretion on the solid floor, affecting hygiene and pen management (Aarnink et al., 2001). Transitioning to fully slatted flooring is possible within the regulatory guidelines for slat dimensions (Federale overheidsdienst volksgezondheid, 2003). However, such a shift raises welfare concerns, as fully slatted floors may not provide the same level of comfort and resting conditions as solid floors.

The material of the floor also plays a crucial role in heat transfer between the pig and its environment. This heat transfer is influenced by two key properties:

1) heat transmission coefficient, the rate at which heat is transferred from or to a surface per unit area and time, and
2) heat capacity, the amount of heat required to change the temperature of a given material by 1°C.

A floor with both a high heat transmission coefficient and high heat capacity will feel cooler, making it more effective in dissipating body heat, particularly during summer. Among commonly used flooring materials, solid concrete floors have the highest heat transfer (199 W/m²), followed by concrete slatted floors (186 W/m²), while cast iron (101 W/m²) and plastic floors (46 W/m²) have the lowest, excluding the potential impact of a slurry pit beneath. When a pig first lies down on a fully solid concrete floor, approximately 40% of its own heat production can be dissipated through non-evaporative heat transfer. This percentage is lower for other flooring types, making concrete the most effective material in providing thermal relief (Pedersen & Ravn, 2008).

2 Consequences of high heat load

High heat loads impact various parameters in pigs, such as altered behaviour, reduced feed intake, and increased T_{rectal}. These changes can be interpreted as adaptive mechanisms to maintain thermoregulation in response to elevated ambient temperatures. This thesis focuses on examining the effects of heat load by analysing how these parameters deviate from their baseline values under thermoneutral conditions, and how they help to enhance normal thermoregulation. However, the precise temperature thresholds at which these changes occur will not be addressed due to the complex interaction of factors influencing thermoregulation, as outlined in Section 3.

2.1 Behaviour

First of all, pigs show behavioural adaptations when temperatures rise. They increase their lying behaviour under higher heat loads (Huynh et al., 2005b) to minimize heat production from movement (thermogenesis due to physical activity), which would otherwise elevate their THP. In addition, to the decreased heat production, lying behaviour will also promote conductive heat loss, a heat transfer mechanism through direct contact between two surfaces, where energy flows from the warmest to the coldest surface. While the contact area between a pig's body and the floor is minimal when standing, it increases significantly when lying, with approximately 20% of the total body surface in contact with the ground (Collier & Gebremedhin, 2015). Furthermore, pigs also modify their postures to enhance heat dissipation (Mount, 1979a). Fattening pigs progressively shift from half-lateral and sternal lying positions to fully lateral lying as ambient temperatures rise. By lying on their sides, pigs maximize their contact with the cooler floor, increasing body surface area exposure (Huynh et al., 2005b). This will increase conductive heat loss even more. Lying locations also shift as heat load increases, with pigs favouring cooler slatted floors over solid ones. Additionally, pigs will avoid physical contact with pen mates (Huynh et al., 2005b), to minimize contact with pigs having the same skin temperature (T_{skin}).

Another natural behavioural adaptation for temperature regulation is wallowing in mud (Keeling & Jensen, 2009). In addition to cooling, this behaviour provides a barrier against insects and protection from harmful ultraviolet rays (McGlone, 1999). Mud stays on the skin for extended periods and retains moisture more effectively than water cooling alone (Ingram, 1965; Keeling & Jensen, 2009). On moderately warm days, pigs may stand in cool water, but as ambient temperatures rise, they will lie with their udders in the mud. Under more extreme heat loads, pigs cover their entire body in mud to enhance heat loss through evaporation. During hot days, 50% to 70% of their body surface may be coated in mud (McGlone, 1999). However, on conventional farms, pigs do not have access to mud. In such environments, they will wallow in their feces and urine to dissipate heat during high-temperature conditions, despite their usual tendency to avoid such behaviour (Huynh et al., 2005b).

2.2 Respiration rate

Pigs exposed to high ambient temperature will increase their RR in the first 24h (Renaudeau et al., 2010). Respiration rate is the first physiological adaptation to heat stress, following behavioural changes (Huynh et al., 2005a; Huynh et al., 2005b). Once pigs move out of their comfort zone, evaporative heat loss begins to increase. At this point, pigs primarily rely on respiratory routes for heat dissipation (Collier & Gebremedhin, 2015), as their ability to dissipate heat through transpiration via the skin is limited due to the limited functional sweat glands. Unfortunately, pigs are also limited in the respiratory evaporation by their small lung capacity (Straw et al., 2013) and indoor housing conditions.

Panting occurs in two phases, each playing a distinct role in thermoregulation:

- Phase 1 involves a controlled increase in respiratory rate accompanied by a decrease in breathing volume. This enhances ventilation in the upper respiratory tract while preserving alveolar ventilation. Increased upper respiratory tract ventilation will ensure evaporative heat loss (Robertshaw, 2006), as non-evaporative heat from the lungs warms the water vapor in the inspired air, effectively removing heat in the form of evaporative moisture (Collier & Gebremedhin, 2015).
- Phase 2 is triggered when the normal evaporative heat loss mechanisms are insufficient, and body temperature begins to rise. At this point, the respiratory pattern shifts: breathing volume increases, and respiratory rate decreases. Animals transition from closed-mouth to open-mouth breathing. Eventually, alveolar hyperventilation will occur. This shift can result in respiratory alkalosis due to excessive removal of carbon dioxide (Collier & Gebremedhin, 2015; Robertshaw, 2006).

2.3 Water intake

During hot periods, water loss increases due to the higher respiratory evaporation as cooling purpose (Patience et al., 2005). At the same time, the water content ingested through feed and generated by metabolism decreases. To maintain effective thermoregulation and water balance, pigs require additional water to support their heat exchange mechanisms (Mroz et al., 1995). Thus, as temperatures rise, the water intake increases. Additionally, water intake is also closely linked to feed intake. However, under conditions of high ambient temperature and RH, water intake becomes more independent of feed intake. This is evident from an increased water-to-feed ratio with rising temperature and/or RH (Huynh et al., 2005a; Mount, 1979b).

2.4 Feed intake

High heat loads negatively impact ADFI in growing and finishing pigs (Le Dividich et al., 1998; Renaudeau et al., 2011). According to some studies, a reduction in feed intake is reported as the primary adaptive response to a high heat load in animals (Collin et al., 2001b; Kemp & Verstegen, 1987; Quiniou et al., 2000). Pigs reduce their ADFI as it is one of the most effective mechanisms to decrease internal heat production, thereby maintaining thermal equilibrium when ambient temperatures approach the UCT (Collin et al., 2001b; Huynh et al., 2005a; Renaudeau et al., 2011). By decreasing feed intake, pigs reduce metabolic heat generated during digestion (heat increment of feeding) (Secor, 2009). However, the degree of reduction in ADFI varies considerably across studies, ranging from 40 to 80 g/day per °C (Renaudeau et al., 2011).

2.5 Skin temperature

During high heat loads, pig will show an increase in their T_{skin}. This is a result of blood repartition towards the skin to maximize radiant heat loss (Mayorga et al., 2019). Heat transfer from the skin to the environment relies on a temperature gradient between the animal and its surroundings (Collier & Gebremedhin, 2015; Mount, 1979c). When ambient temperature increases to approach or exceed the T_{skin}, this gradient diminishes, reducing heat loss and potentially leading to heat gain from the environment (Robertshaw, 1985). Consequently, T_{skin} can fluctuate rapidly, even under moderate environmental conditions (Huynh et al., 2005a).

Skin temperature is influenced by both internal physiological factors and external environmental conditions (Curtis, 1983; Renaudeau et al., 2010). To quantify these interactions, the Thermal Circulation Index (TCI) is a useful tool. The TCI estimates blood flow from the core to the skin and the associated heat transfer between the skin and the environment under specific thermal conditions:

$$TCI = (T_{skin} - T_{ambient})/(T_{rectal} - T_{skin})$$

Where $T_{ambient}$ is the ambient temperature at the time of measurement of T_{skin} and T_{rectal}. A lower TCI indicates a reduced capacity for non-evaporative heat transfer (Renaudeau et al., 2010).

2.6 Rectal temperature

Eventually, the T_{rectal} will increase with excessive heat loads. Rectal temperature, an indicator of core body temperature, is seen as the most relevant parameter to evaluate the animal's heat tolerance, because it specifies the level of homeothermy during heat stress periods (Renaudeau et al., 2010). Normally, core body temperature, in endotherm animals, is maintained constant during a wide range of ambient temperatures. This is done partly by adjusting metabolic heat production (Collier & Gebremedhin, 2015), like increasing the RR (Huynh et al., 2005a) or decreasing feed intake. However, outside the thermoneutral zone, the energy required to maintain homeothermy by adaptive thermogenesis cannot remain constant, as discussed before. This imbalance leads to an increase in body temperature, as indicated by a rise in T_{rectal}.

2.7 Daily gain and feed efficiency

The decline in ADFI contributes to reduced ADG in pigs during heat stress (Huynh et al., 2005a; Renaudeau et al., 2011). Slower tissue growth will reduce energy demands and associated metabolic heat production (Baumgard & Rhoads, 2013). However, reduced feed intake alone does not fully account for the observed decline in ADG. In a study by Pearce et al. (2013a), only one-third of the reduction in ADG during the first 24 hours of heat exposure was attributable to decreased feed intake, as shown in a pair-fed experiment. The remaining decline in ADG resulted from additional factors directly affected by the heat, like water loss, reductions in gastrointestinal tract content, muscle breakdown (high plasma Nπ-methylhistidine), adipose tissue mobilization (high plasma non-esterified fatty acids (NEFA) content) (Pearce et al., 2013a), and impaired nutrient absorption.

Under moderate heat stress conditions (20-30°C), FCR generally improves (Renaudeau et al., 2012b) or remains relatively stable (Renaudeau et al., 2011). The improvement can be attributed to reduced ADFI, where the animals still could maintain ADG (Rauw et al., 2020). When FCR remains stable, the reduction in ADG is proportional to the reduction in ADFI, suggesting that within this temperature range the decrease in ADG is directly linked to the decline in feed intake (Renaudeau et al., 2011). However, when the heat load is too high (for example > 30°C), FCR worsens due to insufficient nutrient absorption, which is primarily driven by a significant reduced feed intake (Renaudeau et al., 2012b). During severe heat stress, nutrient utilization shifts to prioritize maintaining euthermia (Mayorga et al., 2019). At this stage, direct effects of the heat load are observed, such as oxidative stress and increased intestinal permeability, commonly referred to as leaky gut syndrome.

The increased permeability of the intestine is primarily caused by a redistribution of blood flow, as circulation shifts toward the skin to enhance heat dissipation. However, this occurs at the expense of blood supply to the gastrointestinal tract, leading to reduced oxygen availability (hypoxia) in the visceral organs, including the intestines (Lian et al., 2020). Hypoxic conditions in the intestine disrupt the balance between reactive oxygen species (ROS), also called oxidants, and the antioxidant defence system, which is referred to as oxidative stress. Without sufficient antioxidant protection, excess ROS can damage the intestinal epithelial layer (Lambert et al., 2002). At the cellular level, the oxygen imbalance disrupts the tight junctions that regulate the paracellular space between adjacent cells, allowing pathogens and toxins to pass through the intestinal barrier. This disruption triggers an inflammatory response, leading to further detrimental consequences (Lian et al., 2020).

2.8 Metabolic parameters

Exposure to heat induces numerous changes in metabolic parameters in pigs. However, not all of these parameters have been fully identified, and the underlying reasons for some changes remain unclear. Nevertheless, several metabolic parameters have been reported in the literature as potential biomarkers—measurable indicators—to detect signs of heat stress. A short overview of the most commonly studied biomarkers is provided below.

Under high heat load, levels of HSP's increase significantly. Heat shock proteins, including HSP70 and HSP72, are a family of proteins that protect cells from various stresses and serve as generalized repair and stress resistance factors (Volloch & Rits, 1999). The presence of HSP70 is approximately 58% higher in pigs exposed to high heat compared to those in thermoneutral conditions. With prolonged and continuous exposure to high temperatures, HSP70 presence decreases slightly in time but remains significantly higher compared to thermoneutral conditions. This protein appears highly sensitive to even minor fluctuations in body temperature: for instance, pigs under restricted feeding, which consequently had lower body temperatures, showed reduced serum HSP70 levels compared to pigs fed ad libitum in the same thermoneutral environment (Pearce et al., 2013a).

Cortisol is another parameter present during stressful periods in an animal. The hypothalamic-pituitary-adrenal (HPA) axis will be activated and induce an increased cortisol concentration during a stressful condition (Silanikove, 2000). To cope with this stressor, the release of cortisol will provoke an increasing energy availability (Sapolsky et al., 2000), which can be detrimental when there already is a severe heat load present. Different studies showed that an acute heat exposure leads to stimulation of the HPA axis and associated cortisol release while heat acclimation probably will lead to a reduction in cortisol concentrations due to associated, unwanted increasing energy availability (Campos et al., 2017).

The concentration of thyroid hormones, like thyroxine (T_4) and triiodothyronine (T_3), in the pigs' blood may decrease during heat exposure (Campos et al., 2017). Thyroid hormones, produced by the thyroid gland, increase metabolism, which in turn increases metabolic heat production, an undesirable effect under high heat loads. Consequently, during heat acclimation, reduced circulating levels of T_3 and T_4 are observed, helping to diminish metabolic heat production (Bernabucci et al., 2010).

On the first day of heat exposure, plasma levels of NEFA rise significantly, reflecting the mobilization of adipose tissue, a response similarly observed in undernourished animals. This early mobilization of fat stores suggests that adipose tissue breakdown may partially contribute to the decrease in weight gain observed under high heat loads. However, NEFA levels in heat-stressed pigs decline as the duration of the heat exposure increases, reaching levels comparable to those in pigs maintained under thermoneutral conditions (Pearce et al., 2013a). Furthermore, elevated concentrations of N^{π}-methylhistidine, a biomarker indicative of skeletal muscle catabolism (Yoshizawa et al., 1997), have been observed when the duration of heat exposure increases, suggesting that prolonged and severe heat stress may even induce muscle tissue breakdown (Pearce et al., 2013a).

Insulin levels have been found to increase in heat-stressed animals, even when feed intake decreases during hot conditions. This response may represent a survival adaptation to high heat loads (Pearce et al., 2013a) or serve to activate and upregulate HSP's (Li et al., 2006). However, the precise underlying mechanism driving this metabolic change remains poorly understood (Campos et al., 2017).

Another very important sign of a high heat load is the occurrence of respiratory alkalosis. The pH of the blood, or the measured concentration of H^+ (hydrogen ions), must remain constant to ensure optimal metabolic regulation and maintenance (Kellum, 1999). Various processes can disrupt this balance (Kellum, 2000). When the pH decreases (increased H^+ concentration), it leads to acidosis, while an increase results in alkalosis (decreased H^+ concentration), where the blood becomes more basic. The pH of the blood is primarily regulated by the equation present in Figure 5.

On the left hand, the pH can be regulated via the lungs by adjusting the RR: faster, slower, deeper, or more shallow breathing. On the other side, the pH can be influenced by the kidneys, which either excrete HCO_3^- (bicarbonate) or retain H^+. The lungs predominantly regulate pH in the short term, whereas the kidneys play a more significant role over the long term. During a high heat load, a chain reaction occurs (Figure 5) (Kellum, 2000; Liu et al., 2018a):

1. Animals will begin to breathe more rapidly, entering a state of hyperventilation.
2. This rapid RR causes CO_2 to leave the body more quickly than normal, resulting in a decrease in the partial pressure of CO_2 (pCO_2) in the blood.
3. This imbalance reduces the production of H^+, leaving more HCO_3^- in the blood.
4. As a result, the pH increases and becomes more alkaline, leading to what is known as respiratory alkalosis; the increase of blood pH caused by the respiratory system.
5. Over time, the kidneys respond by removing excess HCO_3^- through excretion, to restore the balance.
6. Ultimately, this compensation leads to metabolic acidosis, as the pH will drop due to an indirect increase of hydrogen ions caused by the renal system.

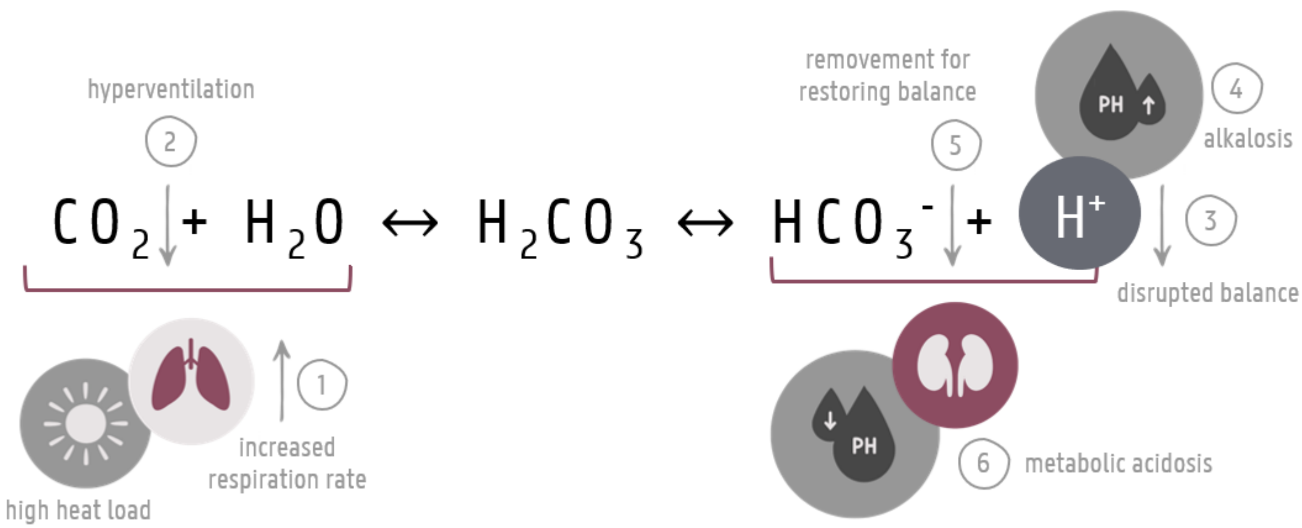

Figure 5: Simplified overview of respiratory alkalosis and metabolic acidosis during a high heat load

2.9 Clinical diseases and sanitary challenges

A high heat load may also influence disease and infection pressure in pigs. Elevated ambient temperatures combined with high RH create favourable conditions for the proliferation of vectors and pathogens (Johnson, 1997, 2012), which will create extra sanitary challenges. Consequently, global warming is expected to promote the development and spread of disease vectors, parasites, and pathogens (Thornton et al., 2009). This will likely lead to increased environmental pathogenic pressure, triggering a more pronounced immune response in pigs.

There are two hypotheses on how a high heat load may affect an animal's sanitary status:

1. The first hypothesis suggests that elevated ambient temperatures negatively impact the immune system during disease (Lacetera, 2012). Both heat stress and inflammatory challenges reduce feed intake and lead to physiological and metabolic disruptions. As a result, pigs may struggle to adapt or cope with these simultaneous stressors (Campos et al., 2017).

2. The second hypothesis suggests that earlier exposure to a high heat load, which leads to acclimation, could help animals better manage subsequent inflammatory challenges (Campos et al., 2014). When pigs experience heat stress, their HSP expression increases (Pearce et al., 2013a). These proteins play a protective role, maintaining cellular and tissue integrity during inflammation (Heidemann & Glibetic, 2005; Hotchkiss et al., 1993).

In summary, we may state that the timing of heat exposure is critical. Initial heat exposure combined with inflammatory challenges may be detrimental. However, if pigs acclimate to heat before facing inflammatory challenges, the impact of these challenges may be reduced due to the adaptive response initiated by the earlier heat exposure.

2.10 Carcass traits

As temperatures rise, carcass composition changes, with lower protein and higher lipid deposition. These changes result not only from reduced feed intake aimed at lowering heat increment but also from the direct effects of heat. Fattening pigs exposed to high heat loads (30°C) show lower protein deposition compared to those kept at thermoneutral conditions (23°C), even at the same feed and metabolizable energy intake levels. This may reflect a direct limitation of maximum protein deposition levels during heat stress (Bellego et al., 2016). These differences arise from variations in energy utilization under hot conditions. Protein deposition requires higher energy expenditure and generates more heat compared to lipid deposition. As a metabolic adaptation to reduce internal THP, pigs under heat stress will increase lipid deposition at the expense of protein deposition due to the more efficient energy use associated with lipid storage. Another factor that may contribute to fatter carcasses is the elevated insulin levels observed during heat stress, inhibiting the mobilization of adipose tissue (Pearce et al., 2013b). However, these differences in lipid and protein deposition may not be apparent in carcasses if the heat load is not severe (Rauw et al., 2020). Additionally, the weight of visceral organs in pigs subjected to high heat loads is reduced compared to thermoneutral pigs, regardless of feed intake. This reduction may be linked to decreased FHP (Koong et al., 1982), which may be an adaptation of heat-stressed pigs because a smaller visceral mass requires less energy than larger ones.

2.11 Meat quality

Acute and chronic heat stress can significantly affect meat quality in pigs. Acute stress before slaughter often leads to a rapid decline in pH while the carcass temperature remains high. This results in PSE (pale, soft, exudative) meat, which is characterized by reduced water-holding capacity, lighter colour, and decreased tenderness (Kowalski et al., 2021). This phenomenon is observed during acute heat stress, such as lairage conditions at 35°C, where the proportion of normal carcasses decreases while PSE carcasses increase. This effect is further amplified by higher RH (Santos et al., 1997). Chronic stress, on the other hand, can result in DFD (dark, firm, dry) meat, characterized by a high ultimate pH (pH_u), dark colour, and increased susceptibility to microbial spoilage (D'Souza et al., 1998; Kowalski et al., 2021). Chronic heat exposure, a stress-inducing condition evidenced by the higher cortisol levels, (Campos et al., 2017), may therefore contribute to the development of DFD meat. The differences in meat quality are also seen in seasonal changes. Pigs fattened in a mild summer have been found to show higher intramuscular fat content (IMF), lower protein content, and reduced water-holding capacity compared to those fattened in autumn (Albert et al., 2024).

3 Heat limits for indoor-raised fattening pigs

The most relevant characteristics affecting the thermoregulation of pigs and the most important consequences of a high heat load have been summarized above. However, other factors undoubtedly influence the lower and upper critical temperatures. This complexity makes determining the precise critical points of the thermoneutral zone challenging, particularly the UCT, which is in the scope of our interest. Furthermore, the UCT lacks a clear definition at the moment (Ramirez et al., 2022). One approach to identifying the UCT is through inflection points (breakpoints) in a broken-line analysis of specific parameters (consequences of a high heat load), such as RR or ADFI. At the breakpoint, a parameter shows a sudden change, signalling the pig's inability to maintain its 'normal' thermal equilibrium, necessitating adaptive responses. For instance, Huynh et al. (2005a) identified breakpoints for RR (22.4°C), T_{rectal} (26.1°C), heat production (22.9°C), and voluntary feed intake (25.5°C) in group-housed fattening pigs (60 kg) during a one-day heat load under varying RH conditions (Figure 6). However, these breakpoints were influenced by the specific experimental settings, including the genetic background of the pigs and environmental conditions, as in all other studies. Furthermore, another critical question arises: does the breakpoint represent an adaptive response or is this already an indication of true thermal discomfort?

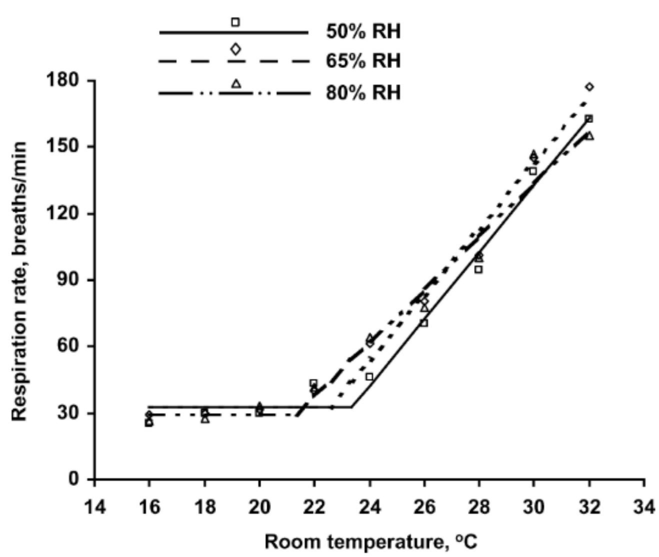

Figure 6: Example of broken-line relationship between ambient temperature and respiration rate during different relative humidity conditions. The breakpoints are between 21.3-22.4 °C, depending on RH (Huynh et al., 2005a)

To address these uncertainties, it is necessary to first define heat stress sensitivity in growing-fattening pigs and try to define the most important changing parameter during high heat loads, which will reflect the end of the thermoneutral zone.

3.1 Definition of 'experiencing heat stress'

In the present thesis we illustrate the effect of heat stress through an increase in body temperature as proposed by Mount (1979c). When an animal uses more energy to maintain homeothermy than can be compensated by heat loss mechanisms, this results in an uncontrolled rise in body temperature, indicating the presence of heat stress. Increased RR is the first physiological response to environmental heat (Huynh et al., 2005a), thus an initial increase in RR should be viewed as an adaptation rather than a sign of heat stress. Panting allows pigs to prevent a 3 to 4 °C rise in T_{rectal} and helps them to maintain a constant feed intake when the ambient temperature rises by a few degrees (Huynh et al., 2005a). Problems start to arise when an increase in RR is accompanied by a rise in T_{rectal}. This is visually seen by a shift from closed to open-mouth breathing, described in Section 2.2 (Collier & Gebremedhin, 2015). Renaudeau et al. (2010) also assert that T_{rectal} is the most relevant parameter for evaluating an animal's heat tolerance, as it indicates the level of homeothermy during high heat load periods. In terms of productivity, feed intake will reduced first with increasing heat load. This is seen as an adaptation to excessive heat, as it is the most effective mechanism due to reduced heat increment (Huynh et al., 2005a). This will eventually reduce daily gain, which is problematic for the farmer. Therefore, we consider in the present thesis that an excessive heat load becomes problematic when it leads to a reduction in growth.

This approach is in alignment with the findings of Brown-Brandl et al. (2001), Huynh et al. (2005a) and Vermeer and Aarnink (2023). Combining these results provides a chronological progression of changes in heat-stress-related parameters, as illustrated in Figure 7.

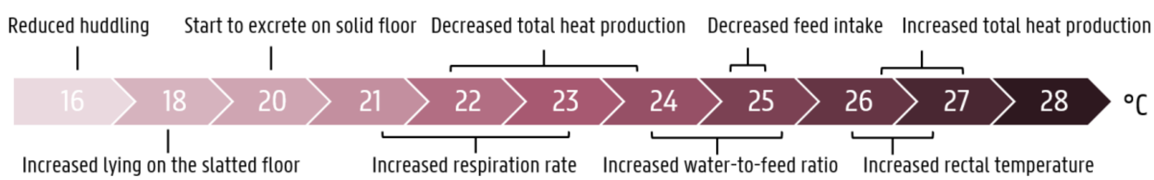

Figure 7: Chronological order of inflection points [°C] of changes in certain heat-stress related parameters. Adapted from Brown-Brandl et al. (2001), Huynh (2005) and Vermeer and Aarnink (2023)

3.2 Upper critical temperature points: what do we learn from literature?

Various methods can be used to evaluate the breakpoint in a broken-line analysis. The parameter of interest may differ, such as RR versus T_{rectal}, as well as co-variables influencing the model, including body weight or genetics. Table 1 provides an example overview of studies that have used broken-line analysis to determine breakpoints for specific parameters.

Additionally, discussions often arise regarding whether the identified breakpoint represents the evaporative or the upper critical temperature. To ensure consistency within this thesis, we categorized the boundaries according to our previously stated definition of 'experiencing heat stress'. The breakpoint for T_{rectal} is always considered the UCT, as changes in this parameter indicate a failure to maintain homeostasis, representing the end of the thermoneutral zone. In contrast, the first breakpoints for parameters such as RR and ADFI are categorized as ECT, since changes in these parameters can temporarily maintain body temperature within a stable range for a few degrees. For heat production (HP), the classification depends on the observed breakpoint. If HP decreases after the breakpoint, it signifies the ECT. However, if HP increases following the breakpoint, it indicates that the system has reached, or surpassed, the thermoneutral zone, representing the UCT.

According to the European project "Monitoring and Optimising Climate in Pig Houses," which collaborated with climate experts from Belgium and the Netherlands, the recommended set-point temperature of the climate system varies throughout the fattening period. On the start of the fattening phase at day 0 (±25 kg), pigs require a set-point temperature of 25°C. By day 5 (±30 kg), this should be adjusted to 22 °C, with a bandwidth of 5–6 °C. For growers at day 50 (±75 kg) and finishers at day 100 (±125 kg), the set-point temperature should be 20 °C, also with a bandwidth of 5–6 °C (Varkensloket, 2025). The set-point temperature marks the beginning of the thermoneutral zone (LCT), while the upper limit of the bandwidth (summed up with the set-point temperature) represents its endpoint (UCT), at which maximum ventilation is required. Based on these recommendations, the summarized values in Table 1 for starters (25–60 kg) and growers (60–90 kg) are comparable, as their LCT and UCT are similar. However, for finisher pigs (90–125 kg), the LCT from literature is lower than the values suggested by climate experts. Overall, literature suggests a thermoneutral zone ranging from 18.2°C to 26.3°C throughout the entire fattening period.

Table 1: Overview of several studies evaluating the inflection points of various parameters under increasing heat load. Empty fields indicate missing information from the respective studies.

Reference	Animal category	Genetics	Housing	Parameter	RH (%)	LCT (°C)	ECT (°C)	UCT (°C)
Verstegen et al. (1987)	Starters (40kg)			ADFI			23.6	
Renaudeau et al. (2011)	Starters (50kg)		Group			19.5		
Quiniou et al. (2001)	Starter-growers (30-90 kg)	Piétrain x Large White	Group		70	23.5	25.0	
Holmes and Close (1977)	Growers (60kg)			HP		18.0		
Renaudeau et al. (2007)	Growers (60kg)	Creole/ Large White	Individually	T_{rectal}	80			27.0
Renaudeau et al. (2007)	Growers (60kg)	Creole	Individually	RR	80		27.5	
Renaudeau et al. (2007)	Growers (60kg)	Large White	Individually	RR	80		25.5	
Brown-Brandl et al. (2001)	Growers (60kg)	Yorkshire x landrace	Individually	RR	40		23.1	
Brown-Brandl et al. (2001)	Growers (60kg)	Yorkshire x landrace	Individually	T_{rectal}	40			26.8
Brown-Brandl et al. (2001)	Growers (60kg)	Yorkshire x landrace	Individually	HP	40			26.6
Adapted from Holmes and Close (1977)	Growers (60kg)		Individually					29.0
Adapted from Holmes and Close (1977)	Growers (60kg)		Group			16.0		
Huynh et al. (2005a)	Gowers (60kg)		Group	RR	50-80		22.4	
Huynh et al. (2005a)	Gowers (60kg)		Group	T_{rectal}	50-80			26.1
Huynh et al. (2005a)	Gowers (60kg)		Group	ADFI	50-80		25.5	
Huynh et al. (2005a)	Gowers (60kg)		Group	HP	50-80		22.9	
Renaudeau et al. (2011)	Finishers (90 kg)			ADG				22.9
Adapted from Holmes and Close (1977)	Finishers (100 kg)		Individually			17.0		29.0
Comberg et al. (1972)	Finishers (100 kg)	German Landrace	Individually	Performance			24.0	
Adapted from Holmes and Close (1977)	Finishers (100 kg)		Group			15.0		
Total mean						18.2	24.6	26.3
Mean starters						21.5	24.3	-
Mean growers						19.2	24.8	26.4
Mean finishers						16.0	24.0	26.0

LCT = Lower Critical Temperature, ECT = Evaporative Critical Temperature, UCT = Upper Critical Temperature, RH = Relative Humidity, ADG = Average Daily Gain, RR = Respiration Rate, T_{rectal} = Rectal Temperature, ADFI = Average Daily Feed Intake, HP = Heat Production

4 Heat stress-reducing measures

There are numerous strategies available to mitigate heat stress in fattening pigs, making it impractical to summarize them all within this thesis. Instead, this section provides an overview of commonly used strategies, either observed on commercial farms or tested on the experimental farm during this PhD research. A key question that frequently arises is: "*Which measure is the most effective for my farm?"*. Unfortunately, there is no universal answer, as the choice of strategy depends on several factors, including the facility's design and infrastructure (Arcidiacono, 2018), the farmer's objectives and the associated costs.

To provide some guidance, Schauberger et al. (2020) attempted to quantify the efficacy of various heat stress mitigation strategies using expert estimations or model-based analyses. It is important to note that these estimations are based on a reference facility with mechanical ventilation and well-insulated walls and roofs. Consequently, the effectiveness of structural adaptations, such as roof and window modifications, may vary depending on the baseline insulation quality of the facility. A summary of the estimated efficacies of the strategies discussed in this thesis is presented in Table 2.

Table 2: Overview of the efficacy of different heat stress reducing strategies using expert estimations or model-based analyses based on a reference facility with mechanical ventilation and well-insulated walls and roofs. From Schauberger et al. (2020)

Heat stress reducing strategies	**Efficacy (%) in reduction of heat stress parameters compared to a reference system**
Management	
Feed adaptations	20-30
Cooled drinking water	5-11
Shifting feeding time	34-51
Increasing space allowance	4-11
Altering genetic selection	13-30
Adapting roof and window properties	3-8
Improving ventilation	23-44
Climate-technical	
Non-evaporative	
Forced air velocity	10-24
Heat exchangers	82-100
Earth-air	93-100
Water-water	82-97
Floor cooling	20-40
Evaporative	
Fogging	42-62
Pad cooling	61-86

4.1 Management measures

There are numerous management measures available to mitigate heat stress in growing-fattening pigs. However, this thesis focuses primarily on low-investment management strategies. Measures requiring complex structural changes, such as modifying floor type, floor material, orientation of the building or insulation of the roof are not included, as these are primarily relevant for newly built facilities. Implementing such changes in existing farms often involves high costs, making them less feasible for short-term use.

4.1.1 Feed adaptations

4.1.1.1 Feed additives

Feed additives can play a role in reducing the negative effects of heat stress in pigs. Antioxidants such as selenium (Se) and vitamin E help combat oxidative stress caused by an imbalance between free radicals and antioxidant capacity, which often results from heat-induced blood redistribution (Collin et al., 2001a; Cottrell et al., 2015). Osmolytes like betaine enhance intestinal health, promote nutrient absorption, and improve water retention in cells, mitigating osmotic stress that accompanies high heat loads (Pearce et al., 2013b; Ratriyanto et al., 2009). While studies have reported promising results with various feed additives, inconsistencies remain regarding their effectiveness, especially concerning dosage, form, and simultaneous supplementation. Additionally, regulatory constraints on permitted doses and the cost of organic alternatives can limit their application in practice. This management strategy is described in detail in Chapter 4A and 4B.

4.1.1.2 Altered chemical composition

Adjusting the chemical composition of feed is another nutritional strategy. The heat increment associated with protein metabolism is higher than that of carbohydrates or fat, making protein reduction a potential method for lowering internal heat production (Morales et al., 2019; Musharaf & Latshaw, 2019). However, maintaining AA balance is essential to prevent performance losses, which can be achieved by supplementing crystalline AA's (Kerr et al., 2003). While altering diet composition has shown positive effects in some studies, challenges remain regarding the balance between heat reduction and maintaining optimal nutrient availability for growth and performance. This measure is further discussed in Chapter 4B.

4.1.1.3 Shifting feeding time

Animal activity, including eating, has a diurnal pattern with peaks in activity often occurring in the evening, which coincides with the hottest part of the day and maximum outdoor temperatures. Reducing activity and feeding during these hours can help minimize energy expenditure, heat production due to physical activity and heat increment of feeding, thereby alleviating heat stress effects. Adjusting the light regime or feeding later in the evening is a practical approach to achieve this. However, implementing such a shift requires adapting the entire facility's schedule to minimize disturbances, including noise, and may lead to additional labour costs (Schauberger et al., 2020). Despite these challenges, this strategy is relatively easy to implement.

4.1.2 Drinking water adaptations

Some studies suggest that providing chilled drinking water can enhance animal performance by reducing body temperature through heat absorption. For instance, a heavy fattening pig (120 kg) can experience an 8.5% reduction in THP when given chilled water. However, this effect depends on adequate water intake (15 L/day) and sufficiently low water temperatures (10°C) (Jeon et al., 2006). Water can be cooled using a heat exchanger, which is explained further. However, this method can require a lot of energy. A more sustainable approach to maintain more constant drinking water temperatures involves insulating pipelines and minimize exposure to indirect solar radiation. There is also a possibility to add certain additives to the drinking water of the pigs, like previously mentioned antioxidants and electrolytes.

4.1.3 Increasing space allowance

Huynh et al. (2005b) recommend increasing the physical space available for pigs as temperature rises, as this allows for lateral lying and reduces contact with other pigs. However, the effectiveness of this strategy during high heat loads remains debated in literature. Schauberger et al. (2019) argue that increasing space by 20–40% has limited effectiveness compared to climate-control systems and results in higher costs due to lost revenue. In contrast, White et al. (2008) found that expanding space by 28% (0.66 vs. 0.93 m^2/pig) could alleviate 50% of the negative effects of heat stress on growth performance. This topic is further discussed in Chapter 5.

4.1.4 Altering genetic selection

Genetic selection on traits which are related to heat-stress tolerance could offer a long-term solution but often comes at the expense of reduced productivity under thermoneutral conditions (Mayorga et al., 2019). For example, the Belgian Piétrain, Belgium's dominant terminal sire line, has higher metabolic heat production due to its lean meat content, making it less heat-tolerant (Renaudeau et al., 2011). In contrast, tropical breeds like Creole and Iberian pigs show better heat stress tolerance but have slower growth and fatter carcasses, limiting their economic viability in temperate climates (Gourdine et al., 2021). Identifying and selecting genes linked to thermoregulation is a complex process that requires time and may introduce unintended trade-offs. The potential of genetic selection to improve heat stress resilience is explored in detail in Chapter 6.

4.1.5 Adapting roof and window properties

Besides complex structural modifications, there are smaller, more easily implementable strategies to reduce both direct and indirect solar radiation on the facility's roof and windows. To minimize indirect solar radiation from a poorly insulated roof, applying white paint can increase reflectivity. Roof irrigation is another option to enhance evaporative cooling (Schauberger et al., 2020), though it requires significant water consumption. Additionally, green roofs can also promote evaporation (Yeom & La Roche, 2017), but their installation and maintenance costs are relatively high. To reduce direct solar radiation through windows, which can contribute up to 3% of additional heat load, green vegetation or other shading methods, like painting the windows white, with solar control coating or foil, may be beneficial (Schauberger et al., 2020).

4.1.6 Improving ventilation

The efficiency of a ventilation system in animal housing depends largely on the inlet design, as it influences air distribution and movement (Bjerg et al., 2002). Ceiling or wall inlets with downward airflow have been shown to effectively reduce heat stress effects in pigs. An optimal design would include a wall inlet with additionally downward flow function, besides the horizontal and rising air flow functions (Bjerg & Zhang, 2013). Downward airflow improves convective heat transfer by delivering air directly to the animal-occupied zone, ensuring higher airspeed near the pigs. Compared to upward inlets, downward inlets do not alter overall temperature distribution but improve airflow patterns, enhancing convective heat transfer of group-housed growing-fattening pigs (Li et al., 2018). Increasing the summer ventilation rate can further enhance convective heat transfer, as higher airspeeds promote air mixing (Li et al., 2018), which is beneficial for climate control. However, increasing the ventilation rate often requires additional investments to adjust fan capacity of the already existing mechanical ventilation system.

4.2 Climate-technical measures

Climate-technical measures involve air treatment systems that regulate the indoor environment. These strategies typically require structural modifications and higher investment costs compared to management-based approaches. However, they offer effective solutions for improving climate control within pig housing.

4.2.1 Non-evaporative cooling

The term non-evaporative or "dry" cooling refers to cooling systems that operate without the use of adiabatic processes. In these systems, no direct humidity is added into the environment to reduce the indoor temperature of the facility. However, indirect humidity may occur in these systems as a result of condensation.

4.2.1.1 Forced air velocity

To enhance convective heat transfer, additional axial fans can be installed within the animal compartment to increase local airspeed. These fans do not affect the overall ventilation rate, but improve airflow at specific locations. However, a potential drawback of this measure is the uneven distribution of airspeed when fans are misplaced, which may create drafts and lead to welfare concerns. To prevent discomfort, it is recommended to not exceed the critical threshold of 0.6 m/s at animal level (Schauberger et al., 2020).

4.2.1.2 Heat exchangers

Heat exchangers come in various designs, but their fundamental working principle remains consistent. As the name implies, a heat exchanger transfers energy in the form of heat between two sources, cooling in the summer or warming in the winter. The 'new' cooled medium flows through small tubes, often located near the air inlet. When warm outside air passes over these cooler tubes, heat is exchanged again: the warm air loses its heat to the cooler tubes, resulting in a reduction in air temperature before it enters the facility (Figure 8). One advantage of heat exchangers is their reversible functionality, allowing them to heat the facility during colder months.

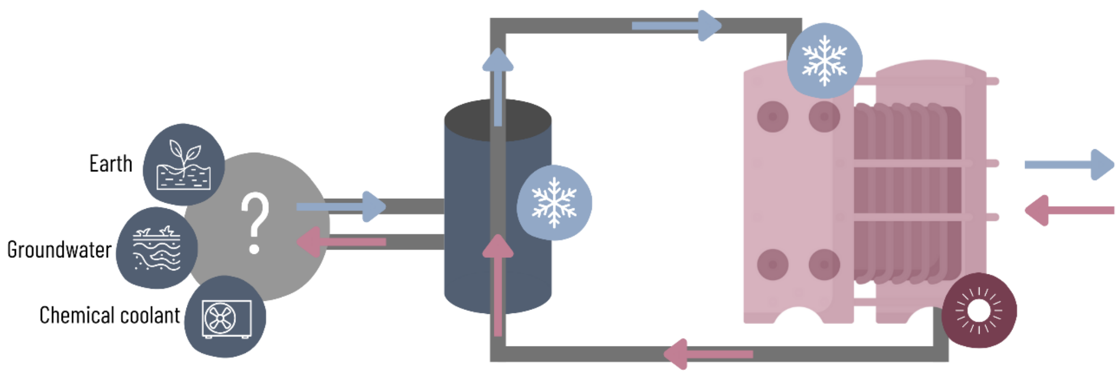

Figure 8: Schematic representation of a heat exchanger, in which a closed circuit is cooled by an external source, such as earth, groundwater, or a chemical coolant. The cooled medium in the circuit (air or liquid) then flows through fine tubes, allowing heat to be exchanged with the surrounding air.

The earth-to-air heat exchanger system utilizes the ground as a natural heat storage medium. Outside air is drawn through long, underground tubes at a significant depth, where it exchanges heat with the surrounding soil. The system's efficiency depends on factors such as the tube diameter, length, and burial depth. Due to the temperature differential, condensation may occur inside the tubes. This system is highly effective: for instance, a model simulation demonstrated a temperature reduction of 10.4°C between the outside air and the inlet air (immediately after passing through the cooling system). Additionally, there was a 1.8 hPa drop in vapor pressure, indicating a decrease in RH (Vitt et al., 2017).

A water-to-water heat exchanger utilizing groundwater is another viable option for cooling (Huynh et al., 2004). Similar to the previously mentioned earth-to-air heat exchanger, this system relies on geothermal cooling but uses groundwater as the primary source. The temperature of groundwater, depending on the depth of the fine coils lying in the groundwater, typically remains close to the annual mean temperature (11°C in Belgium (KMI, 2022)) and remains stable throughout both winter and summer (Samuel et al., 2013). However, a major limitation of this system is groundwater availability, (Schauberger et al., 2020), which may be insufficient during the summer season.

A heat exchanger can also operate with a chemical coolant as the cooling medium, often combined with a buffer tank to store the cooled liquid. This setup allows the cooled water to circulate through small tubes when needed, providing flexibility in heat exchange. Unlike geothermal or groundwater-based systems, this method is not reliant on soil temperature or water availability but used electricity which makes it less environmentally sustainable.

4.2.1.3 Floor cooling

Another method of dry cooling is cooling the solid concrete floor. This approach can help reduce the number of pigs lying on the slatted floors during high ambient temperatures. However, to encourage this behavioural shift, it is crucial to ensure that the cooled solid floor remains at the same or a lower temperature than the slatted floor. Studies have shown that pigs housed with a cooled partly-solid floor had higher feed intake and growth rates compared to those without floor cooling, suggesting a reduction in heat stress effects (Huynh et al., 2004). Floor cooling can be achieved using a heat exchanger system. For instance, Huynh et al. (2004) implemented polyethylene plates and piping beneath the solid floor, circulating cooled water through a water-water heat exchanger, as describes before, to regulate floor temperature effectively.

4.2.2 Evaporative cooling

The term ‘evaporative cooling’ refers to systems that utilize adiabatic cooling, where non-evaporative heat (temperature) is transformed into evaporative heat (humidity) through the process of evaporation, thereby lowering the air temperature (Vitt et al., 2017). These systems often lead to an increase in air humidity due to the addition of water into the environment. Therefore, it is crucial that these systems are equipped with a reliable RH sensor to monitor humidity levels. The sensor should automatically deactivate the cooling system if the RH within the facility or outside reaches high levels. In direct evaporation systems, the air that eventually reaches the animal compartments is directly humidified during the cooling process.

The easiest direct evaporative cooling system is fogging or misting. These systems cool the air through water droplet evaporation, with the evaporation rate primarily depending on droplet size. High-pressure fogging systems generate smaller droplets (10-30 µm) and evaporate more quickly, whereas low-pressure misting systems produce larger droplets (around 60 µm) (Schauberger et al., 2020). Both methods increase indoor air humidity, making it essential to monitor and regulate their use. To prevent excessive humidity and potential higher heat load, the system should be turned off when RH approaches critical levels during high ambient temperatures, such as 80%.

Another form of direct evaporative cooling is the use of cooling pads or pad cooling. In this system, large cellulose (or other materials like plastic or metal) pads with textured surfaces to increase surface area are installed either directly in the walls of the facility or in front of the air inlets. Water continuously flows over these pads, while warm air passes through them. The heat from the air causes the water to evaporate. This energy shift will reduce the air temperature as a result. However, since the air comes into direct contact with the flowing water, its humidity increases. This system is quite effective: for example, a model predicted that the inlet air temperature could drop by 5.2 °C compared to the outside temperature, though this reduction was accompanied by an increase of 3.1 hPa in vapor pressure (Vitt et al., 2017). For optimal performance, proper maintenance is essential, and regular monitoring of water quality is required (Schauberger et al., 2020), as these systems are hotspots for Legionella bacteria and other micro-organisms (Samuel et al., 2013). Additionally, after the system is turned off, the cooling effect may persist for some time due to residual moisture in the pads, which could lead to unintended climate fluctuations. However, compared to fogging systems, properly designed, operated, and maintained pad cooling systems only influence conditions at the air inlet rather than directly at the animal level. Moreover, these systems offer additional benefits, such as improved biosecurity by air filtration as it reduces dust and bioaerosols levels, lower daily temperature fluctuations inside the facility, and reduced water consumption (de Alencar Nääs et al., 2006; Panagakis & Axaopoulos, 2006).

CHAPTER 3 | ARTIFICIAL HEATING PROTOCOL

Adapted from: De Prekel, L., Maes, D., Van den Broeke, A., Ampe, B., & Aluwé, M. (2024). Evaluation of a heating protocol and stocking density impact on heatstressed fattening pigs. *Animal*, *18*(6), 101172. https://doi.org/https://doi.org/10.1016/j.animal.2024.101172

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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. increasing space allowance) 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. Forty 20-week-old barrows weighing 96.5 ± 7.3kg (W_{100}) and forty 17-week-old barrows weighing 72.7 ± 9.9 kg (W_{70}) were housed in two compartments. An artificial heat wave (heat load) was induced for three days. During 3-day periods before, during and after the heat load, physiological parameters (RR, T_{rectal}, T_{skin}) and behaviour) were measured and average daily feed intake was observed. Ambient temperature, RH and THI were monitored. During the heat load, THI reached ≥75 (78.4), even when RH decreased to ±45%. Every physiological parameter showed significant increases during the heat load. 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}. According to the climate data and physiological parameters of the pigs, the protocol for conducting an artificial heat wave was successfully generated and could be replicated effectively, even under varying environmental conditions.

Keywords

Space allowance, Rectal temperature, Respiration rate, Animal welfare, Temperature-humidity index

1 Introduction

Performing induced heat wave trials is a useful way to test various heat stress mitigating measures in a controlled and efficient manner. However, it's 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. Some studies have used artificial heating protocols without testing physiological reactions related to animal welfare such as RR or T_{rectal} in animals subjected to high temperatures (Collin et al., 2001b; 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 UCT drops according to age or body weight (Quiniou et al., 2000). Therefore, accurate evaluation and validation of artificial heat waves is crucial for research. In this study we investigated the suitability and validation of an artificial heating protocol on pigs of different weights.

2 Material and methods

2.1 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 during three days. During three days before (pre-heat), during (heat) and after the heat load (post-heat 1), the physiological parameters of the animals were observed. Fattening pigs of two consecutive weaning batches (three-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) (Figure 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.

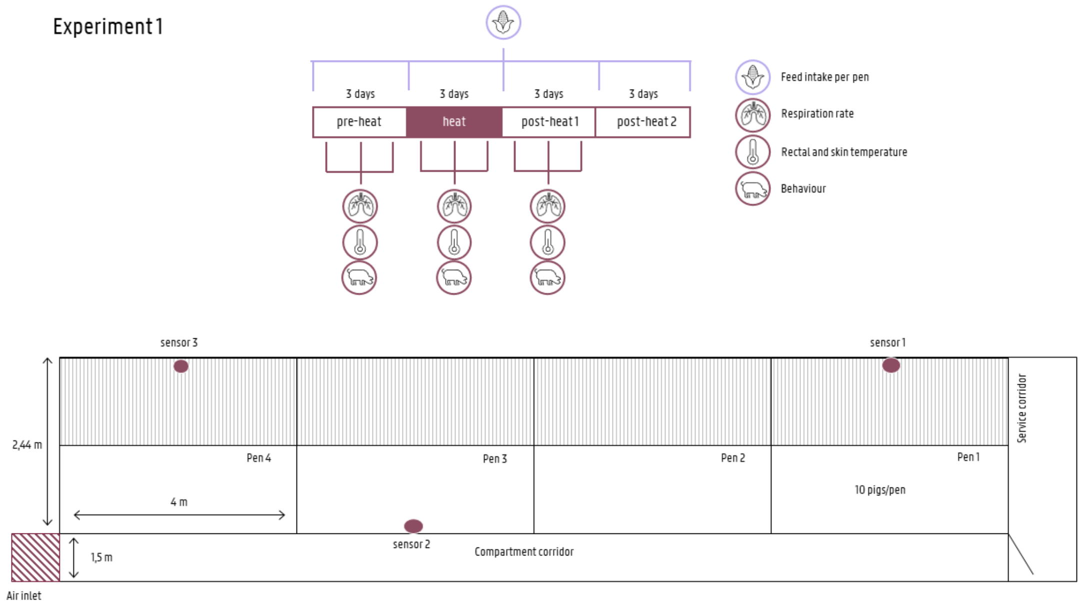

Figure 1: Schematic overview of the experimental design (timeline and follow-up of the different parameters of the fattening pigs) and the compartments.

2.2 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 centre 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 with additional natural light emanating from one window (2.07m²) on the north side of the compartment of W_{70} and on the south side of compartment W_{100}.

2.3 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 30-40% 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 3000 m³ via a heating duct (6m, Ø 0.4m). Throughout the artificial heat wave, the temperature remained consistent at around 31 °C day and night. A more extreme heat condition without cooler nights was deliberately chosen to clearly assess the effect of the tested heat stress-reducing measures within a short time frame. The reasoning was that if a measure is not effective under severe heat stress, it is unlikely to be useful during milder conditions when animals can recover at night. By applying a short but intense heat load, the effectiveness of each measure could be evaluated under the most challenging circumstances. Two climate sensors (FarmConnect™, Stienen, Nederweert, The Netherlands) were placed in the centre 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 RH, ambient temperature, CO_2 and NH_3 concentration every 10 minutes for the entire trial period. CO_2 and NH_3 values were validated before start with a portable sensor (Dräger X-am 700, Drägerwerk AG & Co. KGaA, Lübeck, Germany). The data from the climate sensors was used to calculate the THI. This value combines ambient temperature and RH according to the following formula (1):

$$THI = 0.72\times T_{DB} + 0.72\times T_{WB}+40.6$$

$$T_{WB} = T_{DB}\times \tan^{-1} (0.151977\times \sqrt{(RH + 8.313659)})+ \tan^{-1} (T_{DB}+ RH) - \tan^{-1} (RH - 1.676331)+ 0.00391838 \times RH^{3/2}\times \tan^{-1}(0.023101\times RH) - 4.686035 \quad (1)$$

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 behaviour of pigs during handling and transport, i.e. $75 \leq THI < 79$: warning for heat stress, $79 \leq THI < 84$: danger for heat stress, $THI \geq 84$: great danger for heat stress (NWSCR, 1976).

2.4 Physiological parameters and behaviour

Four reference animals per pen were randomly selected to monitor the physiological parameters individually throughout the study: RR, T_{rectal} and T_{skin}. The same reference animals, who were individually colour marked and tagged, were evaluated throughout the trial. The parameters were measured daily before (pre-heat), during (heat) and after (post-heat) the artificial heat wave. The observations started at 12:00 (until 17:00) and were performed by the same observers. Respiration rate (breaths/min) was scored visually based on the number of flank movements per 30 seconds multiplied by two. Respiration rate was only evaluated when a pig was lying in a resting position. Rectal temperature (°C) was measured in the pen using a digital thermometer (Veterinär-thermometer SC12, Scala electronics GmbH, Stahnsdorf, Germany) for approximately 15 seconds. Observations of pigs with fever due to infected tails or wounds were excluded from the dataset.

Skin temperature (°C) was measured using an infrared (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., 2013a; 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 analysed using Testo software (Testo, Almere, The Netherlands) which calculates the average ($_{Tskin,A}$), 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. (2013a). Behaviour 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 behavioural assessment then continued in the other pens. After evaluating all pens, the observer waited three minutes before starting with the first pen again. In total, the behaviour was evaluated ten times per pen per observation day. Afterwards, the average of all the behaviour results was calculated and converted into a percentage.

Table 1: Definitions of active and inactive behaviour of fattening pigs

Animal behaviour (revised from Ekkel et al. (2003))		
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	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.

2.5 Performance parameters

Feed intake per pen was measured every three days before, during and after the heat load, as well as for an extra 3-day period after the post-heat period (post-heat 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 three days), residual feed was weighed. Based on this data, ADFI per animal was calculated.

2.6 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}), behaviour (passive, active, and eating/drinking) and performance parameters (ADFI), QQ-plots and histograms of the residuals were visually evaluated. No relevant deviations from normality were observed. The effect of the weight group (W_{70} and W_{100}), heat load (pre-heat, heat and post-heat 1 (and post-heat2 for ADFI)) 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 behaviour, active behaviour, eating/drinking behaviour and ADFI), HL = heat load as independent variable (pre-heat, heat, post-heat 1 and post-heat 2), WG = weight group as independent variable (W_{70} and W_{100}), β =vector of the fixed effects, Z = design matrix of random effects (for the physiological parameters: observation date and the pig- identification number (ID); for behaviour 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 $p \leq 0.05$. If the interaction term was significant for a specific variable, it indicated that the weight groups reacted differently to the heat load. Post hoc tests according to the Kenward-Roger degrees of freedom approximation on heat load within the weight group and weight group within heat load were performed when significant *p*-values were found.

3 Results

3.1 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 RH was 46.7 % (min. 41.4 % - max. 51.3 %) in both weight groups. A slight difference in the temperature and RH values was noted among the three sensors (Figure 2). The average RH dropped below 50 % during the heat load, while it was 58.7 % (min. 50.9% - max. 63.3%) in the pre- and post-heat 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 W_{70} and W_{100} (Figure 3). The THI during the heat period of W_{100} (78.8) reached slightly higher values than in W_{70} (78.0). Despite the change in ventilation during the heat load, the CO_2 levels during the heat period remained below 3000 ppm (Figure 4). A single peak in CO_2 occurred at the beginning of the artificial heat wave but dropped relatively quickly. The NH_3 concentration of both compartments during the entire trial period exceeded 25 ppm (Figure 4). However, the NH_3 levels did not show a notable increase during the artificial heat wave compared to the periods before and after heat load when the mechanical ventilation operated under normal working conditions (no restricted ventilation rates).

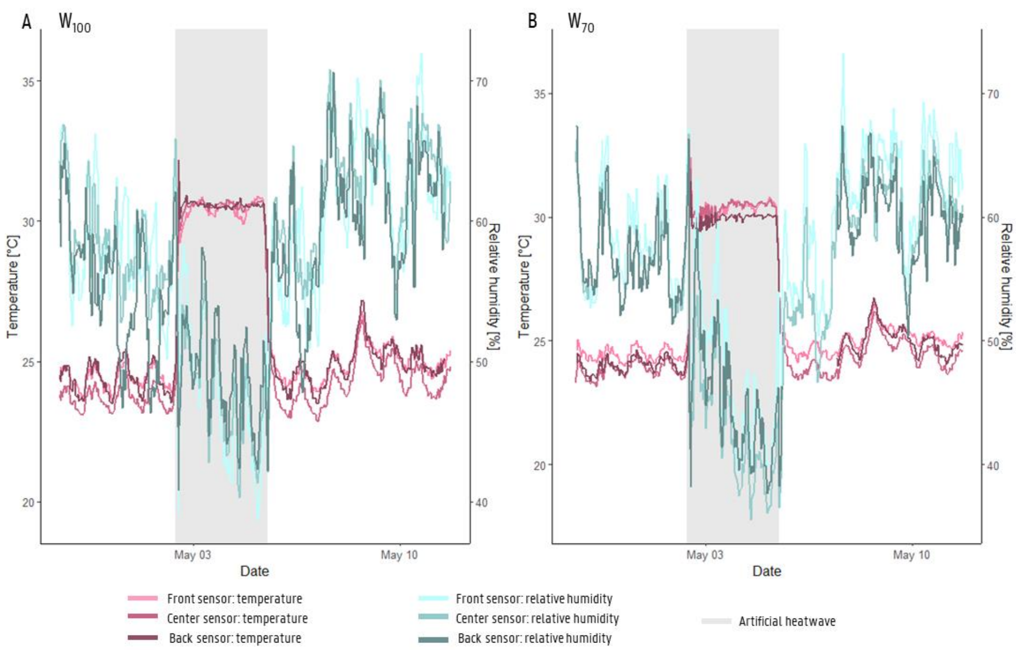

Figure 2: Ambient temperature and relative humidity of the front, centre and back sensors with a ten-minute interval during the trial in the compartment of A) W_{100} (forty 20-week-old fattening pigs of 96.5 ± 7.3 kg) and B) W_{70} (forty 17-week-old fattening pigs weighing 72.7 ± 9.9 kg)

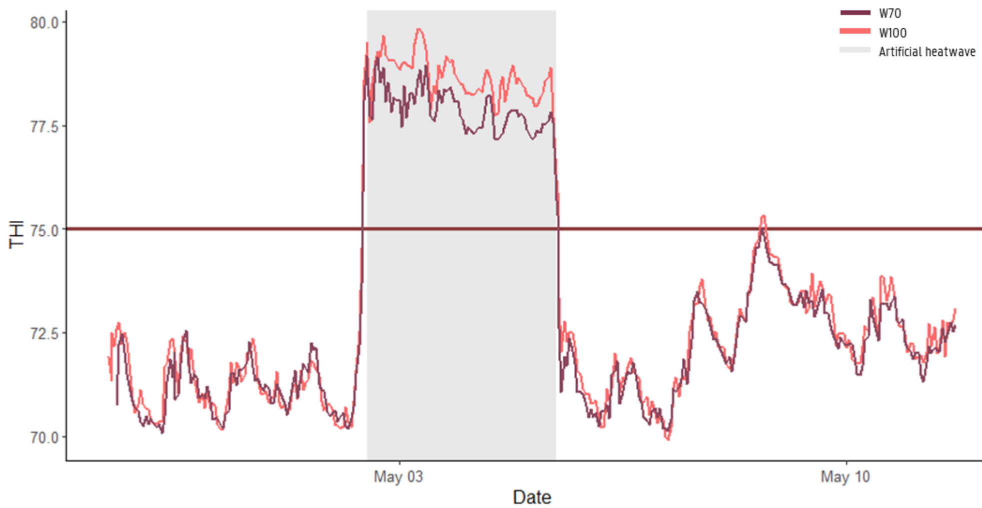

Figure 3: Average Temperature-humidity index (THI) of the front, centre and back sensors with a ten-minute interval during the trial in the compartment of W_{100} (forty 20-week-old fattening pigs of 96.5 ± 7.3 kg) and W_{70} (forty 17-week-old fattening pigs weighing 72.7 ± 9.9 kg)

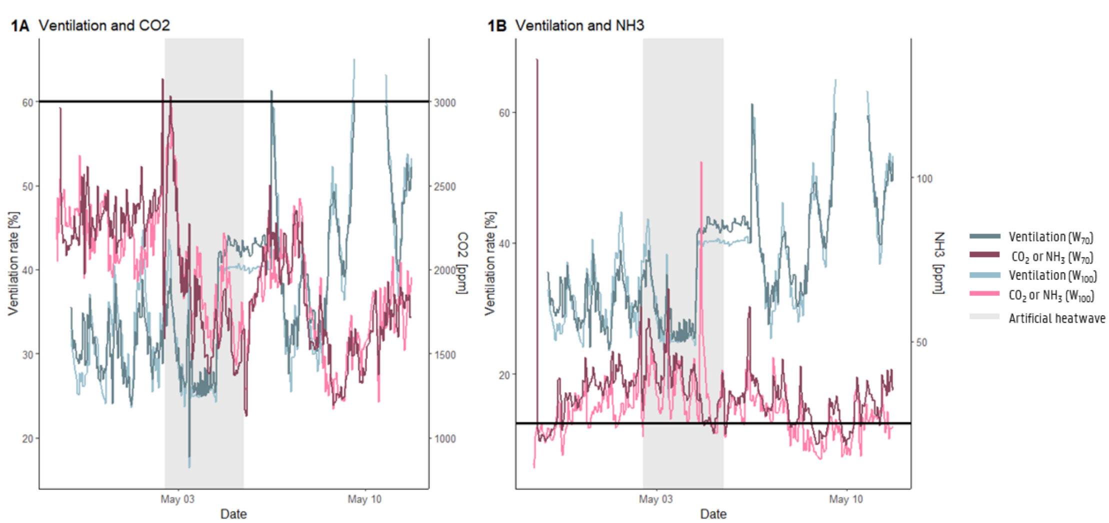

Figure 4: A) Ventilation rate [%] and average CO_2 [ppm] and B) ventilation rate [%] average NH_3 [ppm] with a ten-minute interval during the entire trial in the compartment of W_{100} (forty 20-week-old fattening pigs of 96.5 ± 7.3 kg) and W_{70} (forty 17-week-old fattening pigs weighing 72.7 ± 9.9 kg)

3.2 Physiological parameters and behaviour

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 ($p < 0.001$). For T_{rectal}, no differences between W_{70} and W_{100} 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 W_{100} and W_{70}, 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, $T_{skin,A}$ $p < 0.001$, $T_{skin,C}$ $p = 0.004$ and $T_{skin,H}$ $p = 0.035$). 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 W_{100} was always lower compared to W_{70}, except for $T_{skin,C}$ during the heat load. Pig behaviour differed only slightly: W_{70} tended to be less active (8%) before the heat load compared to W_{100}, while these differences disappeared during and after the heat load (interaction term, $p = 0.082$). For passive and eating/drinking behaviour, 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 behaviour did not significantly alter during the heat load.

3.3 Performance parameters

For ADFI, neither weight groups showed changes in 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).

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), behaviour (passive (%), active (%) and feeding/drinking behaviour (%)) and the feed intake (kg/day per animal) of fattening pigs (estimated means) according to the effect of weight group (W_{70} and W_{100}) and heat load (pre-heat, heat or post-heat period) (n = 4)

Parameter	Heat load	n	Weight group		SEM	*p*-value		
			W_{70}	W_{100}		Weight group	Heat load	Weight × Heat
Physiological parameters								
Respiration rate [bpm]	Pre-heat	95	36^{a}	37^{a}	1.19	0.908	<0.001	0.514
	Heat	96	74^{b}	79^{b}	3.14			
	Post-heat	96	35^{a}	33^{a}	1.31			
Rectal temperature [°C]	Pre-heat	92	38.9^{a}	39.0^{a}	0.09	0.520	0.013	0.403
	Heat	93	39.2^{b}	39.3^{b}	0.07			
	Post-heat	93	38.9^{a}	38.9^{a}	0.11			
Skin temperature: mean [°C]	Pre-heat	95	$32.7^{a,x}$	$31.7^{a,y}$	0.11	<0.001	<0.001	<0.001
	Heat	96	$35.1^{b,x}$	$34.4^{b,y}$	0.09			
	Post-heat	96	$32.4^{a,x}$	$30.8^{a,y}$	0.14			
Skin temperature: cold point [°C]	Pre-heat	95	$29.7^{a,x}$	$28.5^{a,y}$	0.18	0.003	<0.001	0.004
	Heat	96	$32.8^{b,x}$	$32.0^{b,x}$	0.15			
	Post-heat	96	$29.7^{a,x}$	$27.6^{a,y}$	0.21			
Skin temperature: hot point [°C]	Pre-heat	95	$34.2^{a,x}$	$33.5^{a,y}$	0.09	0.001	<0.001	0.035
	Heat	96	$36.3^{b,x}$	$35.7^{b,y}$	0.07			
	Post-heat	96	$34.0^{a,x}$	$32.9^{a,y}$	0.11			
Behaviour								
Passive [%]	Pre-heat	24	75%	70%	0.02	0.234	0.625	0.511
	Heat	24	82%	83%	0.02			
	Post-heat	24	75%	75%	0.03			
Active [%]	Pre-heat	24	$14\%^{x}$	$23\%^{y}$	0.02	0.016	0.452	0.082
	Heat	24	10%	10%	0.01			
	Post-heat	24	18%	18%	0.02			
Feeding & drinking [%]	Pre-heat	24	9%	8%	0.01	0.348	0.776	0.924
	Heat	24	8%	7%	0.01			
	Post-heat	24	7%	7%	0.01			
Feed intake								
Average daily feed intake [kg/day.animal]	Pre-heat	8	2.53	2.79	0.13	0.159	0.933	0.296
	Heat	8	2.26	2.24	0.05			
	Post-heat	8	2.45	2.59	0.08			
	Post-heat 2	8	2.70	2.85	0.07			

W_{70} = Weight group of ±70 kg; W_{100} = Weight group of ±100 kg, NA = not applicable, bpm = breaths/min

$^{x-y}$ Values within a row with different superscript 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

4 Discussion

4.1 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.

A three-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 RH 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 RH values, the THI remained stable between 75 and 79 during the heat wave. In another trial (Chapter 5), 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 trials, the main factor of variance in THI was related to RH. In the present experiment, slightly higher THI values observed in W_{100} may have been due to higher RH values in that specific compartment. The outside RH of both weight groups (both compartments) was the same as they were exposed to heat at the same date. Therefore, it is recommended to test different management strategies within a compartment with sufficient compartment replicates. For the other trial (Chapter 5), one compartment had a higher THI (80.8 in compartment 2) compared to the others (±77.9), despite application of the same heating device and thermostat setting. This difference can be explained by the high outside RH during the artificial heating period in compartment 2 (which took place during a different period than compartment 1, 3 and 4). Outside RH was between ±75-100 % due to rainy and misty conditions, which affected the interior RH 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 RH and possible differences between compartments should be taken into account.

Safety of the indoor climate was tested based on CO_2 and NH_3 values. CO_2 values remained within acceptable limits (Klimaatplatform, 2021; Van Gansbeke et al., 2010). 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. NH_3 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 NH_3 levels, while normally a higher temperature accelerates the ammonia production (Van Gansbeke & 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 ventilation rate at higher ambient temperature can increase ammonia emissions (Van Gansbeke & Van den Bogaert, 2020). Regardless, it is important to note that high NH_3 levels can negatively affect the health of animals and humans. For humans, 25 ppm NH_3 is the maximum permissible concentration in the workplace. For pigs, this concentration causes higher susceptibility for respiratory diseases. NH_3-concentrations above 50 ppm reduce the pig's general health status and zootechnical performance (Van Overbeke et al., 2010). When high NH_3 levels are measured, measures should be taken to mitigate pen fouling or limit ammonia emissions (Klimaatplatform, 2021).

4.2 Validation through assessment of changes in physiological, behaviour and performance parameters

The artificial heat wave protocol can also be confirmed to induce a heat stress response in the animals, as evident from 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, increased during the heat load. Respiration rate, which is the first physiological adaption to heat stress (Huynh et al., 2005a), doubled. Rectal temperature, the most relevant parameter for determining the level of homeothermy during heat stress (Renaudeau et al., 2010), also significantly increased by approximately 0.25 °C during the heat load. At lower RH 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., 2005a). Even under the trial conditions of a low RH 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 (da Fonseca et al., 2020; Zhang et al., 2019). 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., 2024a) 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, 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 behaviour and position, the chosen emission coefficient and the degree of soiling on the skin (Petry et al., 2017). Another important consideration is the accuracy of different infrared devices, especially given the wide range in cost between models. A review or meta-analysis comparing devices across price categories would be valuable to evaluate their reliability. The use of artificial intelligence may also support this process by enabling continuous monitoring and identifying deviations from normal temperature patterns. However, even with advanced methods, measurements can still be affected by external factors. For example, when ambient temperatures rise, it becomes more difficult to detect surface temperature changes using IR cameras, as the temperature difference between the pig's body and its environment decreases. For these reasons and due to the time-consuming nature of these measurements, T_{skin} was not included in further chapters (except for Chapter 4A). In the first experiment, the occurrence of passive (lying) behaviour increased to a non-significant degree in both weight groups by ±10 % during the heat load, mainly at the expense of active behaviours. Other studies also showed a significant increase in lying behaviour (3-5 %) when fattening pigs were exposed to high ambient temperatures (Hillmann et al., 2023; Huynh et al., 2005b). As the effect of heat stress on these main behavioural classes was relatively limited when using the applied scan sampling method, it was excluded in further chapters (except for Chapter 4A and 6).

An important adaptation during a period of heat stress is reduced feed intake, which effectively lowers the animal's internal heat production (Collin et al., 2001b; Huynh et al., 2005a; Kemp & Verstegen, 1987; Quiniou et al., 2000). In this study, a numerical but non-significant decrease in feed intake (10 % for W_{70} and 17 % for W_{100}) was observed. Collin et al. (2001b) did find a significant reduction of 30 % in ADFI of young pigs (±21 kg) during a relatively short (4 days) incremental rise in temperature (from 25 to 33 °C) at a RH 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 this study. Other studies found also significant decreases in ADFI when pigs were exposed to a longer heat load period (Huynh et al., 2005a; Nienaber et al., 1996; Quiniou et al., 2000). In another trial (Chapter 5), when the heat load was extended from 3 days to 7 days, we did observe a significant reduction in ADFI during the heat load (15 %). This validated our prolonged heating protocol for the measurement of ADFI.

4.3 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 T_{skin} and active behaviour. As noted above, T_{skin} 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 (Li et al., 2018; Quiniou et al., 2000). The current study therefore suggests that this weight effect is not, or is at least less relevant within the weight range of 70 to 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 & 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 (Brown-Brandl et al., 2013a; Simmons et al., 2011). 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 ADFI compared to W_{70}, although not significant (p > 0.05). We expected to see a significant difference in ADFI between the different weight groups, as feed intake is influenced by age, body weight (Kanis & Koops, 2010; Quiniou et al., 2001) and increasing ambient temperature (Close, 2018; Rinaldo & Le Dividich, 1991). Important to note is that feed intake was only measured over a period of three days, which may be too brief to show significant results, since we indeed found significant differences when applying a 7-day heat load (Chapter 5).

5 Conclusions

A successful artificial heating protocol was established, as the THI remained above the threshold of 75 even when RH decreased. Every physiological parameter of the barrows was altered during the period of heat load. The altered heating protocol of seven days led to a significant drop in ADFI. The present study showed that, aside from T_{skin} and active behaviour, weight within the studied range of 70 to 100 kg had no significant impact on the evaluated parameters. This suggests that barrows with an average weight of ±70 kg are equally affected by higher heat loads in terms of RR, T_{rectal}, and passive behaviour compared to barrows that are 3 weeks older and 20 kg heavier.

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CHAPTER 4 | EFFECT OF FEED ADAPTATIONS

CHAPTER 4A | EFFECT OF FEED ADDITIVES

Adapted from: De Prekel, L., Maes, D., Van den Broeke, A., Ampe, B., & Aluwe, M. (2024). Effect of Simultaneous Dietary Supplementation of Betaine, Selenomethionine, and Vitamins E and C under Summer Conditions in Growing-Finishing Pigs. *Veterinary Sciences*, *11*(3), 110. https://doi.org/10.3390/vetsci11030110

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Abstract

Heat stress in pigs negatively affects welfare, health, and performance. Osmoprotectants and antioxidants may alleviate oxidative damage during hot periods. We investigated whether an antioxidant-enriched feed can reduce negative effects in pigs during summer conditions. Sixty growing-finishing pigs were allocated into two groups: the control and enriched group. The control group contained 0.4 mg/kg inorganic Se and 100 ppm vitamin E, while the enriched group contained 0.3 mg/kg inorganic Se, 0.1 mg/kg selenomethionine, 200 ppm vitamins E and C, and 0.2 % betaine. Feed was offered ad libitum. Respiration rate, rectal and skin temperature, behaviour, and weight gain were assessed weekly. Daily measurements of these parameters were performed during a 3-day heat wave (THI ≥ 75) and during an artificial heating period. Individual average daily water intake (ADWI) and feed intake were measured using RFID systems. Carcass traits and meat quality were evaluated after slaughter. The ADWI of the enriched group did not change during heat load while it increased for the control group, while this was the other way around for water-to-feed ratio. No differences between diet groups in carcass traits or meat quality were found. Independent of dietary treatment, increases in ΔTHI or THI were accompanied by significant increases in both RR and T_{skin}. In conclusion, dietary supplementation of betaine, selenomethionine, vitamin E, and vitamin C did not significantly alter the physiological and performance parameters of growing–finishing pigs raised under the tested summer conditions.

Key words

Precision livestock farming, Respiration rate, Rectal temperature, Meat quality, Temperature–humidity index

1 Introduction

Some feeding strategies show potential to mitigate the negative effects of heat stress. These feeding strategies may include alterations in the chemical composition of the diet (Kerr et al., 2003; Spencer et al., 2005) or the addition of functional feed additives. Antioxidants are frequently used as feed additives to mitigate the effects of heat stress in different animal species, as they prevent osmotic stress caused by free radicals and oxidants. Non-enzymatic antioxidants, such as vitamin E and vitamin C, can improve the efficiency of the antioxidant system when used with the synergetic cooperation of enzymatic antioxidants, such as glutathione peroxidase. Selenomethionine plays an important role in this process (Cottrell et al., 2015). Betaine, which has been tested extensively in pigs and poultry during thermoneutral conditions, functions as an osmoprotectant and methyl group donor and thus may also reduce the adverse effects of heat stress (Eklund et al., 2005; Ratriyanto et al., 2009). Furthermore, the addition of some of these feed additives can positively influence carcass traits and pork meat quality (Dugan et al., 2004; Eklund et al., 2005; Ellis & McKeith, 1999; Lahučký et al., 2005; Ngapo & Gariepy, 2008; Ratriyanto et al., 2009; Rosenvold & Andersen, 2003).

The supplementation of individual additives like betaine, selenomethionine, vitamin E or vitamin C has already been investigated under thermoneutral conditions, but the effects of concurrent supplementation of different additives are less clear (Cottrell et al., 2015). In addition, the effect of Se during high heat load periods has been studied in poultry (Habibian et al., 2015) but less in growing–finishing pigs. A larger number of reports on betaine supplementation in poultry during high heat loads (Ratriyanto et al., 2009) is available in comparison to similar studies on pigs. Various studies also supplied additives outside of the regulatory limits, limiting their applicability for practical use. 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 yet been tested.

The aim of this study was to investigate the effect of dietary supplementation of betaine, vitamin E, vitamin C, and selenomethionine in growing–finishing pigs under summer conditions. Concentrations of the tested additives were within legal limits. Physiological parameters, animal welfare, carcass and meat quality, and individual performance parameters were evaluated to determine the possible effects of dietary supplementation on symptoms of heat stress.

2 Material and Methods

2.1 Experimental setup and animals

A total of 60 mixed-sex growing–finishing pigs (hybrid sow × Piétrain) were divided into a control and an enriched group, with two pens of 15 animals per treatment. The distribution was made so that each pen had about the same average weight and an equal distribution of barrows and gilts. The diets were provided in two phases: a starter diet and a grower diet starting at 15 weeks of age. In both phases, the enriched diet was supplemented with selenomethionine, betaine, vitamin C, and vitamin E (Table 1). Additive levels were implemented based on 1) literature data about the effectiveness of feed additives as a measure against heat stress (Cottrell et al., 2015; Gan et al., 2014; Liu et al., 2018b; Liu et al., 2017; Liu et al., 2016; Lv et al., 2015; Ratriyanto et al., 2009; Stewart et al., 2015; Tang et al., 2019), and 2) the advice of the feed expert group of the research project Coolpigs. Feed and water were provided ad libitum to both groups. The trial started on 30 June 2021 at 10 weeks of age (31.0 ± 2.9 kg) and ended on 12 October 2021 at 25 weeks of age and when the pigs had reached slaughter weight (120.6 ± 11.3 kg). When the outside temperature was predicted to exceed 25 °C for a minimum of three consecutive days, physiological parameters and animal behaviour were observed more intensely (Figure 1). Additionally, an artificial heat wave was induced for three days in the last week of September when the animals were 23 weeks old. Three pigs were removed from the trial: one due to lameness and two due to stress from having been stuck in the feeding system. All three came from the same pen (control pen 1) and were not included in the data set for statistical analysis.

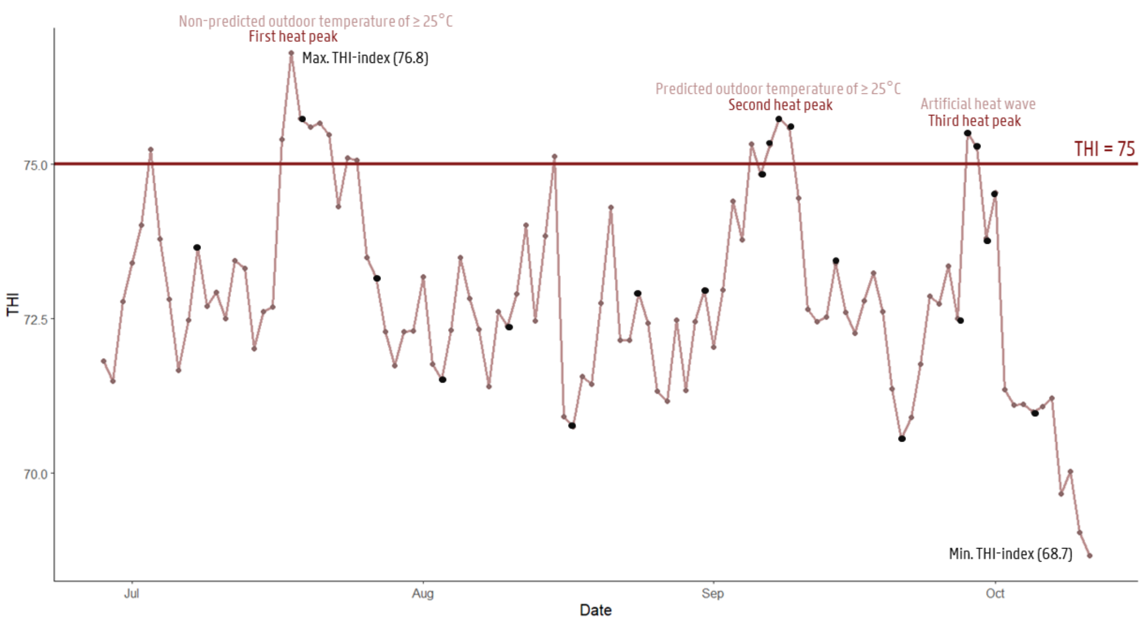


Figure 1: Evolution of the daily maximum temperature–humidity index (THI) and indication of the three heat peaks and min (68.7) and max (76.8) THI during the entire growing–finishing period. (● = days where physiological parameters and animal behaviour were observed between 13:00 and 17:00; ▬ = a THI of 75 indicates a warning for heat stress).

2.1.1 Housing

The trial was performed at ILVO's experimental farm (Melle, Belgium). The stable was comprised of eight pens (Figure 2), four of which were used in the trial. A Nedap feeding system (Nedap Prosense©, Nedap N.V., the Netherlands) was located in the right front corner of each pen with one drinking nipple in the centre back of the pen. The other four pens were not used in this trial as they were not equipped with a Nedap system. Those pens were populated with pigs kept according to standard farm management. All pens in the compartment had a partially slatted floor with a total pen surface of 19.11 m² (1.27 m²/animal). The compartment was artificially lit from 08:00 to 16:30 plus natural light from one window (90 × 80 cm) on the left side (southwest) of the compartment and six windows (90 × 60 cm each) on the right (north–east). The air inlet for the mechanical ventilation system (110 × 35 cm) was located under the six windows.

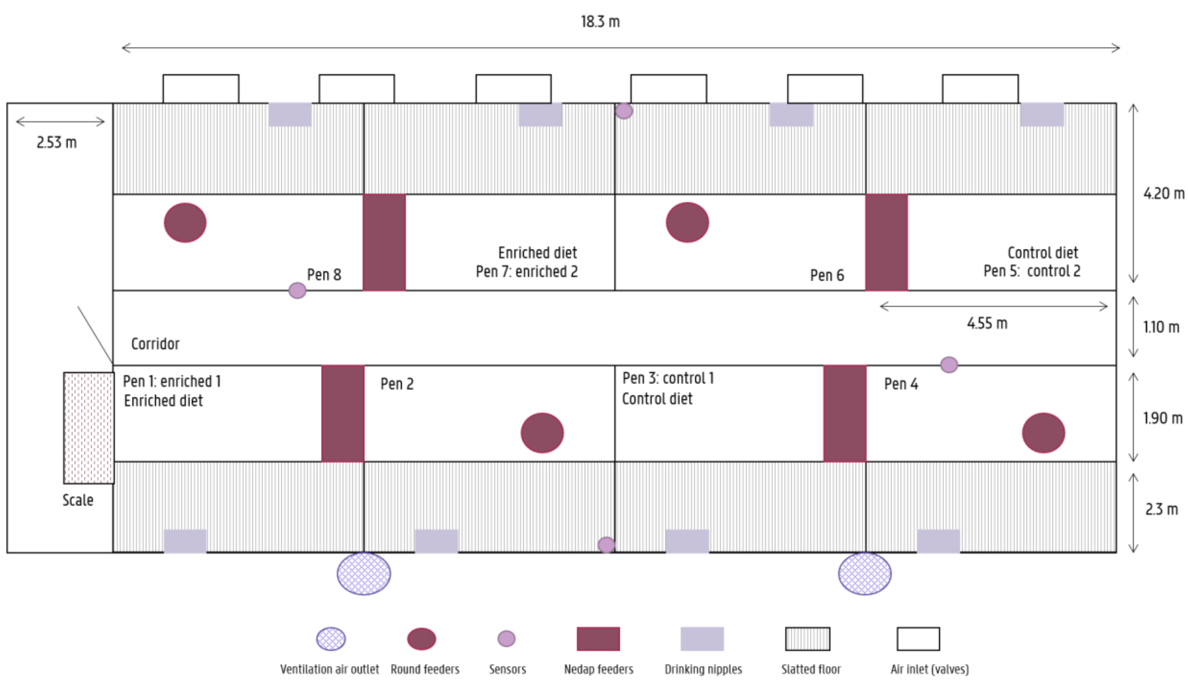


Figure 2: Schematic overview of the entire compartment.

Table 1: Ingredients and analysed chemical composition of the control and enriched diet [%] of the starter and grower phases.

	Diet			
	Starter Phase (10–15 Weeks of Age)		Grower Phase (15–25 Weeks of Age)	
Ingredients and Composition	**Control**	**Enriched**	**Control**	**Enriched**
Ingredients [%]				
Wheat	31.094	31.365	36.902	36.386
Barley	20.000	20.000	20.000	20.000
Maize	5.000	5.000	-	-
Soybean meal (48 % crude protein)	13.096	13.339	6.500	6.500
Biscuits	8.000	8.000	7.500	7.500
Corn flakes	3.000	3.000	3.000	3.000
Wheat middlings	0.000	0.000	3.700	3.606
Wheat gluten	8.590	5.317	13.000	13.000
Palm oil	1.636	1.831	0.900	0.900
Beet molasses	2.000	2.000	2.700	2.641
Palm kernels	2.000	2.000	0.835	0.835
Beet pulp	1.500	3.085	1.500	1.500
Feed chalk	1.472	1.392	1.474	1.403
Table salt	0.473	0.165	0.543	0.167
DL-Methionine	0.203	0.208	0.131	0.130
L-Valine	0.052	0.055	0.005	0.000
L-LYS (50 %)	0.799	0.792	0.715	0.700
Tryptophan (25 %)	0.167	0.172	0.046	0.038
vL-Threonine	0.202	0.198	0.200	0.160
Betaine[1]	0.000	0.667	0.000	0.667
Vitamin E[2]	0.020	0.030	0.020	0.030
Vitamin C[3]	0.000	0.057	0.000	0.057
Sodium bicarbonate[4]	0.000	0.450	0.000	0.400
Magnesium oxide	0.121	0.128	0.109	0.110
Monocalcium phosphate	0.119	0.246	0.067	0.067
Organic acid mix	0.300	0.300	-	-
Premix control diet[5]	0.150	0.000	0.150	0.000
Premix enriched diet[6]	0.000	0.200	0.000	0.200
Phytase	0.006	0.003	0.003	0.003
Analysed chemical composition				
Crude protein (N × 6.25) [%V]	16.4	16.0	15.3	15.7
Crude fat [%V]	4.9	5.1	4.6	4.6
Crude ash [%V]	5.2	5.2	5.5	5.0
Crude fibre [%V]	4.5	4.6	4.5	4.8
Water [%V]	10.4	10.9	10.1	10.9
LYS [g/kg] [7]	10.6	10.5	9.0	8.9
NE [MJ/kg] [7]	9.6	9.6	9.4	9.3

[1] The enriched diet contained 2000 mg/kg more betaine (anhydrous) than the control diet. [2] The enriched diet contained 100 mg/kg more vitamin E than the control diet. [3] The enriched diet contained 200 mg/kg more vitamin C than the control diet. [4] The enriched diet contained sodium bicarbonate instead of table salt. [5] The premix of the control diet contained 0.4 mg/kg of inorganic selenium, [6] The premix of the enriched diet contained 0.3 mg/kg inorganic selenium and 0.1 mg/kg selenomethionine. [7] Calculated composition. NE = Net Energy.

2.1.2 Climate Control

The stable climate was automatically controlled with a climate computer (Hotraco Agri©, Hotraco Group, the Netherlands) during the trial. The same heating protocol as described in Chapter 3 was used, where during the artificial heat wave, the temperature was kept constant at approximately 31 °C between 07:00 and 22:00. However, between 22:00 and 07:00, the temperature was reduced to 26 °C. These limits were based on temperatures achieved in the stable in the summer of 2020 during a naturally occurring heat wave. Two climate sensors (Monnit®, Monnit Corporation, South Salt Lake, UT, USA) were placed in the corridor at a height of 125 cm. Two additional sensors were placed in the back of the pens in the middle of the compartment at a height of 110 cm (Figure 2). These sensors logged the RH and ambient temperature in the stable every two hours for the entire trial period. During the artificial heat wave, four additional sensors (HOBO onset®, Bourne, MA, USA) were placed at the treatment pens in the corridor at a height of approximately 150 cm. They measured RH and temperature every five minutes. The Monnit sensors were used for analysis.

Depending on the measured parameter and RH, pigs can start showing signs of heat stress at different temperatures (Huynh et al., 2005a). To predict the potential level for heat stress experience, the THI was used, as described in Chapter 3.

2.2 Measurements at farm level

2.2.1 Physiological parameters and animal behaviour

Physiological parameters monitored were RR, T_{rectal} and T_{skin}. Per pen, seven randomly selected reference animals were chosen for physiological measurements. The reference animals did not change during the entire trial. Animal behaviour was measured at pen level. The parameters were measured weekly during the entire trial period. In addition, the parameters were evaluated daily during a predicted natural heat wave (three consecutive days ≥ 25 °C). During the artificial heat wave, the parameters were measured during the three days of heating, one day before and one day after heating. The observations always started at 13:00 and were conducted by the same group of observers. Treatments were blinded for the observers. Respiration rate, T_{rectal}, T_{skin} and behaviour were measured according to the same method as described in Chapter 3

2.2.2 Correlations

A correlation coefficient between T_{rectal} and T_{skin} was calculated in the second heat period and during the entire growing–finishing period to verify the reliability of T_{skin} as non-invasive indicator of heat stress. Also, the correlation with ambient temperature was taken into account to check the reliability of T_{skin} as a physiological parameter.

2.2.3 Performance Parameters

Pigs were individually tagged with a low-frequency Radio Frequency Identification (RFID) ear tag. The feeding system (Nedap Prosense, Nedap livestock management©, Groenlo, the Netherlands) registered every animal's visit to the feeder and the individual's number and feed intake. In addition, all pigs were weighed individually 1 × /week. Based on this data ADFI, ADG and FCR were calculated. Each pig was also tagged with a high-frequency tag to register the drinking pattern at the nipple. Animal number, daily drinking time, number of drinking visits, and ADWI were registered as described by Maselyne et al. (2014) and Maselyne et al. (2016). Drinking time was measured by the time of the same pig's first and last registered visit. Registrations shorter than three seconds (too short to drink) or longer than 180 s (too long to drink; probably lying or exploring in front of the nipple) were removed from the dataset. The daily water intake was measured with a flow meter (FT210-Turboflow, Gems Sensors and Controls Inc., Plainvilles, CT, USA) installed in front of the drinking nipple that registered the flow during each drinking visit. The pigs could partly regulate the flow rate by biting down more or less forcefully on the nipple.

2.3 Measurements in the Slaughterhouse

2.3.1 Observations in the lairage area

All pigs were transported to a commercial slaughterhouse at 25 weeks of age. Upon arrival at the slaughterhouse, the same selected reference pigs were observed in the lairage area. Heat stress score (HSS) was assessed upon entry to lairage and again one hour later. The HSS is based on a Tagged Visual Analogue Scale (TVAS) ranging from 0 to 150 mm, as shown in Figure 2. The purpose of a HSS is to quickly and visually assess signs of heat stress when animals are standing, resting, in the stable or at the abattoir. This scoring system was consistently applied by a group of observers who were trained. Observers tagged a line on the scale, and the distance from the zero mark to the drawn line was measured (mm), resulting in a score between 0 and 150.

Examples of HSS scores are (Figure 3):

- Score 0: No panting, respiration movements are barely visible, no signs of heat stress.
- Score 15: Respiration movements are easily seen, but RR remains within the normal range, typically observed in pigs lying down and resting under thermoneutral conditions.
- Score 30: Accelerated RR without open mouth or drooling, entire body is not moving.
- Score 60: Accelerated RR without open mouth or drooling but additional body movement.
- Score 90: All previous signs are present, and the mouth was sometimes open, with drooling appearing.

- Score 120: Open mouth and drooling are present. The pig can show red-white spots on the skin, as shown in Figure 4.
- Score 150: The animal is at risk of dying due to excessive heat load.

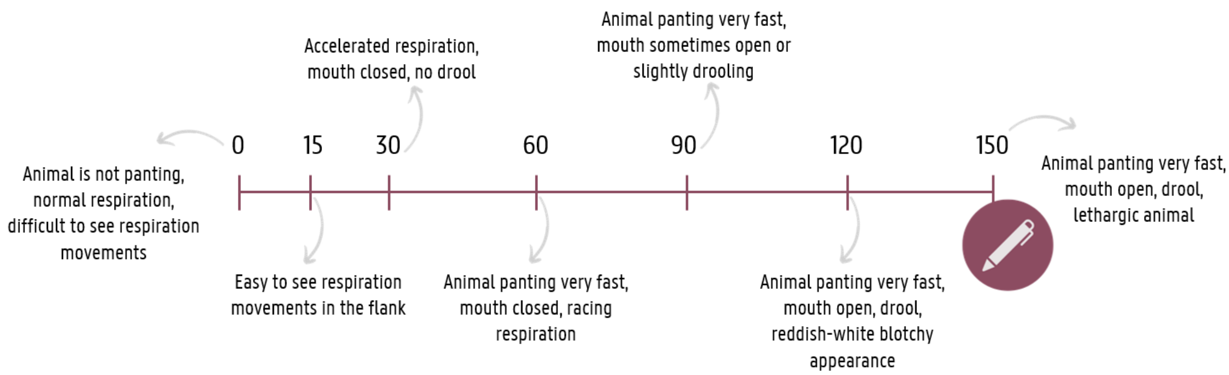


Figure 3: Tagged Visual Analogue Scale (TVAS) from 0-150 mm of heat stress related signs. Adapted from Van Laer et al. (2015)

Figure 4: Example of a pig with a Heat Stress Score (HSS) > 120: respiration was very high (not visible on photo), open mouth for the whole observation time and a reddish-white blotchy skin

Presence of panting, open mouth and drooling were also observed (yes/no) at the time of heat stress scoring. Skin colour (pink, red or red-spotted) and skin lesions (on a ordinal scale from 0 (0% of the body of the pig is covered with injuries) to 4 (100% of the body is covered with injuries)) were also assessed at the same time as heat stress scoring.

For assessing climate in the slaughter house, one HOBO sensor was installed above the middle pen in the row where the pigs were housed in the abattoir. The THI was calculated using the same method as previously described.

2.3.2 Carcass traits

Pigs were slaughtered by exsanguination after carbon dioxide stunning. The 'AutoFOM III™' system (Frontmatec, Denmark) was used to define different carcass traits, as described by Kowalski et al. (2020). Warm carcass weight, cold carcass weight, carcass lean meat content, fat thickness, muscle thickness, dressing yield and lean tissue growth were determined.

2.3.3 Meat quality: instrumental

At 35 minutes post-mortem, the initial pH (pH_i) (Type HI98163 electrode FC2323, Hanna Instruments®, Temse Belgium) of all 57 animals was measured in the *M. longissimus thoracis et lumborum* next to the 7th rib (starting from the rostral side of the carcass). About 22h after slaughter, loin samples of the left side of all test animals were collected in the slaughterhouse. Twenty-four hours post-mortem, the pH_u was measured at the bottom, the middle and the top of the loin sample. Then the *M. longissimus thoracis et lumborum* was cut into meat slices and visible connective tissue and fat were removed. Water holding capacity was evaluated according to the gravimetric EZ-drip loss method of Christensen (2003), where two slices of 2.5 cm were used. From each meat slice, a circular sample (Ø 25 mm) between 9.5-10.5 g was cut with a circular knife and weighed. Then, each sample was put into an EZ-drip loss container and stored for 48 h at 4°C. The sample was weighed after 48h and the drip loss percentage 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 minutes and cooled down for a minimum of 15 minutes 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 250N, Mecmesin, UK). Afterwards, the average of all ten measurements per animal was calculated. Intramuscular fat content was measured according to the Bligh and Dyer method (Hanson J & Olley, 1963). First, two meat slices of 2.5 cm were homogenised with a meat grinder and an ULTRA-TURRAX (ULTRA-TURRAX®, IKA®-Werke GmbH & Co. KG, Germany). Then, 100 g of the homogenised sample was put in the NIRS device (NIRS DS2500 L™, FOSS, Denmark). 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, USA) after 30 minutes of blooming at 9 °C. Hue (colour vividness) and chroma (colour saturation index) were calculated according to the following formulas (King et al., 2023):

$$Hue = \tan^{-1}(b^*/a^*)$$

$$Chroma = \sqrt{(a^{*2} + b^{*2})}$$

With L*: lightness, a*: redness and b*: yellowness

When collecting the loin samples for meat quality, two carcasses were misplaced and were not included in the dataset.

2.3.4 Meat quality: sensory

Sensory evaluation of the loin samples was performed by a trained panel. Five sessions were organised in which 11 samples were served per session, with five or six samples per diet group. Each sample was scored by six trained panellists (according to Arildsen Jakobsen et al. (2014)) on different characteristics (fried odour, piggy odour, softness, juiciness, fried flavour, piggy flavour and acidic flavour) by a visual analogue scoring from 0-100. Between each sample, the panellists cleansed their palate by drinking water and eating an unsalted cracker. Each sample (slices of 2.0 cm) was grilled on a clean pan (Tefal Ultra Compact Comfort GC3060, Tefal, Rumilly, France) to an inner temperature of 72 °C. No herbs, salt or fat were added. Each slice was divided into six pieces and covered by a plastic cup to retain the aroma.

2.4 Statistical Analysis

Statistics were calculated in R® software (version 4.1.1) (RStudio, R Core Team, Auckland, New Zealand), where the effect of the diet, ΔTHI, and the interaction of diet × ΔTHI were evaluated based on the independent physiological parameters (RR, T_{rectal} and T_{skin}) and animal behaviour. Interaction terms of the fixed effect with p-values > 0.05 were excluded from the final model. Differences were considered significant if $p \leq 0.05$. A broken line model based on the collected data with a pre-set cut-off THI value of 75 (as based on the limit of potential signs of heat stress by NWSCR (1976)) was used to determine the slope of the animal parameters of the diet groups under greater ΔTHI. All THI values lower than 75 had ΔTHI = 0, while THI values higher than 75 had a ΔTHI = 75-THI. When the data did not fit the broken line model, the data were fitted with the THI increase (no pre-set cut-off) instead of ΔTHI. In the model, age of the pigs was used as co-variable. Observation date and pig ID within the pen were used as random variables. The effect of the diet, the period of heat load (before, during, and after), and the interaction of diet × period on ADWI, daily drinking visits, drinking time, and ADFI were determined using linear mixed model. This was established by evaluating these parameters before, during, and after a heat peak totalling 16 days (number of days within the three peaks; Figure 1). The same number of days before and after a heat peak were implemented in the analysis. In the model, the heat peak (first, second, or third peak) was used as co-variable, and pig ID within the pen was used as random variable. The effect of diet and observation time point during lairage (upon arrival and one hour after) on the HSS and skin lesions was evaluated using a linear mixed model with the pen as a random variable. The effect of diet on the different parameters of carcass traits, instrumental meat quality and sensory evaluations was also evaluated by a linear mixed model with pen as random variable for carcass traits and instrumental meat quality and panel time as random variable for the sensory meat quality. A post hoc was performed when significant differences were found. The pen was considered as experimental unit.

3 Results

3.1 Temperature-humidity index

The average maximum daily THI during the trial was 72.9 (Figure 1). Only 16 days had a maximum daily THI above 75; the highest maximum daily THI was 76.8. The first THI peak above 75 (17 to 25 July) was not forecast as a period of at least three days at ≥ 25 °C. During that period, performance parameters were registered, but daily observations of physiological parameters were not performed. The second peak (5 to 9 September) had been accurately forecast, so four daily observations were implemented. Of these, only three observation days met the requirement of a maximum daily THI ≥ 75. The last small peak occurred when the artificial heat wave was induced from 28 to 30 September, where only two observation days had a maximum daily THI ≥ 75. In total, there were 21 observation days, of which only six took place when the maximum daily THI was ≥ 75. The average maximum daily THI of the three heat peaks was 75.4.

3.2 Measurements at Farm Level

3.2.1 Physiological Parameters and Animal Behaviour

There was no effect of diet or diet × ΔTHI interaction on the RR and T_{rectal} of the growing–finishing pigs (Table 2). A significant effect ($p < 0.001$) of ΔTHI on RR was found, where the RRs for both diet groups increased with an increasing THI (34.5 breaths per min, per ΔTHI above the baseline of THI = 75).

Table 2: Effect of diet (control and enriched), ΔTHI (temperature–humidity index), and its interactions on physiological parameters and animal behaviour of growing–finishing pigs housed during the summer season. The slope indicates the increase/decrease in the parameter when THI increases with 1 value from a baseline THI of 75.

Parameters	Diet				*p*-Value		
	Baseline at THI = 75		Slope [1] per ΔTHI or THI [2]				
	Control	Enriched	Control	Enriched	Diet	ΔTHI	Diet × ΔTHI
Physiological parameters							
Respiration rate [breaths/min]	46.3	45.7	34.5	34.5	0.904	<0.001	n.s.
Rectal temperature [°C]	39.6	39.6	0.129	0.129	0.869	0.216	n.s.
Skin temperature [2] [°C]	35.1	35.2	0.294	0.294	0.875	<0.001	n.s.
Animal behaviour [%]							
Active behaviour	43	43	−14	−14	1.000	0.079	n.s.
Standing	11	11	−5	−5	0.975	0.186	n.s.
Exploring	25	23	−5	−5	0.669	0.108	n.s.
Sitting	7	8	−4	−4	0.204	0.084	n.s.
Inactive behaviour	47	49	15	15	0.649	0.056	n.s.
Sternal lying	37	35	0	0	0.659	0.965	n.s.
Semi-sternal lying	3	5	4	4	0.334	0.281	n.s.
Lateral lying	8	8	7	17	0.971	0.298	0.086

n.s. = no-significant interaction; THI = temperature –humidity index. [1] The slope increase of the two treatments is the same if no interaction or other significant difference was found between treatments. [2] Skin temperature according to THI (linear) instead of ΔTHI, due to its linear progression.

The broken line model was not used for T_{skin} due to the linear progression of the rising T_{skin} with an increasing THI, where no clear inflexion point could be seen. Also, no effect of diet or the diet × THI interaction on T_{skin} was found (Table 2). Nevertheless, the THI had a significant effect ($p < 0.001$) on T_{skin}, as the T_{skin} for both groups increased when the THI also increased (0.294 °C per THI). No significant diet effect or diet × ΔTHI interaction was found on the different behaviour parameters. For lateral lying behaviour, a trend of interaction was found between diet and ΔTHI ($p = 0.086$) (Table 2). This implies that the enriched group had a steeper slope of lateral lying than the control group with an increasing ΔTHI (17 and 7 % per ΔTHI, respectively). Furthermore, with an increasing ΔTHI, sitting ($p = 0.084$) and active behaviour ($p = 0.079$) tended to decrease, while inactive behaviour ($p = 0.056$) tended to increase.

3.2.2 Correlations

When comparing T_{rectal} and T_{skin} during the second heat period, a low and negative correlation coefficient of −0.19 ($p = 0.323$) was found. During the entire growing–finishing period, the correlation coefficient between T_{rectal} and T_{skin} was 0.12 ($p = 0.004$), while the correlation was much higher for T_{skin} and ambient temperature (0.48, $p < 0.001$).

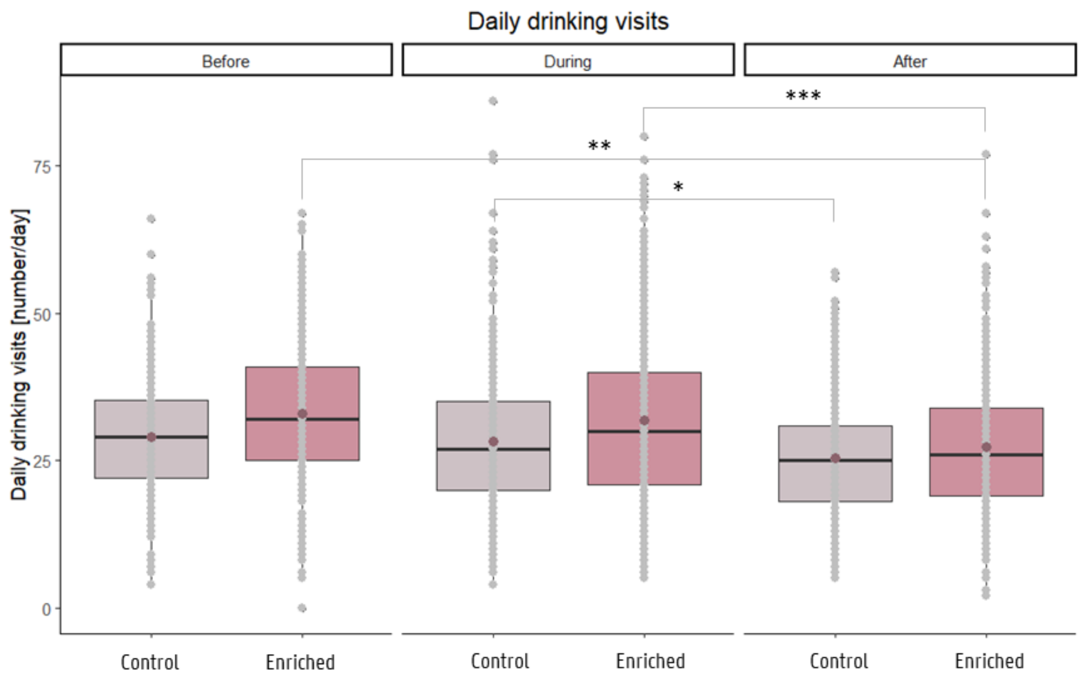
3.2.3 Performance Parameters

The ADFI, ADG, and FCR between the control and enriched groups did not significantly differ in the starter or the grower phases ($p > 0.05$). Nevertheless, we observed that the ADFI of the enriched group was numerically lower in the starter and grower phase than the control group (starter: 1562 g/day vs. 1645 g/day; grower: 2472 g/day vs. 2518 g/day, respectively). Also, the ADG of the enriched group was numerically lower than in the control group for both phases (starter: 787 g/day vs. 856 g/day; grower: 882 g/day vs. 905 g/day, respectively) (Table 3).

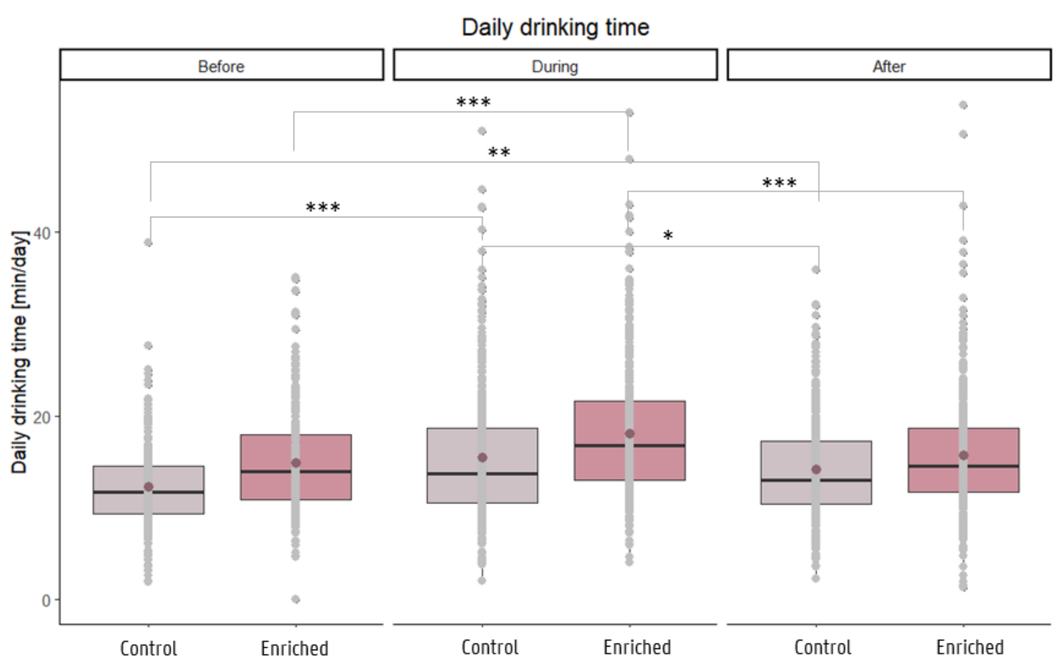
Table 3: Effect of diet (control and enriched) per phase (starter- (10-15 weeks of age) and grower phase (15-25 weeks of age)) on average daily feed intake, average daily gain and feed conversion ratio of growing-finishing pigs housed during the summer season.

Parameter	**Phase**	**Diet**		**SEM**	***p*-value**
		Control	**Enriched**		**Diet**
Average daily feed intake [g/day]	Starter	1645	1562	32.1	0.328
	Grower	2518	2472	48.6	0.690
Average daily gain [g/day]	Starter	856	787	16.8	0.165
	Grower	905	882	13.3	0.478
Feed conversion ratio	Starter	1.92	1.99	0.01	0.310
	Grower	2.78	2.81	0.03	0.446

For daily drinking visits, daily drinking time, and ADWI, a significant interaction between diet × period of heat load (before, during, or after a higher heat load period) was found ($p = 0.013$, $p = 0.03$ and $p = 0.013$, respectively) (Table 4). During a period of higher heat load, the control and enriched groups showed a significantly higher daily drinking time compared to the period before ($p < 0.001$ and $p < 0.001$, respectively) and after a higher heat load ($p = 0.048$ and $p < 0.001$, respectively). For the control as well as the enriched group, the number of daily drinking visits dropped significantly after a higher heat load period ($p = 0.024$ and $p < 0.001$, respectively), as seen in the post hoc analysis (Figure 5). Furthermore, the control group had a significantly higher ADWI during a period of higher heat load compared to the previous period ($p = 0.009$), while this was not the case for the enriched group. Interestingly, the enriched group generally had more drinking visits and longer drinking times than the control group, while the total daily water intake of the enriched group was lower ($p < 0.05$). No diet effect or diet × period of heat load interaction was found on ADFI. Nevertheless, there was an effect of heat load for both diet groups ($p < 0.001$). The ADFI of both diet groups was higher in the week after compared to the week before the higher heat load (Table 4).


Daily drinking visits
Before
During
After
Daily drinking visits [number/day]
0
25
50
75
***
**
*
Control
Enriched
Control
Enriched
Control
Enriched

a


Daily drinking time
Before
During
After
Daily drinking time [min/day]
0
20
40
***
**
***
***
*
Control
Enriched
Control
Enriched
Control
Enriched

b

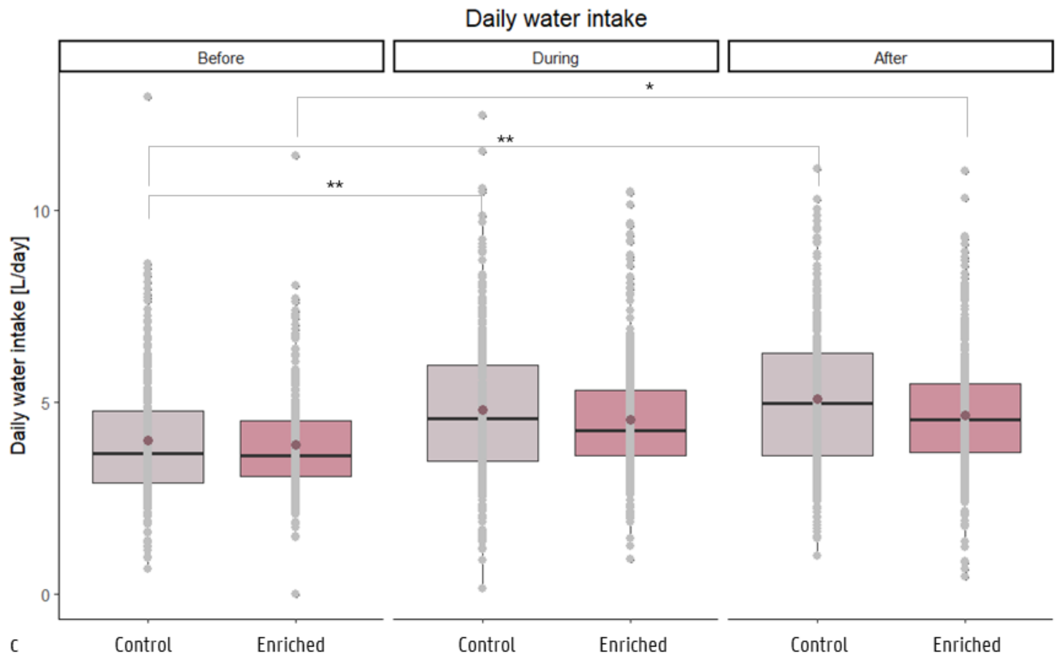


Figure 5: (a) Daily drinking visit [number/day], (b) daily drinking time [min/day], and (c) daily water intake [L/day] per diet group (control and enriched) before, during, and after a period of heat load. Significant differences between diet groups and period of heat load are implemented by * ($p \leq 0.05$), ** ($p \leq 0.01$), or *** ($p \leq 0.001$). (✸ = mean of the parameter of the diet group.)

Table 4: Effect of diet (control and enriched), the period of heat load (before, during, and after), and their interactions on drinking parameters and daily feed intake of growing–finishing pigs housed during the summer season. Each period (before, during, and after a heat peak) consists of 16 days.

Parameter	**Heat Load**	**Diet**		**SEM**	***p*-Value**		
		Control	**Enriched**		**Diet**	**Heat load**	**Diet × Heat**
Daily drinking visits [number/day]	Before	29 ^{abcd}	33 ^{bd}	0.43	0.150	0.008	0.013
	During	28 ^{cd}	32 ^{bd}	0.45			
	After	26 ^{ab}	27 ^{ac}	0.37			
Daily drinking time [min/day]	Before	12 ^{ab}	15 ^{ace}	0.20	0.099	<0.001	0.036
	During	15 ^{ef}	18 ^{bdf}	0.24			
	After	14 ^{cd}	16 ^{ace}	0.20			
Daily water intake [L/day]	Before	4.0 ^{ac}	3.9 ^{ab}	0.06	0.730	<0.001	0.013
	During	4.8 ^{bd}	4.6 ^{abcd}	0.05			
	After	5.1 ^{bd}	4.6 ^{cd}	0.06			
Daily feed intake [g/day]	Before	2088 ^{ac}	2007 ^{ab}	25.7	0.503	<0.001	n.s.
	During	2144 ^{abcd}	2085 ^{abcd}	18.5			
	After	2296 ^{bd}	2234 ^{cd}	19.8			

n.s. = non-significant interaction. $^{a–f}$ Values within a row (daily drinking visits, daily drinking time, daily water intake, and daily feed intake) with different superscripts differ significantly at $p < 0.05$ for diet × heat load interaction.

In addition, the water-to-feed intake ratio for each period was calculated based on the results represented in Table 4. For the control group, this ratio was 2.3, 2.2, and 2.2 L/kg for the periods before, during, and after the heat load, respectively. For the enriched group, the values were 1.9, 2.2, and 2.1 L/kg, respectively.

3.3 Measurements in the Slaughterhouse

3.3.1 Observations in the Lairage Area

No significant interaction between diet and observation time for HSS or skin lesions was found (Table 5). Also, no significant differences were found between the two diets for both parameters. However, for both diets, observation time showed a significant difference ($p < 0.001$) where the HSS was lower after an hour of lairage compared to the observation upon arrival at the slaughterhouse. There were no significant differences in panting, open mouth, drooling, and skin colour between the groups ($p > 0.05$).

Table 5: Effect of diet (control- (and enriched), observation time point in the lairage area (upon arrival and one hour after) and its interactions on heat stress score and skin lesions of growing-finishing pigs housed during the summer season

Parameter	**Observation time point**	**Diet**		**SEM**	***p*-value**		
		Control	**Enriched**		**Diet**	**Time point**	**Diet x time point**
Heat stress score	Upon arrival	27.7^a	26.2^a	1.63	0.587	<0.001	n.s.
	One hour after arrival	19.0^b	17.5^b	1.58			
Skin lesions	Upon arrival	0.4	0.4	0.11	0.913	0.255	n.s.
	One hour after arrival	0.6	0.6	0.12			

n.s. = non-significant interaction

a- b Values within a row with different superscripts differ significantly at $p < 0.05$ for diet × time point interaction.

3.3.2 Carcass Traits and Meat Quality

Carcass parameters (warm carcass weight, cold carcass weight, carcass lean meat content, fat thickness, muscle thickness, dressing yield, and lean tissue growth) as well as meat quality (pH, water holding capacity, shear force, IMF content, and CIE-L* a*b* colour determinants) did not significantly differ between the control and enriched groups ($p > 0.05$). Furthermore, no significant differences were observed in the different sensory evaluation parameters (fried odour, 'piggy' odour, tenderness, juiciness, fried flavour, 'piggy' flavour, and acidic flavour) between the diet groups ($p > 0.05$) (Table 6).

Table 6: Effect of diet (control and enriched) on carcass traits and meat quality (instrumental and sensory) of growing-finishing pigs housed during the summer season.

Parameters	Diet		SEM	*p*-value
	Control	Enriched		Diet
Carcass traits				
Fasted weight [kg]	119.0	116.0	1.49	0.476
Warm carcass [kg]	97.2	94.6	1.24	0.409
Cold carcass [kg]	95.3	92.8	1.21	0.409
Lean meat content [%]	63.8	63.4	0.38	0.637
Fat thickness [mm]	8.4	8.6	0.37	0.787
Muscle thickness [mm]	67.6	65.5	0.66	0.243
Dressing yield [%]	80.6	79.8	0.20	0.503
Lean tissue gain [g/day]	449.0	429.0	4.90	0.172
Meat quality: instrumental				
pH_i	6.5	6.5	0.04	0.545
pH_{24}	5.6	5.6	0.01	0.346
Drip loss [%]	7.3	6.3	0.26	0.439
Cooking loss [%]	30.6	28.3	0.29	0.217
Shear force [N]	30.4	32.4	0.91	0.553
Intramuscular fat [%]	1.9	2.1	0.09	0.363
Lightness L*	57.1	57.4	0.27	0.603
Redness a*	6.5	6.2	0.08	0.332
Yellowness b*	14.9	15.1	0.07	0.480
Hue	66.6	67.6	0.25	0.172
Chroma	16.3	16.3	0.08	0.975
Meat quality: sensory				
Fried odour	30	28	2.50	0.681
'Piggy' odour	18	16	0.94	0.298
Tenderness	49	44	2.13	0.218
Juiciness	45	48	1.25	0.253
Fried flavour	22	20	2.01	0.550
'Piggy' flavour	25	26	0.84	0.541
Acidic flavour	11	12	0.78	0.611

4 Discussion

The aim of the present study was to evaluate an all-round strategy, i.e., concurrent addition of different additives throughout the entire growing–finishing period during the summer to mitigate the effects of heat stress. This approach was chosen for its practicality, namely applying a one-time adjustment during the entire summer growing–finishing period versus reacting only when heat load increased. Moreover, concurrent supplementation of the various antioxidants and osmolytes over the entire period represented the maximum potential of this strategy on all evaluated variables. In our study, the enriched group showed no need to increase water intake during a higher heat load.

Despite relatively limited high heat load conditions, in this study, there was an increase in RR. Pigs primarily depend on evaporative heat loss, and an elevated RR is therefore the first physiological adaptation to the effects of heat stress (Huynh, 2005). This change may be indicative of potential impacts on production outcomes, which are of economic importance. Since farmers need to monitor easily noticeable parameters that change in the early stages of heat stress, this non-invasive parameter can be conveniently tracked by them. Feed additives were also considered for their potential positive effects on meat quality, irrespective of the presence of heat stress, in comparison to the control feed.

4.1 Measurements at Farm Level

4.1.1 Physiological Parameters and Behaviour

For both the control and enriched groups, RR and T_{skin} increased significantly with an increasing ΔTHI (above 75) or daily THI, respectively, while this effect was not significant for T_{rectal}. This finding also reinforces that RR is the first physiological change to an increasing heat load (Huynh et al., 2005a). It is possible that the THI was too low to reach the inflexion point of T_{rectal} (Huynh et al., 2005a).

The effect of a higher heat load on physiological parameters did not differ between the two diets despite the anticipated effects based on literature. For example, Liu et al. (2018b) found a less steep increase in T_{rectal} during a heat load period of 8 days when feeding an organic Se supplementation (1.0 ppm). Furthermore, Gabler et al. (2013) found a decrease in RR in pigs fed a betaine-supplemented feed. Chauhan et al. (2014) also found a decreased RR and T_{rectal} in sheep with vitamin E and Se supplementation, and Attia et al. (2009) found a decreased T_{rectal} in betaine-fed growing chickens. In agreement with our study, no effects of vitamin E and organic Se on T_{rectal} could be demonstrated when the high heat load period only lasted 2 days (Liu et al., 2016). The heat load in our study may have been too mild and/or too short to observe possible beneficial effects of the additives. It should be noted that heat stress severity and duration differ among studies which complicates a comparison of study results. In addition, little is known about the combined effect of the different additives.

One study that included the simultaneous combination of Se, vitamin E, and betaine in sows under heat stress did not find alterations in RR (Liu et al., 2017). A study that combined Se, vitamin E, and betaine in a diet for broilers found that the reduction in RR during heat stress due to betaine supplementation was less pronounced when Se and vitamin E were also added (Shakeri et al., 2018). These results were confirmed in a second study by Shakeri et al. (2019), who had found a trend in a decreasing T_{rectal} in broilers when betaine was supplemented but not under concurrent supplementation of betaine, Se, and vitamin E. The same study noted that for RR, a significant decrease was found when supplementing betaine that did not decrease further when Se and vitamin E were supplemented in addition to betaine. This indicates a need for further research to evaluate the effects of concurrent supplementation of these nutrients in growing–finishing pigs during more extreme heat loads.

It is well-known that pigs increase their lateral lying behaviour when the heat load increases. When pigs lie on their side, the contact area with the cooler floor increases (Huynh et al., 2005b). These findings were also found in the present study. However, the increase in lateral lying behaviour with an increasing ΔTHI also differed between diets, as indicated by the diet × ΔTHI interaction. Pigs fed the enriched diet increased their lateral lying behaviour by 10% more than the control group with an increasing THI. Other behavioural parameters were not significantly affected by the dietary treatments.

4.1.2 Correlations

Rectal temperature and RR are considered good parameters to evaluate responses to a high heat load at the animal level. These parameters are time-consuming to measure, however. Skin temperature could be an interesting non-invasive proxy for T_{rectal} and an indicator for heat stress in practice. Brown-Brandl et al. (2013a) state that thermal images can be a tool to indicate thermal comfort. However, the correlations between the T_{rectal} and T_{skin} of the flank indicates that T_{skin} is not reliable for this purpose, especially because the correlation coefficient during the entire growing–finishing period was positive, while this was a negative coefficient when examining only the data of the second and highest heat peak. The poor correlation between the T_{rectal} and T_{skin} of the flank was also found by Schmidt et al. (2013), who observed that the surface temperature of a sow's thigh obtained with an IR was not in close agreement with T_{rectal}. In addition, the results of Dewulf et al. (2003) showed that T_{skin} and T_{rectal} had a linear relationship ($p < 0.01$), with a minimal slope of 0.044 °C increasing in T_{rectal} with an increase of 1 °C in T_{skin}, but that T_{skin} cannot replace or predict T_{rectal}. A relatively strong correlation was found between T_{skin} and ambient temperature, reinforcing the idea that T_{skin} may not be a reliable physiological parameter. This indicates that T_{skin} of the flank with IR technology cannot replace the measurement of T_{rectal}.

4.1.3 Performance Parameters

Performance results did not show significant differences, but the enriched group had numerically lower values for ADFI and ADG during the starter and grower phases. These numerical differences are relevant and are difficult to clarify. The flavour of certain additives may decrease ADFI with a resulting decrease in ADG. A review study on the function of betaine in pigs summarised that only 2 out of 41 studies showed significant adverse effects of betaine on ADFI in growing–finishing pigs (Lawrence et al., 2002; Matthews et al., 2001). A total of 15 studies found no differences, while all the other studies showed positive results on performance parameters (Ratriyanto et al., 2009). In recent studies, betaine did not affect performance parameters (Lan & Kim, 2018; Mendoza et al., 2017). For Se, a meta-analysis indicates that Se supplementation increases ADG and feed efficiency (Quisirumbay-Gaibor et al., 2020). Based on the literature, it seems unlikely that Se or betaine would negatively influence ADFI. Another explanation may be related to the number of pigs per feeder. In one of the two pens of the control group (control 1), three animals had to be excluded from the trial at the beginning of the growing–finishing period. Therefore, the feed competition at the Nedap feeding system was probably lower due to increased space allowance, which may have led to a higher ADFI of the individuals in that pen and consequently a higher mean ADFI of the control group. This was also found by Hyun and Ellis (2001), where feed intake and growth rates were lower for groups with 12 growing–finishing pigs in the starter phase as compared to groups of 2, 4, 6, or 8 pigs, while this was not the case for growing–finishing pigs in the grower phase (Hyun & Ellis, 2002). As the numerical difference between the control and enriched groups was more pronounced in the starter phase, the stocking density and/or feed competition may partly explain these findings. The diet composition might also contribute to a lower ADFI. Despite the efforts to maintain constant values, there are some small, unintended differences in the analysed diet composition. Typically, slight increases in crude protein content do not impact ADFI (Prandini et al., 2013), but an increase in crude fibre may slightly reduce ADFI (Kyriazakis & Emmans, 1995b; Zhang et al., 2013). However, this seems unlikely, as the percentage increases in diet composition in the enriched diet were rather small.

The significant interactions in diet × period for daily drinking visits, daily drinking time, and ADWI imply that animals on the enriched diet behave differently during a higher heat load period. Water intake during a period of elevated THI increased significantly in the control group while it was not changed in the enriched group. Regardless of the treatment, all pigs suffered from a higher heat load, as reflected by the significant increase in RR (and T_{skin}.) Previous studies indicate that pigs normally increase their ADWI during a period of high heat load (Huynh et al., 2005a). Nevertheless, as the enriched group showed no significant change in ADWI during a heat period, it may indicate that the enriched pigs were less affected by the higher heat load than the control group. To our knowledge, no study has yet shown an effect of dietary Se or betaine addition on the water intake of growing–finishing pigs subjected to heat load. Because betaine functions as an osmoprotectant (Ratriyanto et al., 2009), also called compatible osmolyte (Landfald & Strom, 1986), it can increase the water-binding capacity of the intestinal cells (Kettunen et al., 2001) and protect cell components from denaturisation due to high ionic strength (Kempf & Bremer, 1998). This may result in a lower need to increase water intake during periods of more intense heat. Noticeably, the enriched group had an overall higher number of drinking visits and spent more drinking time than the control group, while their overall daily water intake was lower. This can be explained by the relatively higher average flow rate in the control group (0.812 L/min) compared to the enriched group (0.716 L/min). In contrast with the previous hypothesis, the water-to-feed ratio revealed other insights. Interestingly, this ratio was similar between the enriched and control groups during the heat load. Within the enriched group, however, a lower water-to-feed ratio was observed before and after the heat stress period. This may suggest that during heat exposure, pigs in the enriched group increased their water intake more independently of feed intake, or alternatively, that their feed intake decreased more while water intake remained stable. The latter seems less likely, as feed intake slightly increased during the heat load period. However, the total water intake per pig per period, regardless of the amount of feed intake remains stable in the enriched group.

The ADFI between the diet groups before, during, and after a heat load period showed no significant differences. Surprisingly, the ADFI for both groups increased significantly during a higher heat load period compared with the period before. Usually, a decrease in feed intake is expected during a heat load period (Collin et al., 2001b; Kemp & Verstegen, 1987; Quiniou et al., 2000), as this is one of the primary mechanisms in animals to reduce heat production (Huynh et al., 2005a). According to Huynh et al. (2005a) the inflexion point where voluntary feed intake starts to decrease is situated around 25.5 °C at a RH of 65% (average RH in this trial was 62%), which corresponds to a THI of 74 according to the formula of Lucas et al. (2000). During our trial, the average THI during the three heat peaks was higher (75.4) and therefore a decrease in ADFI can be expected. Possibly other factors such as stocking density played a role. The stocking density in our study (1.27 m²/pig) was lower than in the study of Huynh et al. (2005a) (1.1 m²/pig). This may lower the heat stress effects and/or increase the threshold value for a decreased ADFI. Because the period of a high heat load is associated with the week of the growing–finishing period, the increasing ADFI over the three periods can also be regarded as an age effect because feed intake is correlated with live weight (Kanis & Koops, 2010; Labroue et al., 1994).

4.2 Measurements in the Slaughterhouse

4.2.1 Observations in the Lairage Area

The enriched feed had no effect on the HSS in the lairage area, but it should be noted that the pigs were not subject to heat stress during lairage, as the THI was 60.6 at the time. The mean HSS after arrival in the lairage was only around 27, which is relatively low on a tagged visual analogue scale of 150. When staying in the lairage the HSS dropped further regardless of the diet group.

4.2.2 Carcass Traits and Meat Quality

No significant differences were found between diet groups for carcass traits and meat quality, which is in contrast to most studies of betaine on carcass traits. Two review studies stated that betaine supplementation increases lean meat content and decreases carcass fat content (Eklund et al., 2005; Ratriyanto et al., 2009). Furthermore, a study on the supplementation of vitamin E and Se (and soy isoflavone) found a decreased back fat thickness at the last rib, an increased yellowness b* value, and decreased drip loss (Zhu et al., 2022). Also, supplementation of vitamin E and vitamin C may positively affect pH and drip loss (Lahučký et al., 2005). In addition, vitamin E has been found to increase water-holding capacity and improve the colour of the loin (Dugan et al., 2004; Ellis & McKeith, 1999; Ngapo & Gariepy, 2008; Rosenvold & Andersen, 2003). Selenium, on the other hand, would contribute very little to meat quality (Dugan et al., 2004).

This trial had certain weaknesses. The main objective was to investigate the different feed additives during periods of high heat load during a naturally warm summer. During the summer of the trial, there were no naturally occurring heat waves and the maximum daily THI remained relatively low throughout the trial. The stocking density was also lower than field conditions on a conventional farm. The pigs therefore had more space to cope with heat stress according to conductive and radiative heat transfer. In addition, although most parameters were measured at the individual level, statistically, the number of repetitions per diet treatment was only two due to the practical limitation that only one diet could be supplied per pen.

5 Conclusions

Dietary supplementation of betaine, selenomethionine, vitamin E, and vitamin C did not significantly alter the physiological and performance parameters of growing–finishing pigs raised under the tested summer conditions. Carcass and meat quality also showed no significant differences between the two diets. Pigs in the enriched group had no altered total water intake during the warm periods, but their water-to-feed ratio increased during the heat load. Future research should focus on the effect of various additives in different proportions at higher heat loads.

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CHAPTER 4B | EFFECT OF CHEMICAL COMPOSTION & ANTIOXIDANTS IN THE FEED

Adapted from: De Prekel, L., Maes, D., Van den Broeke, A., & Aluwe, M. (2025). Effect of antioxidant and osmolyte enriched or energy-dense diet on heat-stressed fattening pigs. Animal, 19(6), 101514. https://doi.org/10.1016/j.animal.2025.101514

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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 crude protein, 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 ± 80kg. The control diet comprised 15% crude protein, 3.6% crude fat (CF), 9.1 MJ/kg net energy (NE), 0.4 mg/kg inorganic 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% crude protein, 6.6% CF, and increased energy (9.7 MJ/kg) and lys content. The lys:energy ratio of all 3 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 T_{rectal} 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 FCR 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 supplementation of antioxidants and osmolytes numerically ameliorated daily gain over the entire trial.

Key words

Heat wave, Blood gas parameters, Carcass and meat quality, Rectal temperature, Performance

1 Introduction

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 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 energy source as when carbohydrates or fats are used (Musharaf & Latshaw, 2019). 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 AA's 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., 2016), 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 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., 2001a). These antioxidants alleviate heat-induced oxidative stress by neutralizing 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 (Gabler et al., 2013; Morales et al., 2018; Pathak et al., 2018; Ratriyanto et al., 2009), 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., 2019; Shakeri et al., 2018). 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. For vitamins, there is no legal restriction, but for Se, European legislation sets the maximum permitted added level of active substance in animal feed at 0.5 mg/kg of feed (Europees Parlement, 2003). Betaine is considered safe at a maximum supplementation rate of 2,000 mg betaine/kg complete feed (Rychen et al., 2018). Furthermore, natural forms of additives are often costlier or unavailable.

In the present study, we investigated the effect of an additive-enriched (like Chapter 4A) 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.

2 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).

2.1 Study design

A total of 32 pens of mixed-sex (3 gilts and 3 barrows) fattening pigs (two consecutive batches of 96 pigs) (Topigs TN70 x Belgian Piétrain) were randomly divided into 3 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 ± 11.9kg) with a one-week acclimatization period and ended at slaughter at 25 or 26 weeks of age (116.4 ± 9.5kg) (Figure 1). Two artificial heat waves were induced for 7 days, i.e. the first heat wave at 20 weeks of age (87.4 ± 9.3kg) and the second at 22 weeks of age (98.7 ± 9.4kg).

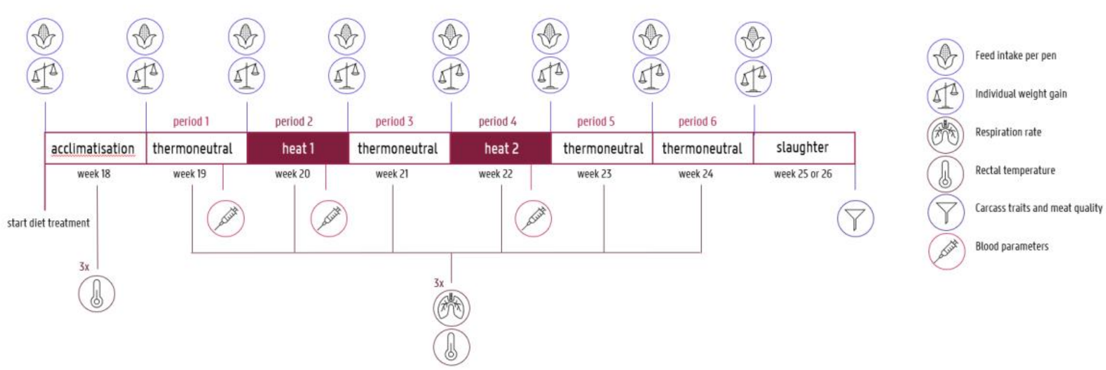

Figure 1: Visual representation of the trial design

Table 1: Ingredients, premix composition and chemical composition of the control, enriched and E-dense diet provided 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-LYS 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]				
Crude protein (N x 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
Dry matter [g/kg]		890	890	894
NE [MJ/kg][2]		9.1	9.1	9.7
SID LYS: NE ratio [g/MJ][2]		0.86	0.86	0.87
EB [meq/kg][2]		182.5	182.5	220
Composition premix[7]				
Vitamin E [mg/kg]	Calculated	100.0	200.0	100.0

	Analysed (DL-alfa-tocopherol)[3]	99	195	NA
Vitamin C [mg/kg]	Calculated	0.0	200.0	0.0
	Analysed (ascorbic acid)[4,5]	<5	51.7	NA
Betaine hydrochloride [mg/kg]	Calculated	0.0	1306.7	0.0
	Analysed (total)[4,6]	1400	2300	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]				
LYS	Calculated (SID)	7.8	7.8	8.4
	Calculated (total)	9.0	9.0	9.4
	Analysed (total)	8.8	8.8	9.5
MET	Calculated (SID)	2.9	2.9	3.3
	Calculated (total)	3.2	3.2	3.6
	Analysed (total)	3.3	3.2	3.8
THR	Calculated (SID)	5.4	5.4	5.7
	Calculated (total)	6.5	6.5	6.6
	Analysed (total)	6.3	6.3	6.4
TRP	Calculated (SID)	1.6	1.6	1.7
	Calculated (total)	1.9	1.9	2.0
	Analysed (total)	1.9	2.0	2.0
ILE	Calculated (SID)	4.4	4.4	4.3
	Calculated (total)	5.3	5.3	5.0
	Analysed (total)	5.5	5.3	5.2
LEU	Calculated (SID)	8.9	8.9	8.4
	Calculated (total)	10.8	10.8	9.9
	Analysed (total)	10.9	10.6	9.8
VAL	Calculated (SID)	5.4	5.4	5.6
	Calculated (total)	6.7	6.7	6.7
	Analysed (total)	7.0	6.6	6.9
HIS	Calculated (SID)	2.9	2.9	2.6
	Calculated (total)	3.5	3.5	3.1
	Analysed (total)	3.5	3.5	3.3
PHE	Calculated (SID)	5.5	5.5	4.7
	Calculated (total)	6.6	6.6	5.6
	Analysed (total)	6.6	6.5	5.7
M+C:LYS	Calculated (SID)	0.62	0.62	0.60
	Calculated (total)	0.66	0.66	0.63
	Analysed (total)	0.68	0.66	0.65
THR:LYS	Calculated (SID)	0.66	0.66	0.65
	Calculated (total)	0.72	0.72	0.70
	Analysed (total)	0.72	0.72	0.67
TRP:LYS	Calculated (SID)	0.20	0.20	0.19
	Calculated (total)	0.21	0.21	0.21

	Analysed (total)	0.22	0.23	0.21
ILE: LYS	Calculated (SID)	0.54	0.54	0.49
	Calculated (total)	0.59	0.59	0.53
	Analysed (total)	0.63	0.60	0.55
LEU:LYS	Calculated (SID)	1.15	1.15	0.99
	Calculated (total)	1.20	1.20	1.05
	Analysed (total)	1.24	1.20	1.03
VAL:LYS	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: standardized ileal digestible, NE: Net Energy, EB: Electrolyte Balance, NA: not applicable

[1]Analysed by the ILVO ANIMALAB, Melle, Belgium, [2]Formulated composition, [3]Analysed by Ecca, Merelbeke, Belgium, [4]Analysed by Eurofins Food Testing Belgium, Nazareth, Belgium, [5]Analysed one year after production which can influence the concentration of vitamin C, [6]Total betaine calculated as total betaine hydrochloride, [7]Calculated and produced by DSM, Deinze, Belgium, [8]Analysed amino acid by FFQ Laboratorium, Merksem, Belgium

2.2 Housing and management

The trial was performed in 4 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 three-week intervals between each batch, were divided into 2 compartments each. Each compartment consisted of 8 pens of 6 pigs with a random distribution of the 3 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 m². The compartment was artificially lit from 07:30 to 15:30 plus natural light from a window (2.07m²) on the south side for batch 1 (compartments 1 and 2) and on the north side for batch 2 (compartments 3 and 4).

2.3 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 3 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% crude protein, 3.5% crude fat (CF), NE of 9.1 MJ/kg, electrolyte balance (EB) of 182.5 meq/kg and SID Lys 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, 1307 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 crude protein content of 13.6% and a higher CF content of 6.6%, NE of 9.7 MJ/kg, EB of 220 meq/kg and SID Lys of 8.4 g/kg. The E-dense diet was provided with extra synthetic AA's according to the recommendations of CVB (2023) to compensate the decrease in crude protein content (Table 1). The SID lysine (LYS): NE ratio of all 3 diets was almost the same. Diets were formulated based on 1) literature to identify various feed additives/ strategies that could mitigate heat stress (Cottrell et al., 2015; De Prekel et al., 2024a; Gan et al., 2014; Haydon et al., 1990; Liu et al., 2018b; Liu et al., 2017; Liu et al., 2016; Lv et al., 2015; Ratriyanto et al., 2009; Stewart et al., 2015; Tang et al., 2019) and (2) the advice of the feed expert group of the research project Coolpigs, and were formulated to meet optimal nutrient requirements and AA levels of fattening pigs between 80-120 kg (CVB, 2023). Feed and water were provided *ad libitum* to all groups.

2.4 Climate control

The compartment was mechanically ventilated by channel ventilation, meaning that the incoming air entered the compartment 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). Two seven-day heat waves were applied, as during the previous diet experiment with feed additives (Chapter 4A,), the heat stress conditions were relatively mild. To ensure that a high heat load was present in order to properly test the effectiveness of the diets, an altered duration of the validated heating protocol of Chapter 3 was applied. Furthermore, the initial three-day heating protocol did not lead to consistent changes in average daily feed intake, while a seven-day heat wave in an earlier conducted study (Chapter 5), showed clear effects on performance parameters. 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 12:00 on the first day of the heat wave with increases of 2 °C per half-day, ending around 31 °C at 18:00 on the second day of the heatwave. During the artificial heatwave, the temperature remained constant during the day and night (Figure 2). 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 RH and ambient temperature in the compartment in 10-minute intervals during the entire trial period. The data from the climate sensors was used to calculate the THI, as mentioned in Chapter 3.

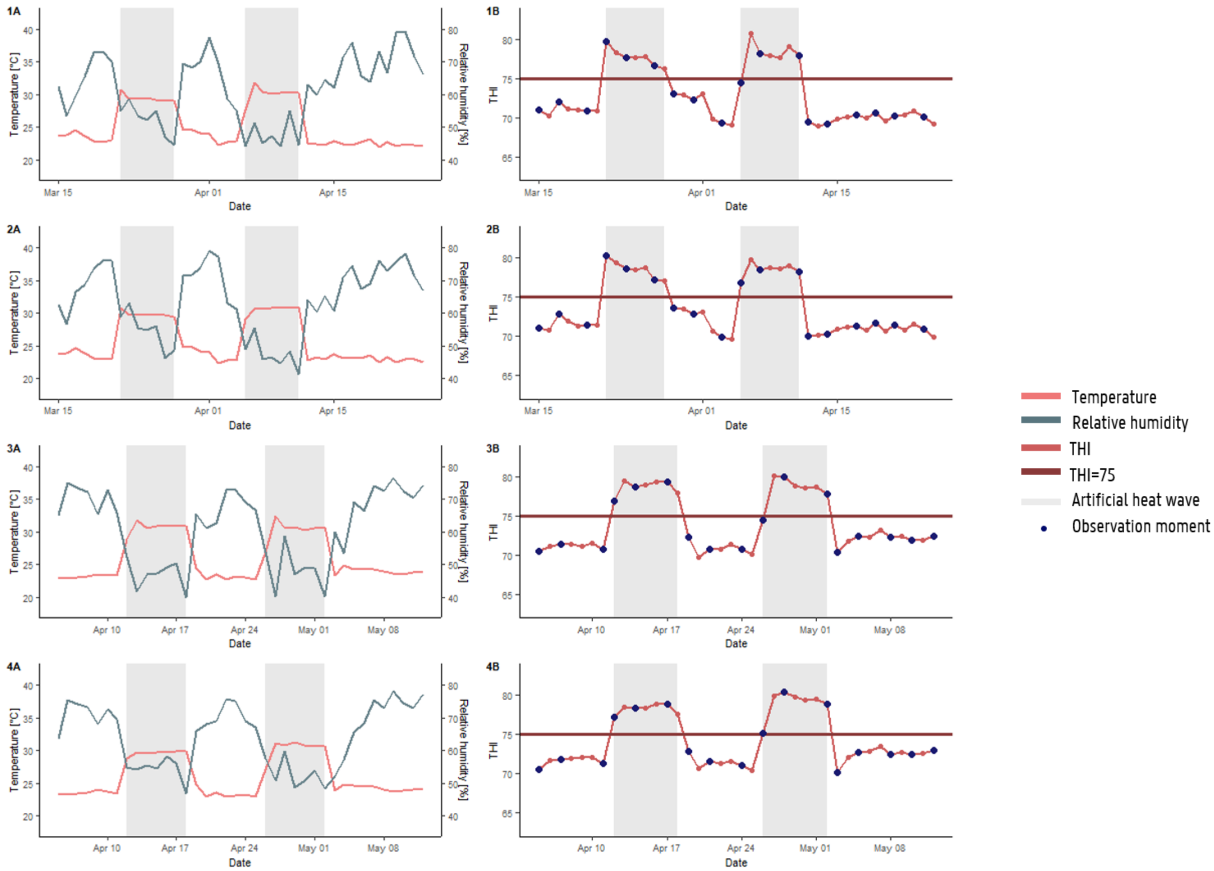

Figure 2: Evolution of the ambient temperature and relative humidity (A) of the highest Temperature-humidity index between 14:00-17:00 (THI) (B) during the trial for each of the 4 compartments (1, 2, 3 and 4) as measured during the study. The grey areas indicate the artificial heat waves, while the white areas indicate the thermoneutral weeks.

2.5 Description of critical methods

2.5.1 Physiological parameters

Three reference animals (2 barrows and 1 gilt, or 2 gilts and 1 barrow) per pen were randomly selected to monitor the physiological parameters throughout the study: RR and T_{rectal}. The same reference animals were evaluated throughout the trial. The parameters were measured 3 times per week in the afternoon (between 13:00 and 15:00) starting at 19 until 24 weeks of age (Figure 1). Respiration rate and rectal temperature were measures according to the same methods as described in Chapter 3. During the acclimatization 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. The treatments were blinded for the observers.

2.5.2 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 (Figure 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 Se 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 hours after sampling for 10 minutes at 4 °C and 1500 g. After this, plasma was divided into 4 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 Se 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 (220µl) was put on a CG8+ cartridge which analysed pH, ionized calcium, Sodium (Na), Potassium (K), glucose, haematocrit, haemoglobin, oxygen partial pressure (pO_2), carbon dioxide partial pressure (pCO_2), bicarbonate (HCO_3^-), total carbon dioxide, oxygen saturation and base excess.

2.5.3 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, ADFI, ADG and FCR were calculated per week, per pen 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.

2.5.4 Carcass traits and instrumental meat quality

The pigs were transported to a commercial slaughterhouse after a fasting period of minimum 16h. Each batch of pigs was sent to the slaughterhouse in two groups: the first group, with an average pen weight of ≥110kg 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 minutes post-mortem, the pH_i (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 post-mortem, the pH_u was measured by 3 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 (2 slices), 3) cooking and shearing, 4) vitamin E, 5) colour and marbling, 6) Se, 7) drip loss and IMF (2 slices), and 8) colour and marbling determinations. Water holding capacity was evaluated according to the drip loss method of Honikel (1987) using 2 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 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 minutes and cooled down for a minimum of 15 minutes by tap water. Then 5 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 250N, Mecmesin, UK). Afterwards, the 2 most extreme values were removed from the dataset and the average of the remaining 8 measurements per animal was calculated. Intramuscular fat content 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 J & Olley, 1963). First, 2 meat slices of 2.5 cm thickness were homogenised using a meat grinder and 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 minutes of blooming at 9 °C. From the same meat slices, marbling (IMF content) was visually evaluated by a 7-point marbling level scale by 2 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).

2.6 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 acclimatization period for the measurement of physiological parameters was incorporated. pH meter calibration occurred initially in the slaughterhouse, followed by a 22-hour 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 standardized using a black and white panel.

2.7 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.

To evaluate the effects of dietary treatment-in reaction on the heat load on different parameters, 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 (week 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, ionized 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 (week 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:

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

Where $Y_{meat\text{-}carcass}$ = dependent variables (pH_i, pH_u, drip loss, cooking loss, total fluid loss, shear force, IMF, 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 $p \leq 0.05$. Post-hoc tests according to the Kenward-Roger degrees of freedom 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 post-hoc test according to the Kenward-Roger degrees of freedom 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 post-hoc tests is not given. The pen was considered as the experimental unit.

3 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 was 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.

3.1 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).

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 x Week
Respiration rate [breaths/min]	19 (thermoneutral)	288	48^{a}	45^{a}	49^{ab}	0.816	0.554	<0.001	0.728
	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			
	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	0.621	<0.001	0.386
	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			
	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.

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, $p = 0.018$) (Figure 3). 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).

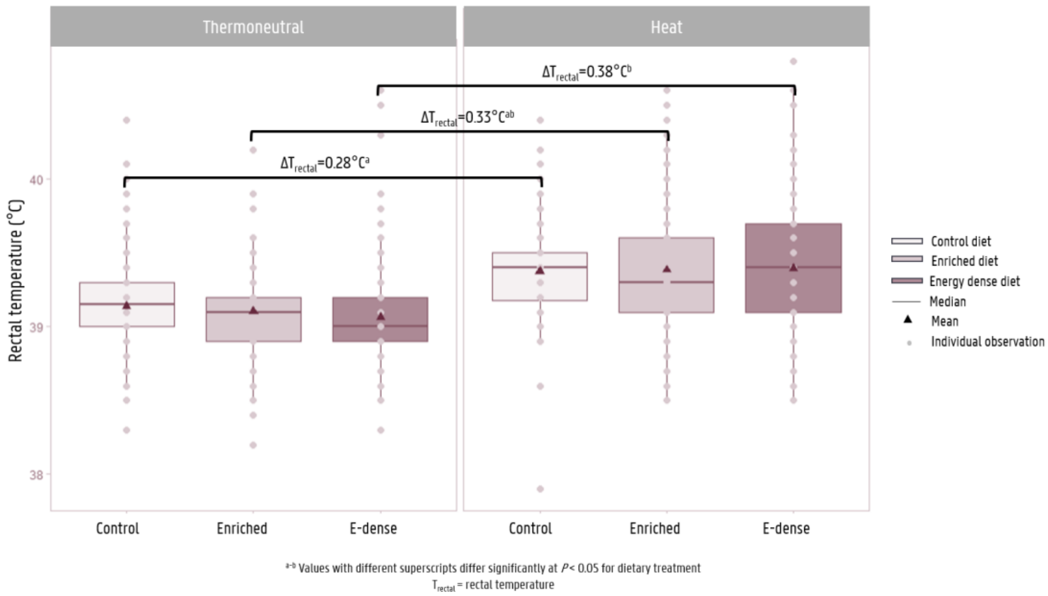

Figure 3: Mean rectal temperature (T_{rectal}) of the three dietary treatment groups (control, enriched and E-dense) during all thermoneutral weeks and the 2 heat waves, and differences in T_{rectal} between these means, representing ΔT_{rectal}

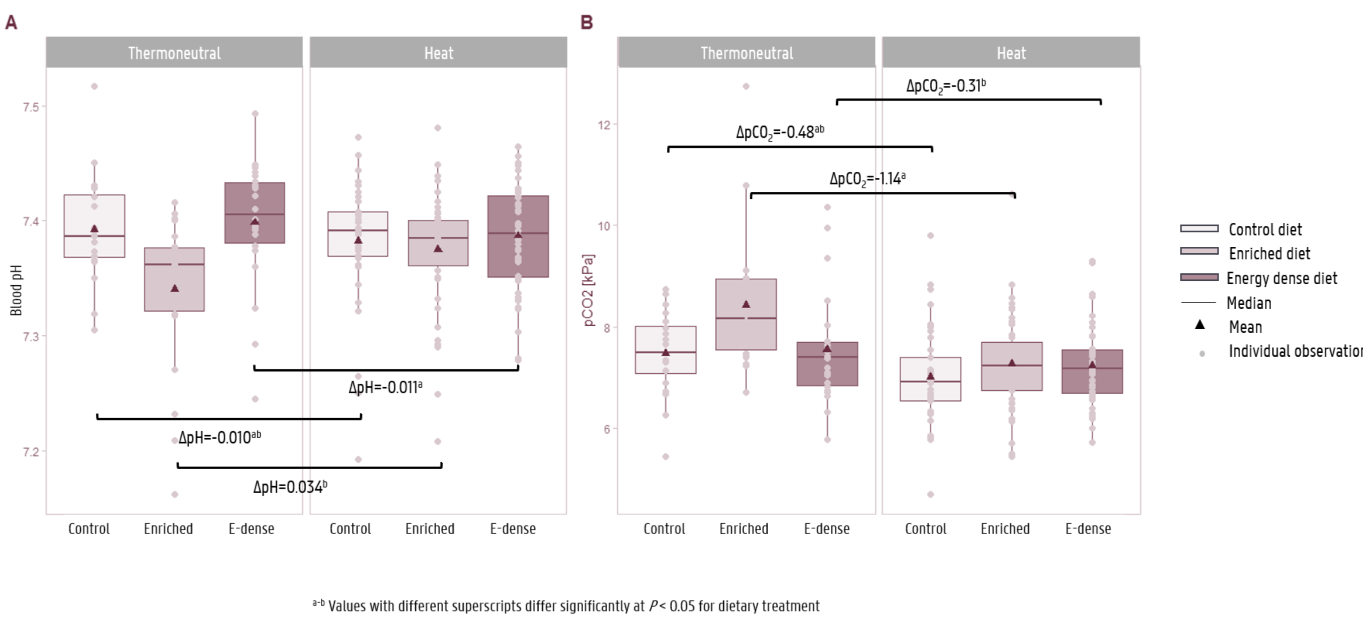

Figure 4: Mean blood pH (A) and carbon dioxide partial pressure (pCO_2) (B) of the 3 dietary treatment groups (control, enriched and E-dense) during the first thermoneutral week (week 19) and the 2 heat waves, and differences of these parameters between the means, representing ΔpH and ΔpCO_2

3.2 Blood (gas) parameters

The average change in blood pH during heat load (ΔpH) was significantly different between the enriched and E-dense group (Figure 4). 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, $p = 0.035$) (Figure 4A). Furthermore, ΔpCO_2 was lower for the E-dense than for the enriched group (-0.31 vs -1.14, $p = 0.032$) (Figure 4B); 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 Table 3.

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, $p = 0.004$).

3.3 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 4). 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. Post hoc 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 ($p = 0.090$).

Table 3: Blood gas parameters of fattening pigs (estimated means) according to the effect of dietary treatment (control, enriched, E-dense) and the heat week (thermoneutral week and heat 1 and heat 2)

Parameter	Week	n	Diet			SEM	*p*-value		
			Control	Enriched	E-dense		Treatment	Week	Treatment x Week
pH	19 (thermoneutral)	64	7.39[y]	7.34[a,x]	7.40[y]	0.01	<0.001	0.694	0.087
	20 (heat 1)	64	7.38	7.37[ab]	7.38	0.01			
	22 (heat 2)	63	7.39	7.38[b]	7.40	0.01			
pCO_2 [kPa]	19 (thermoneutral)	64	7.45[x]	8.42[y]	7.55[x]	0.15	0.002	0.100	0.128
	20 (heat 1)	64	7.09	7.39	7.39	0.11			
	22 (heat 2)	63	6.85	7.18	7.08	0.11			
pO_2 [kPa]	19 (thermoneutral)	64	3.72	3.81	3.94	0.08	0.646	0.611	0.428
	20 (heat 1)	64	3.92	3.53	3.53	0.10			
	22 (heat 2)	63	3.72	3.63	3.65	0.10			
HCO_3	19 (thermoneutral)	64	34.0[b]	33.9[b]	34.6[b]	0.28	0.504	<0.001	0.756
	20 (heat 1)	64	31.3[a]	31.9[a]	32.4[a]	0.27			
	22 (heat 2)	63	30.8[a]	31.5[a]	32.6[a]	0.31			
BE	19 (thermoneutral)	64	9.05[b]	8.14	9.91[b]	0.33	0.109	<0.001	0.549
	20 (heat 1)	64	6.20[a]	6.50	7.23[a]	0.32			
	22 (heat 2)	63	5.72[a]	6.40	7.73[a]	0.35			
SO_2	19 (thermoneutral)	64	49.3	47.4	53.5	1.32	0.303	0.803	0.477
	20 (heat 1)	64	51.9	45.2	45.6	1.76			
	22 (heat 2)	63	49.3	47.3	47.8	1.76			
tCO_2	19 (thermoneutral)	64	35.6[b]	35.8[b]	36.5[b]	0.29	0.509	<0.001	0.801
	20 (heat 1)	64	32.9[a]	33.5[a]	34.1[a]	0.28			
	22 (heat 2)	63	32.2[a]	33.1[a]	34.2[a]	0.33			
Na	19 (thermoneutral)	64	141.0	141.6	140.9	0.17	0.195	0.122	0.579
	20 (heat 1)	64	140.1	140.5	139.6	0.18			
	22 (heat 2)	63	140.5	140.2	139.7	0.18			
K	19 (thermoneutral)	64	4.79	4.80	4.77	0.06	0.975	0.096	0.997
	20 (heat 1)	64	4.50	4.52	4.50	0.05			
	22 (heat 2)	63	4.54	4.62	4.55	0.06			
iCa	19 (thermoneutral)	64	1.39[x]	1.44[y]	1.42[xy]	0.01	0.022	0.717	0.881
	20 (heat 1)	64	1.40[x]	1.44[y]	1.42[xy]	0.01			
	22 (heat 2)	63	1.40	1.43	1.41	0.01			
Glu [mmol/L]	19 (thermoneutral)	64	5.53	5.70	5.96	0.08	0.140	0.740	0.429
	20 (heat 1)	64	5.40	5.68	5.61	0.08			
	22 (heat 2)	63	5.55	5.42	5.81	0.10			
Hct	19 (thermoneutral)	64	34.7[a]	35.4	33.9	0.38	0.311	0.057	0.084
	20 (heat 1)	64	36.0[ab]	34.7	35.3	0.40			
	22 (heat 2)	63	36.9[b,y]	34.6[xy]	34.1[x]	0.42			
Hb* [g/L]	19 (thermoneutral)	64	118[a]	120	115	1.28	0.313	0.055	0.084
	20 (heat 1)	64	122[ab]	118	120	1.37			
	22 (heat 2)	63	125[b,y]	118[xy]	116[x]	1.43			

pCO_2=carbon dioxide partial pressure, pO_2=oxygen partial pressure, BE=base excess, sO_2=oxygen saturation, tCO_2=total carbon dioxide, iCa=ionized calcium, Glu=glucose, Hct=haematocrit, Hb*=haemoglobin, n= number of observed fattening pigs

[a-b] Values within a column with different superscripts differ significantly at $P < 0.05$ for heat period within a diet group

[x-y] Values within a row with different superscripts differ significantly at $P < 0.05$ for diet group within a heat period

Table 4: 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 x 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
	19 (thermoneutral)	32	2627^{c}	2504^{b}	2667^{bc}	44			
	20 (heat 1)	32	2115^{a}	2134^{a}	2183^{a}	35			
	21 (thermoneutral)	32	2506^{bc}	2566^{b}	2743^{bc}	41			
Daily feed intake [g/day]	22 (heat 2)	32	2030^{a}	2053^{a}	2099^{a}	39	0.204	<0.001	0.461
	23 (thermoneutral)	32	2399^{b}	2457^{b}	2572^{b}	43			
	24 (thermoneutral)	32	2634^{c}	2603^{b}	2807^{c}	53			
	Total	32	2376	2380	2505	34	0.123	NA	NA
	19 (thermoneutral)	32	1081^{d}	1043^{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			
Daily gain [g/day]	22 (heat 2)	32	458^{a}	532^{a}	486^{a}	35	0.504	<0.001	0.437
	23 (thermoneutral)	32	955^{cd}	970^{bc}	1050^{d}	31			
	24 (thermoneutral)	32	884^{bcd}	807^{b}	750^{bc}	37			
	Total	32	891	925	851	14	0.090	NA	NA
	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			
Feed conversion ratio [g/g]	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

$^{a-d}$ 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

3.4 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 5). 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.

Table 5: 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						
pH_i	96	6.46	6.45	6.38	0.02	0.400
pH_u	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

pH_i = initial pH after 35 min postmortem, pH_u = ultimate pH after 22h post-mortem

[1]visual score of intramuscular fat on a seven point-marbling scale (1-7)

n = number of observed carcasses/meat loin

$^{x-y}$ Values within a row with different superscripts differ significantly at p < 0.05 for dietary group

4 Discussion

4.1 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 T_{rectal} and/or RR during heat stress (minimum 7 days of cyclic heat periods) in various animal species (Chauhan et al., 2014; Liu et al., 2018b; 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 (De Prekel et al., 2024a; Liu et al., 2017). 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, neutralizing 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 crude protein 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 crude protein 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 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 NE than those used in our study, including the difference between the two levels. In our study, the differences in crude protein and NE 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.

4.2 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., 2018a; Liu et al., 2018b; Liu et al., 2016). An increase in blood pH and a decrease in pCO_2 were expected during the heat weeks due to elevated RR's. In this study, we observed higher respiratory rates, but no signs of respiratory alkalosis. The utilization of venous blood instead of arterial blood for blood pH measurements does not affect the results (Augustinsson & 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 & 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 EB in the diet (Kellum, 2000). As the E-dense diet had a higher calculated EB (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 EB's around the optimal level of 250 mEq/kg (National Research Council, 2012).

4.3 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 Se 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 to 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, AA 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 Se.

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 AA's (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 AA to LYS, since the digestible LYS:NE ratio is almost constant. This effect might have been exacerbated by the numerically higher NE intake in the E-dense pigs. Normally, a low-protein diet formulated with a relatively constant digestible LYS:NE (Lebret, 2008) and a digestible essential AA:LYS that respects the ideal protein should not impair performance. However, our diets were based on the AA profile for fatting pigs (gilts and barrows) from 80-120 kg following CVB (2023) recommendations. Also, no major differences in the essential digestible and total AA:LYS ratio of the formulated and analysed diet were observed that could explain the variations in FCR and lipid deposition, although some amino acids ratios, like isoleucine and leucine to lysine, were close to the lower limit (DSM, 2023). Another possible explanation for the worse results of the E-dense group could be the asynchrony in the availability of energy and AAs. Fat was mainly used as an energy source in the E-dense feed. This possibly leads to slower absorption and energy metabolization than rapidly available carbohydrates (Wang et al., 2018). In turn, the supplementation with crystalline AA's may be absorbed faster than AA's bound in protein (Chung & Baker, 1992). van den Borne et al. (2007) showed that performance may decrease when the availability of AA's 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. In addition, the combination of slightly lower AA to LYS ratios and asynchrony of the energy source could also limit the performance results.

4.4 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 pH_u and colour, depending on the experimental setting (Dugan et al., 2004; Lahučký et al., 2005; Ngapo & Gariepy, 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 60-70 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 100g serving of enriched meat can provide an additional 10µg 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 AA's 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 NE 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).

5 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 T_{rectal} and less favourable FCR 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 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.

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CHAPTER 5 | EFFECT OF SPACE ALLOWANCE

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Abstract

The impact of space allowance on fattening pigs during an artificial heat wave was investigated as measure to mitigate the effects of heat stress. In total, 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 seven days when the pigs weighed 88.1± 12.2 kg. Respiration rate and T_{rectal} were observed before, during and after the heat load. Average daily gain and average daily feed intake were also noted. During the heat load, the THI reached ≥75 (78.6), even when RH decreased to ±45%. Every physiological parameter showed significant increases during the heat load. The prolonged heating protocol of seven days also provoked significant decreases in ADFI (15%) and ADG (19%) for all treatment groups. We found that 0.8 m²/pig nearly 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 metabolic heat production. However, RR and performances was not significantly affected by the 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.

Key words

Space allowance, Rectal temperature, Respiration rate, Animal welfare, Heat stress

1 Introduction

A commonly proposed, simple and low-cost method for mitigating heat stress in fattening pigs is to lower the stocking density, which increases the space available for pigs. The minimum floor surface for housing fattening pigs between 85-110 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 increasing space allowance 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 space allowance 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 (Dewulf et al., 2007; Huynh, 2005; Mount, 1979a). 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 Schauberger et al. (2019) have shown that increasing the space allowance 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 increase in space allowance 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 (Hillmann et al., 2023). 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 this study we assessed the impact of different space allowances on fattening pigs during periods with a high heat load induced by an artificial heat wave. The possible impact of an increase space allowance on heat stress was determined by evaluating physiological and performance parameters of the animals.

2 Material and Methods

2.1 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 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 seven days when the animals were 21 weeks old (88.1 ± 12.2 kg). In the week before (pre-heat period), during and after the artificial heat wave (post-heat 1), the physiological parameters of the animals were observed (Figure 1). As ten pigs were removed from the trial due to illness or lameness before the pre-heat period, some $SD_{0.8}$ pens (6 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 20-23 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.

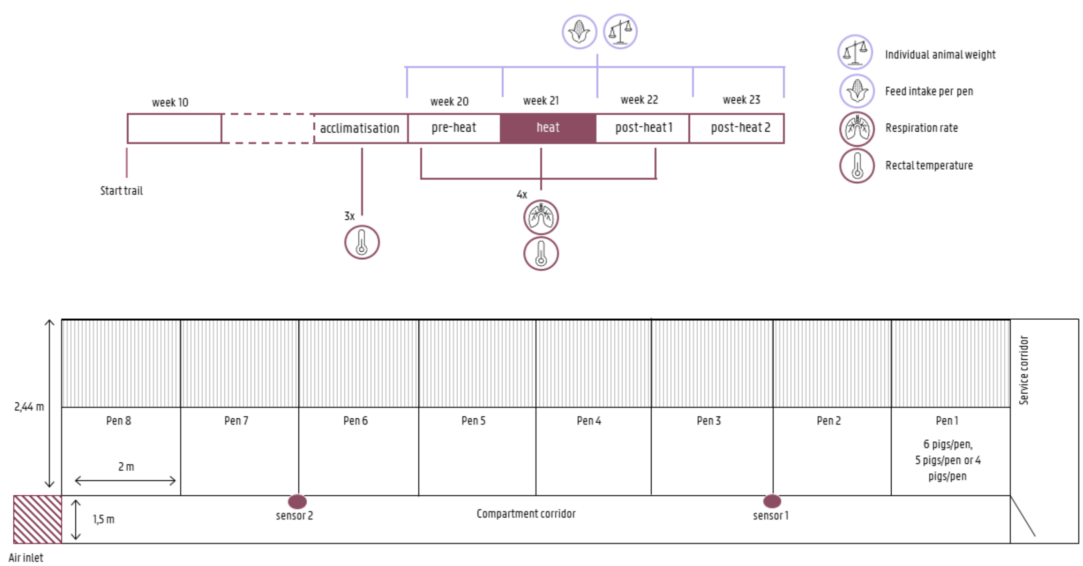

Figure 1: Schematic overview of the experimental design (timeline and follow-up of the different parameters of the fattening pigs) and the compartments (all compartments were identical, some were mirrored)

2.2 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 m^2. The compartment was artificially lit from 07:30 to 15:30 plus natural light from one window (2.07 m^2) on the south side of compartments 1, 3 and 4 and on the north side of compartment 2.

2.3 Climate control

The same heating protocol as described in Chapter 3 was used, 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 RH and ambient temperature in the compartment every 10 minutes for the entire trial period. The data from the climate sensors was also used to calculate the THI as describes in Chapter 3. Also, the periods before, during and after the heat load lasted seven days instead of three days.

2.4 Physiological parameters

Four reference animals per pen were randomly selected to monitor the physiological parameters RR and T_{rectal} individually as described Chapter 3. The parameters were measured daily for four days during pre-heat, heat, and post-heat 1. Before the start of the pre-heat period, T_{rectal} was measured for three 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 behaviour and T_{skin} were not evaluated in this experiment.

2.5 Performance parameters

Individual animal weight and feed intake per pen were measured weekly for the pre-heat, heat, and post-heat 1 period, as well as for an extra period during the second week after the heat load (post-heat 2 period). Based on the data, ADFI, ADG and FCR were calculated.

2.6 Statistical analysis

Statistics were calculated in R® software version 4.1.1. QQ-plots and histograms of the physiological (RR, T_{rectal}) and performance (ADFI, ADG and FCR) 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 (pre-heat, heat and post-heat 1 (and post-heat 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}, ADFI, ADG and FCR), HL = heat load as independent variable (pre-heat, heat, post-heat 1 and post-heat 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. Post hoc tests according to the Kenward-Roger degrees of freedom 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}, ΔADFI, ΔADG and ΔFCR were calculated by the differences between the average of the parameters on control days (pre- and post-heat load) and days with a high heat load for every stocking density. Afterward, a post hoc test according to the Kenward-Roger degrees of freedom 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 post hoc test is not given.

3 Results

3.1 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 RH of the highest THI per day during the observation time (14:00 - 17:00) is given for every compartment, which took place during different periods (Figure 2). 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.

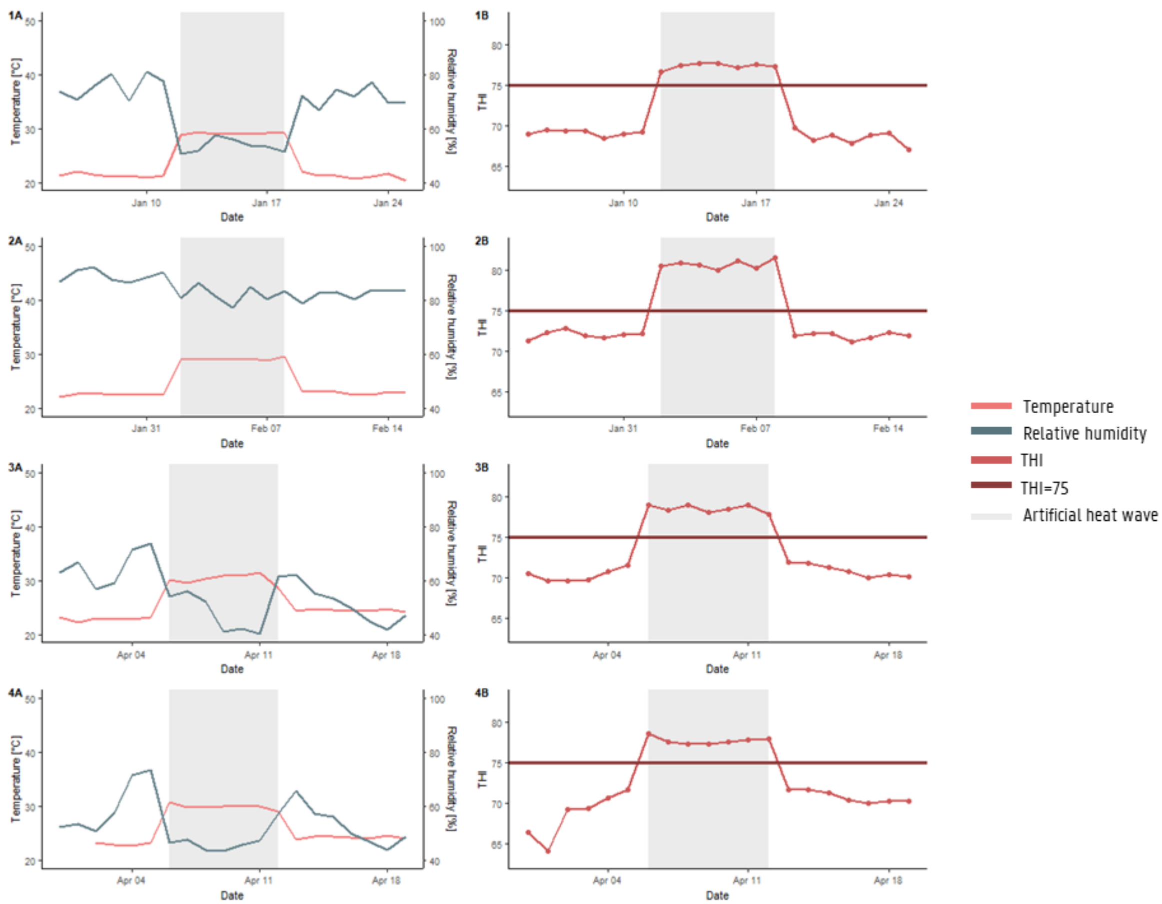

Figure 2: Ambient temperature and relative humidity (A) of the highest Temperature-humidity index (THI) (B) during the observation time from 14:00 to 17:00 in every compartment of the housed fattening pigs

3.2 Physiological parameters

For RR, the stocking density groups did not react differently to the heat load (interaction term, p = 0.532) (Table 1), but independent of the different stocking densities, RR increased by ±18 breaths/min during the heat load in comparison to the pre-heat 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 ΔT_{rectal}, however, revealed notable differences for the three stocking density groups during the heat load: $SD_{0.8}$ had a higher ΔT_{rectal} than $SD_{1.0}$ (p = 0.033) and tended to be higher than $SD_{1.3}$ (p = 0.053) (Figure 3). This means that T_{rectal} in the $SD_{0.8}$ 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.

Table 1: 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 (pre-heat, heat or post-heat period)

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
Respiration rate [breaths/min]	Pre-heat	48^{a}	50^{a}	50^{a}	0.649	0.001	0.544	0.532
	Heat	69^{b}	70^{b}	68^{b}	1.022			
	Post-heat	50^{a}	53^{ab}	51^{ab}	0.670			
Rectal temperature [°C]	Pre-heat	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			
	Post-heat	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

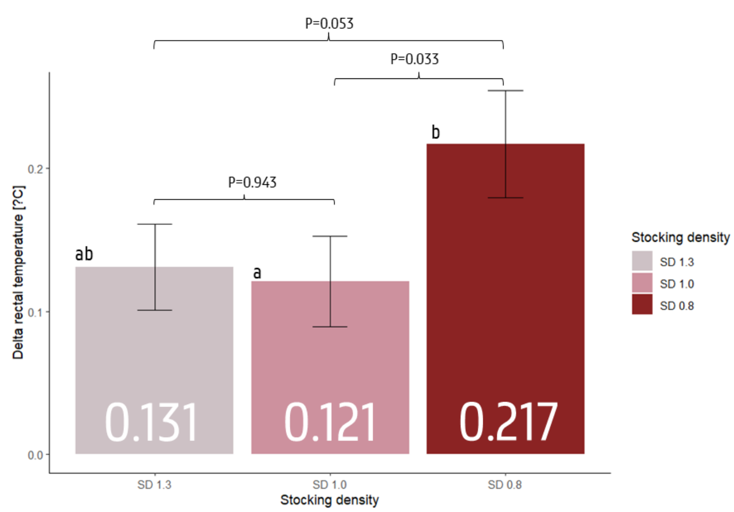

Figure 3: Effect of stocking density ($SD_{1.3}$ = stocking density at 1.3 m²/pig; $SD_{1.0}$ = stocking density 1.0 m²/pig; $SD_{0.8}$ = stocking density at 0.8 m²/pig) on ΔT_{rectal} (delta rectal temperature) of fattening pigs between control (pre- and post-heat period) and the heat load period

3.3 Performance parameters

For ADFI, 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 2), but the heat load did influence the feed intake within the stocking density groups ($p < 0.001$). The ADFI decreased significantly during the heat load, after which all groups recovered by increasing their ADFI. Notably, the $SD_{1.3}$ group showed significant increases in ADFI after the heat load and continued to increase it significantly by ± 350 g/day, measured 2 weeks after the heat period (post-heat 2).

For ADG, the stocking density groups all reacted similarly to the heat load (interaction term, $p = 0.758$), with the heat load causing a lower ADG ($p = 0.001$). However, ADG of $SD_{1.3}$ was numerically always higher than $SD_{1.0}$ and $SD_{0.8}$ In addition, when examined across all four weeks of the trial, ADG of $SD_{1.3}$ tended to be higher (977 g/day) than ADG of $SD_{1.0}$ (893 g/day) and $SD_{0.8}$ (905 g/day) ($p = 0.073$) (data not given in table). For FCR, no significant effects were found.

Table 2: 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 (pre-heat, heat or post-heat period)

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
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]	Pre-heat	2697^{b}	2592^{b}	2647^{b}	57	<0.001	0.653	0.258
	Heat	2321^{a}	2259^{a}	2208^{a}	40			
	Post-heat 1	2637^{b}	2654^{b}	2713^{b}	61			
	Post-heat 2	3017^{c}	2784^{b}	3002^{b}	69			
Average daily gain [g/day]	Pre-heat	1019^{b}	937	973^{ab}	26	0.001	0.428	0.758
	Heat	820^{a}	784	764^{a}	26			
	Post-heat 1	1024^{b}	950	1016^{b}	24			
	Post-heat 2	1041^{b}	912	866^{ab}	37			
Feed conversion ratio [g/g]	Pre-heat	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			
	Post-heat 1	2.59	2.83	2.71^{a}	0.08			
	Post-heat 2	2.93^{x}	3.14^{x}	$3.81^{b,y}$	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

4 Discussion

Increasing the space allowance did not decrease the impact on RR or significantly improve ADFI, ADG and FCR during higher heat loads. However, ΔT_{rectal} increased more in the lowest space allowance, suggesting that the effects of heat stress may be reduced to a slight degree by increasing space allowance. Further, pigs with more space allowance showed a better recovery in ADFI and ADG after the heat load.

Pigs housed at stocking density of 0.8 m²/animal showed nearly twice the increase in ΔT_{rectal} compared to those housed at 1.0 m²/animal or more space allowance. This may be explained by the radiant heat emitted from pen mates, as heat loss of pigs depends on the emissivity of radiating surfaces (Fialho et al., 2016). Further, pigs stocked at lower space allowance 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 m²/animal or more space allowance already had a lower ΔT_{rectal} compared to those housed at 0.8 m²/animal, suggesting that stocking density of ≥ 1.0 m² per pig may have already allowed the pigs to cope with the effects of heat stress created within the settings of this trial.

Increasing space allowance for fattening pigs did not significantly affect ADFI during the heat load. In contrast to our study, White et al. (2008) found a significant reduction in ADFI of pigs housed under 0.66 m²/pig compared to 0.93 m²/pig during the heat load. Another study found that pigs housed individually at 2.0 m²/pig had a higher feed intake in the week after the heat load than those housed at 1.0 m²/pig (Kerr et al., 2005). This suggests that regardless of the presence of pen mates, additional available floor space can minimize the decrease in ADFI during the heat load by compensation of feed intake after the heat load. We also observed that the group with 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 second week after the heat load. This might suggest that pigs with more space allowance recover faster from a heat wave.

The ADG of pigs was not affected by stocking density during the heat load. However, the pigs housed with the highest space allowance had a numerically higher ADG compared to those housed with less space allowance, even during high heat loads, and tended to show a higher ADG across the entire trial period. White et al. (2008) found that lower space allowance and increased temperature independently reduced ADG and suggested that providing pigs with a 28 % increase in space allowance (from 0.66 m²/pig to 0.93 m²/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 ADG one 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 ADG in the two weeks following heat exposure compared to those with less space allowance. Typically, an increase in ADG can be attributed to increased ADFI, for example due to less feeding competition at a lower number of pigs per feeder (Hyun & Ellis, 2001) as can be applied in the study by White et al. (2008). However, several studies (Edilson et al., 2020; Jensen et al., 2012; Kerr et al., 2005; Pearce & Paterson, 1993) have not revealed any significant difference in ADFI, while the ADG tended to change (in the present study across the entire trial period) or significantly changed. Observed lower values of ADG in pigs with low space allowance, in the present study, might be due to stress induced by less space allowance, 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 ADG may be due to more efficient feed utilization (Kerr et al., 2005).

5 Conclusions

A lower space allowance did result in a doubling of the increase of T_{rectal} during 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 lower space allowances (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. Regarding to performance, no significant differences were observed between the stocking densities. Our recommendations for pig welfare under conditions of high head loads, based on measurements of the increase in T_{rectal} during increased heat load within the current trial design, are to increase space allowance to 1.0 m²/animal as this appears to be sufficient to mitigate some negative effects of heat stress.

6 Acknowledgments

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CHAPTER 6 | EFFECT OF TERMINAL SIRE LINE SELECTED FOR OPTIMAL GROWTH RATE OR OPTIMAL CARCASS QUALITY

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Abstract

Management of heat stress in animal husbandry is becoming more important as the number and intensity of heat waves increases due to climate change. Pigs are more sensitive to heat than other animals but show individual differences. Could these differences be traced to sire lines used in Europe? In the present study the influence of terminal sire line on heat stress related parameters was investigated to better understand genotype-environment interactions with the aim of improving heat stress resilience in commercial growing-finishing pigs. In total, 360 fattening pigs in three consecutive batches were divided at weaning into pens of five piglets of the same genetic background. The animals were progeny of TN70 sows x a terminal sire line selected for either optimal growth rate (S_{growth}, homozygous stress negative MaxiMus sire line) or optimal carcass quality ($S_{carcass}$, homozygous stress positive Belgian Piétrain sire line). Pigs were monitored from 10 weeks of age (25.2 ± 0.4 kg) until slaughter age (118.4 ± 0.7 kg) during the summer of 2022. Respiration rate, T_{rectal} and passive behaviour were evaluated during days with a high heat load (predicted outdoor temperature ≥ 28 °C, Temperature-Humidity index (THI) ≥ 75) and thermoneutral days (THI < 75). Blood parameters were evaluated in batch 3 during an artificially induced 7-day heat wave at 21 weeks of age. The expected phenotypic differences between the terminal sire types could be confirmed, with faster growth in S_{growth} pigs (1003 vs. 923 g/day, p <0.001) and better carcass quality in $S_{carcass}$ pigs (67.3 vs. 61.7 mm lean meat thickness, p < 0.001). Rectal temperature and RR showed no significant differences among sire lines as heat load increased (ΔTHI × sire line, p > 0.05). Regardless of genetic background, physiological parameters were influenced by weight: heavier pigs showed faster increases in T_{rectal} and RR with rising THI in comparison to lighter pigs (interaction term ΔTHI × weight, p < 0.001). Daily gain of $S_{carcass}$ pigs dropped as heat load duration increased, whereas daily gain of S_{growth} pigs either remained fairly stable or increased (sire line × NDT (number of days when THI ≥ 77 within a week), p = 0.020). Future research should focus on identifying a terminal sire line that combines steady growth under high heat loads with optimal performance traits such as FCR and carcass quality, without compromising the wellbeing of the animal.

Keywords

Genetics, Belgian Piétrain, Performance, Behaviour, Lean meat thickness

1 Introduction

The most-used terminal sire line in Belgium is the Belgian Piétrain, largely due to its excellent FCR and high lean meat yield. In 2023, the Belgian Piétrain represented 29% (536,702 of 1,837,229) of all terminal sire line sperm doses used nationwide. The total doses of all Piétrain breeds (i.e., Belgian, French, and German variants) represented 42% of the total sperm doses in Belgium. Another 48% of sperm doses came from other breeds and hybrid sire lines, with MaxiMus alone comprising 4% of the total usage (Kerkhove et al., 2024). This distribution underscores the significant role of lean Piétrain genetics in Belgian pig production. However, Renaudeau et al. (2011), reported that pigs selected for high lean growth efficiency cope less well with heat stress in comparison to other genotypes. In a meta-analysis, the most recent publications reported a greater effect of increasing temperature on ADG and FCR, suggesting that genetic selection for high lean meat content reduced the resilience against heat stress (Renaudeau et al., 2011). Additionally, Brown-Brandl et al. (2001) found increased heat production in modern moderate-lean growth genetic lines compared to sire lines from four decades earlier. Other breeds such as Creole (Gourdine et al., 2007; Gourdine et al., 2021; Renaudeau et al., 2007) and Iberian pigs (Rauw et al., 2020; Silió, 2000) have demonstrated greater heat stress tolerance compared to typical commercial breeds selected for optimal growth and lean meat carcasses raised in temperate climates. This is due to their lower metabolic heat production, which results from reduced productive potential in terms of growth. However, the financial viability of tropical breeds may be less interesting due to higher production costs attributed to slower growth and fatter carcasses (Gourdine et al., 2021; Rose et al., 2017).

According to Gourdine et al. (2021) current pig breeding programs do not include traits associated with thermoregulation. One of the important factors for successful selection for better resistance against heat stress is the genotype × environment interaction, as different genotypes may react differently to variable climate conditions. However, only few studies in fattening pigs have included the interaction between genotype × heat (environment) load (Gourdine et al., 2021; Rose et al., 2017). Therefore, exploring the heat tolerance potential of existing commercial breeds in temperate environments can be investigated by assessing the genotype × heat load interaction.

In the present study, we investigated the effect of heat stress-related parameters in fattening pigs from two terminal sire lines, selected either for optimal growth rate or maximal carcass quality during high heat loads. The aim was to assess the responses of the fattening pigs during natural and artificially induced heat waves, expressed by a sire line (genotype) × heat load (environment) interaction. Physiological, behavioural, blood (respiratory alkalosis) and performance parameters were used.

2 Material and Methods

The Ethics Committee for Animal Experiments at Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) approved all experimental procedures during the trial (number 2022/411).

2.1 Study design

A total of 72 pens of fattening pigs (total of 360 pigs) over three consecutive batches were divided at weaning into pens of five pigs with the same genetic background (terminal sire line). The animals were crosses of a TN70 sow x a terminal sire line selected for either optimal growth rate (S_{growth}, homozygous stress negative MaxiMus sire line, 9 boars) or optimal carcass quality ($S_{carcass}$, homozygous stress positive Belgian Piétrain sire line, 9 boars). Boars selected for optimal growth rate were chosen based on the highest estimated breeding values for daily gain, while $S_{carcass}$ boars were selected for their highest estimated breeding values related to slaughter quality and the lowest estimated breeding values for daily gain. Pigs with the same sex (barrows and gilts) and weight class (light, middle and heavy) were blocked per pen. Treatments were randomly divided over the compartments. The trial started on 3 May 2022 and ended on 4 October 2022. Pigs were monitored from 10 weeks of age (25.2 ± 0.4 kg) until slaughter age (average pen weight of 118.4 ± 0.7kg). One pen was removed from the trial due to the presence of an uncastrated male; all others were barrows. The pen was considered as the experimental unit.

2.2 Housing and management

The trial was performed in nine compartments of the Pig Campus, the experimental pig housing of ILVO, Ghent University (UGent) and University College Ghent (HOGENT) in Melle, Belgium. Three consecutive batches were housed in three compartments each. Each compartment consisted of eight pens of five pigs. A feeder was located at the right or left front corner of each pen, with one drinking nipple at the left or right back of the pen. All pens of batch 1 and 2 had a fully slatted floor with a total pen surface of 4.88 m^2; the pens of batch 3 had partially slatted floors. The compartments were artificially lit from 07:30 to 15:30 plus natural light from one window (2.07m^2) on the south or north side of the compartments. Feed and water were provided *ad libitum* to all groups.

The pigs were fed a standard grain-based starter diet up to ±50 kg and a grower diet up to ±80 kg (Table 1). From 80 kg onward, pigs were fed with one of two finisher diets for another trial. To avoid complex interactions, only pigs from the control diet in the finisher phase (36 pens in total) were included for this study.

Table 1: Analysed and calculated chemical composition of the starter, grower and finisher diet during the trial period

Chemical composition	**Starter (25-50 kg)**	**Grower (50-80 kg)**	**Finisher (80-118 kg)**
Number of pens followed	71	71	36
Analysed chemical composition[1] [%]			
Crude protein (N x 6.25)	17.5	15.8	13.8
Crude fat	3.3	3.7	3.9
Crude ash	5.5	5.4	5.1
Crude fibre	4.4	4.7	5.0
Sugar	5.2	5.0	4.7
Starch	35.3	36.6	38.1
Water	10.5	10.5	10.1
Calculated chemical composition			
Net energy [MJ/kg]	9.3	9.3	9.3
SID LYS [g/kg]	10.6	9.5	7.8

[1]Analyzed by ILVO-ANIMALAB, Melle, Belgium

SID: standardized ileal digestible

2.3 Climate control

The compartments were mechanically ventilated by channel ventilation, meaning that the incoming air entered the compartment via the slats of the compartment corridor. During the entire trial, the indoor climate was automatically monitored and controlled by a climate computer (Hotraco Agri©, Hotraco Group, Hegelsom, The Netherlands). One sensor (HOBO MX2301A, Onset®, Bourne (MA, USA) was placed in the corridor at a height of 150 cm in every compartment. These sensors logged the RH and ambient temperature in the compartment every 10 minutes during the entire trial period. The data from the climate sensors was used to calculate the THI as described in Chapter 3.

In batch 3, an artificial heat wave was induced for seven days when the animals were 21 weeks old (Figure 1). During the artificial heat wave, the heating protocol of Chapter 3 was used, with a constant temperature (± 31 °C) night and day.

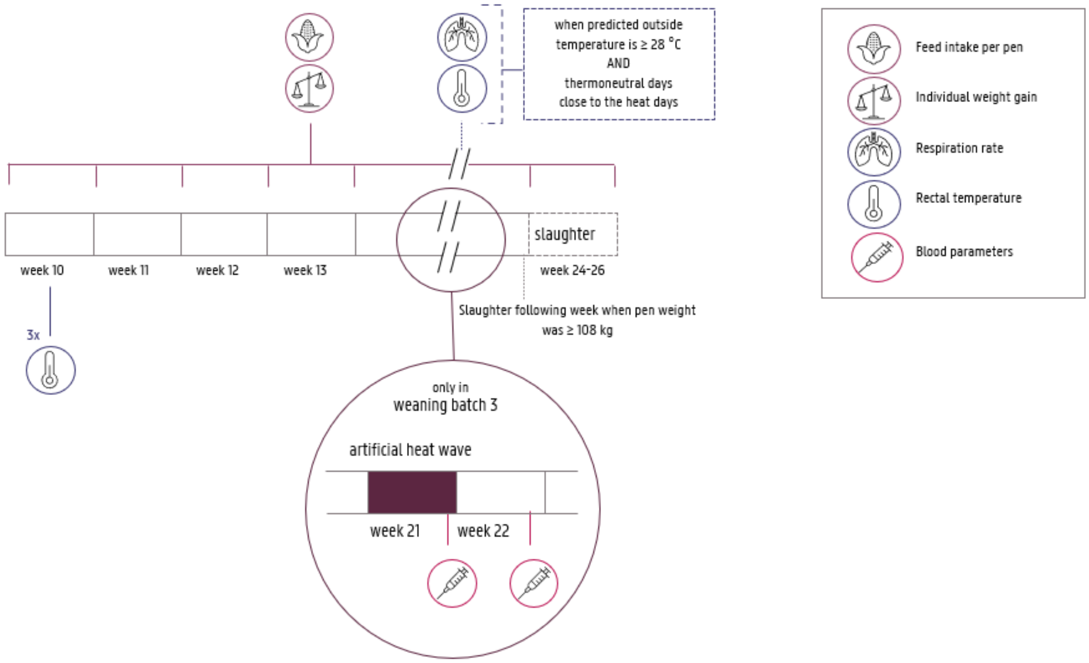


Figure 1: Schematic overview of the trial design

2.4 Physiological parameters and behaviour

Three reference animals per pen were randomly selected to monitor the following physiological parameters throughout the study: RR and T_{rectal}. The same reference animals were evaluated throughout the trial. Measurements were conducted on two or more consecutive days when the predicted outdoor temperature exceeded 28 °C (expected THI ≥ 75), along with two thermoneutral days adjacent to the heat days (before or after) as control (expected THI < 75, Figure 1). This approach ensured adequate representation of observations across various THI values. Respiration rate and rectal temperature were measured according to the same methods as described in Chapter 3. Rectal thermometers were validated every three weeks using a calibrated thermometer. When the pigs reached 10 weeks, T_{rectal} had already been measured for three 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. Animals with elevated T_{rectal} due to lameness or infected lesions by tail biting were excluded from the dataset (27 of 2289 observations).

Behavioural observations were conducted at pen level using scan sampling on the same days as RR and T_{rectal} measurements. The observations started with the first pen, where each animal's activity was classified as either active (supported by ≥ 3 legs, such as eating, drinking, standing, or upright playing) or passive (supported by ≤ 2 legs, such as lying, sitting, or sitting while playing). The behavioural assessment then proceeded sequentially across pens. After evaluating all pens, the observer waited 3 min before starting with the first pen again. Behaviour was recorded 10 times per pen per observation day. Afterward, the average of all the behaviour results was calculated and converted into a percentage. The treatments were blinded for the observers.

2.5 Blood parameters

Whole blood was collected for batch 3 from the three reference animals per pen at two time points: during the second last day of the artificial heat wave and on a thermoneutral day (e.g., 3 days after the artificial heat wave) (Figure 1). Blood was taken to evaluate the heat stress level (e.g. signs of respiratory alkalosis) from both terminal sire lines. 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). Using a 200 µL pipet, blood was placed on a CG8+ cartridge for analysis of pH, ionized Ca, Na, K, glucose, haematocrit, haemoglobin, O_2 partial pressure, CO_2 partial pressure, HCO_3^-, total CO_2, oxygen saturation and base excess. Outliers of O_2 partial pressure values (twice the average of the population) were excluded from the dataset due to the possibility that they represented arterial blood rather than venous blood samples.

2.6 Performance parameters and carcass traits

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. Extra feed provided within the same week was also recorded. At the end of each week, residual feed was weighed. Based on these data, average ADFI and ADG were calculated at pen level for each week (week 10 through week of slaughter). Each week was assigned an NDT number (i.e., number of days with THI > 77 per week), with a high NDT indicating a prolonged heat load period (i.e., a high number of days within that week where the THI exceeded 77). The threshold value of 77 was selected based on a mid-range value within the "heat stress warning" category as mentioned above. During the summer of 2022, the average RH was reported at 65% (KMI, 2022), corresponding to a temperature of 28 °C, which results in a THI of 77. This temperature aligns with the observation days (for physiological parameters) chosen for days of increased heat load, which were determined based on the forecasted outdoor temperatures (see above). Feed conversion ratio was only calculated at pen level for each phase (starter, grower and finisher) due to the instability of FCR per week.

All pigs housed in one pen were transported to a commercial slaughterhouse when the average weight reached ≥ 108kg the week before slaughter. Pigs were slaughtered by exsanguination after carbon dioxide stunning. The 'AutoFOM III™' system (Frontmatec, Kolfing, Denmark) was used to define different carcass traits as described by Kowalski et al. (2020): fat thickness, lean meat thickness, and warm carcass weight. Daily lean meat gain was calculated as described by Kowalski et al. (2020):

Daily lean meat gain = 1000 × (((cold carcass weight × carcass lean meat content × 0.01)-(start weight × 0.45)) / number of days in trial)

2.7 Statistical analysis of data

Statistics were performed in the R® software (version 4.3.1). QQ-plots and histograms of the residuals of the models for the physiological, behavioural, blood and performance parameters were evaluated to check the normality of the residuals. No relevant deviations from normality were observed. To validate an effective difference between S_{growth} and $S_{carcass}$ pigs, a linear mixed model was used to determine the effect of terminal sire line, phase, and their interactions:

$$Y_{performance} = SL \times \beta_{SL} + P \times \beta_{P} + SL \times P \times \beta_{SL\times P} + SW \times \beta_{SW} + Sex \times \beta_{Sex} + Z \times \mu + \varepsilon$$

Where $Y_{performance}$ = dependent variables (ADFI, ADG, FCR and end weight), SL = terminal sire line as independent variable (S_{growth} and $S_{carcass}$), P = phase of diet as independent variable (starter, grower and finisher phase), SW = start weight as independent variable, Sex = 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. For the performance parameters during the total trial period, the same model was used, except for the phase as independent variable and the use of batch as random variable instead of pen (number of observations is equal to the number of pens). Furthermore, carcass traits were analysed using a linear mixed model to determine the effect of terminal sire line:

$$Y_{carcass} = SL \times \beta_{SL} + Sex \times \beta_{Sex} + SW \times \beta_{SW} + Z \times \mu + \varepsilon$$

Where $Y_{carcass}$ = dependent variables (daily lean meat gain, lean meat thickness and back fat thickness), SL = terminal sire line as independent variable (S_{growth} and $S_{carcass}$), Sex = sex as independent variable (barrow and gilt), SW = start weight as independent variable, β = vector of the fixed effects, Z = design matrix of random effects (batch), μ = vector of the random effects and ε = vector of random errors. Slaughter date was removed as random effect as the variance was near zero.

To evaluate the terminal sire lines for possible differences in different parameters as a reaction to the heat load, the following models were calculated. For the physiological parameters, a linear mixed model was fitted to determine the effect of terminal sire line, weight, ΔTHI, and their interactions:

$$Y_{physiological} = SL \times \beta_{SL} + \Delta THI \times \beta_{\Delta THI} + SL \times \Delta THI \times \beta_{SL\times\Delta THI} + W \times \beta_{W} + W \times \Delta THI \times \beta_{W\times\Delta THI} \; (+ Sex \times \beta_{Sex}) + Z \times \mu + \varepsilon$$

Where $Y_{physiological}$ = dependent variables (RR and T_{rectal}), SL = terminal sire line as independent variable (S_{growth} and $S_{carcass}$), ΔTHI = THI excess above a baseline of 75 (ΔTHI = 0 if THI ≤ 75, and ΔTHI = THI-75 if THI > 75) as independent variable, W = weight (individual) at the time of observation as independent variable, β = vector of the fixed effects, Z = design matrix of random effects (individual animal and batch), μ = vector of the random effects and ε = vector of random errors. Sex was only included as independent variable in the model of T_{rectal} due to its significant effect ($p < 0.001$). For behaviour, a linear mixed model was fitted to determine the effect of terminal sire line, weight, ΔTHI, and their interactions:

$$Y_{behaviour} = SL \times \beta_{SL} + \Delta THI \times \beta_{\Delta THI} + SL \times \Delta THI \times \beta_{SL\times\Delta THI} + W \times \beta_{W} + Sex \times \beta_{Sex} + Z \times \mu + \varepsilon$$

Where $Y_{behaviour}$ = dependent variables (% passive behaviour), SL = terminal sire line as independent variable (S_{growth} and $S_{carcass}$), ΔTHI = THI excess above a baseline of 75 (ΔTHI = 0 if THI ≤ 75, and ΔTHI = THI-75 if THI > 75) as independent variable, W = weight (pen level) at the time of observation as independent variable, 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. For the blood parameters, a linear mixed model was used to determine the effect of terminal sire line, heat load, and their interactions:

$$Y_{blood} = SL \times \beta_{SL} + HL \times \beta_{HL} + SL \times HL \times \beta_{SL\times HL} + Sex \times \beta_{Sex} + Z \times \mu + \varepsilon$$

Where Y_{blood} = dependent variables (analysed pH, ionized Ca, Na, K, glucose, haematocrit, haemoglobin, O_2 partial pressure, CO_2 partial pressure, HCO_3^-, total CO_2, oxygen saturation and base excess), SL = terminal sire line as independent variable (S_{growth} and $S_{carcass}$), HL = heat load as independent variable (thermoneutral and heat), Sex = sex as independent variable (barrow and gilt), β = vector of the fixed effects, Z = design matrix of random effects (individual animal), μ = vector of the random effects and ε = vector of random errors. For the performance parameters, a model was calculated using linear mixed model to determine the effect of terminal sire line, the number of days when THI ≥ 77 per week, and their interactions:

$$Y_{performance} = SL \times \beta_{SL} + NDT \times \beta_{NDT} + SL \times NDT \times \beta_{SL\times NDT} + W \times \beta_{W} + SL \times W \times \beta_{SL\times W} + NDT \times W \times \beta_{NDT\times W} + Sex \times \beta_{Sex} + Z \times \mu + \varepsilon$$

Where $Y_{performance}$ = dependent variables (ADFI and ADG), SL = terminal sire line as independent variable (S_{growth} and $S_{carcass}$), NDT = number of days with THI ≥ 77 within a week as independent variable, W = weight (mean pen weight) on the start of the week as independent variable, Sex = sex as independent variable (barrow and gilt), β = vector of the fixed effects, Z = design matrix of random effects (week in fattening period and pen), μ = vector of the random effects and ε = vector of random errors. For ADFI, the interaction term of sire line × weight was excluded from the model since the effect was negligible ($p = 0.8$). Batch was also excluded from the model of ADG and ADFI as random effect as the variance was near zero. The cut-off of the NDT was set to a THI of 77 to allow use of a more sensitive model. Differences were considered significant if $p \leq 0.05$. Tukey's post hoc tests were performed when significant *p*-values were found.

3 Results

3.1 Climate control

Throughout the entire trial period, observations were carried out during 32 days when the THI ≥ 75, and 12 days when THI was less than 75 (Figure 2). For batch 3, all observations were conducted when THI conditions exceeded 75, despite the attempts to include observation days with THI values below 75. Overall, the highest recorded indoor THI during observation days was 81.9, corresponding to a temperature of 31.5 °C and RH of 64%, while the lowest THI recorded was 72.5, corresponding to a temperature of 25.4 °C and RH of 53%. Throughout the observations, all other THI values fell within this range, ensuring sufficient variation of observations across different THI values.

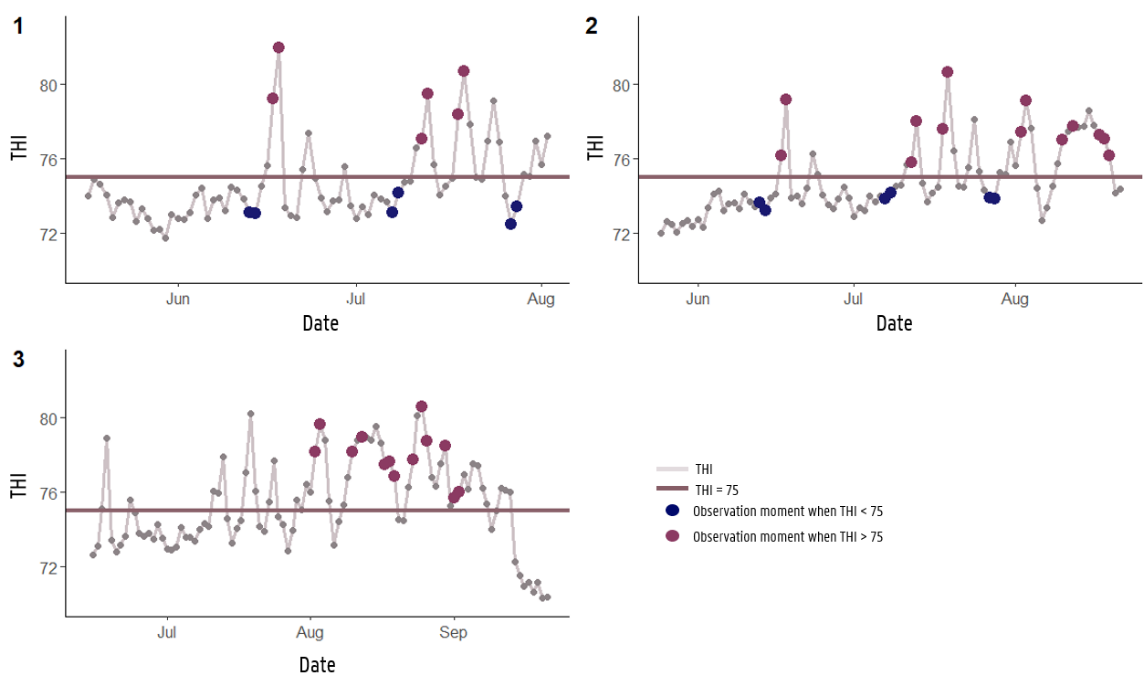


Figure 2: Evolution of the highest Temperature-humidity index between 12:00-18:00 (THI) during the trial for each of the 3 batches (1, 2 and 3) as measured during the study.

3.2 Validation of difference in terminal sire line

At the beginning of the growing-fattening phase, S_{growth} pigs had a higher initial weight compared to $S_{carcass}$ pigs (26.8 vs. 23.5 ± 0.43 kg) (Table 2). Additionally, S_{growth} spent fewer days in the growing-fattening phase (92 vs 104 days), indicating they reached the preferred slaughter weight more quickly.

Table 2: Descriptives (number of days in phase, start weight and slaughter weight) of fattening pigs (estimated means) according to terminal sire line ($S_{carcass}$ and S_{growth}) and phase (starter, grower and finisher) (360 pigs)

Parameter	Terminal sire line		SEM
	$S_{carcass}$	S_{growth}	
Start weight [kg]			
Starter	23.5	26.8	0.43
Grower	51.5	52.5	0.31
Finisher	82.6	82.3	0.31
Number of days in phase			
Starter	31.9	26.8	1.01
Grower	31.9	28.3	0.68
Finisher	40.5	37.1	0.94
Slaughter weight	119.0	118.5	0.74

S_{growth} pigs had a significantly higher total ADFI than $S_{carcass}$ pigs (2392 vs. 2129 g/day, $p < 0.001$) (Table 3). This was also reflected in total ADG where S_{growth} pigs had a significantly higher growth (1006 vs. 923 g/day, $p < 0.001$). Feed conversion ratio was higher in S_{growth} pigs (2.38 vs. 2.31, $p = 0.044$). In addition, $S_{carcass}$ pigs had a significantly higher carcass lean meat thickness compared to S_{growth} pigs (67.3 vs. 61.7 mm, $p < 0.001$), while daily lean meat gain was not significantly different, although a tendency towards higher lean meat gain in S_{growth} pigs was found (481 vs 454 g/day, $p = 0.068$).

Table 3: Performance parameters (average daily feed intake, average daily gain, feed conversion ratio, daily lean meat gain, lean meat thickness, fat thickness) of fattening pigs (estimated means) according to the effect of terminal sire line ($S_{carcass}$ and S_{growth}) and phase (starter, grower, finisher and total)

Parameter	n	Terminal sire line		SEM	*p*-value		
		$S_{carcass}$	S_{growth}		Sire line	Phase	Sire line x phase
Average daily feed intake [g/day]							
Starter	71	1468^{a}	1657^{b}	24			
Grower	71	2182^{c}	2439^{d}	28	<0.001	<0.001	0.436
Finisher	36	2646^{e}	2889^{f}	44			
Total	36	2129^{x}	2392^{y}	35	<0.001	NA	NA
Average daily gain [g/day]							
Starter	71	871^{a}	973^{b}	11			
Grower	71	981^{b}	1051^{c}	11	<0.001	<0.001	0.119
Finisher	36	933^{b}	983^{b}	12			
Total	36	923^{x}	1006^{y}	12	<0.001	NA	NA
Feed conversion ratio [g/g]							
Starter	71	1.69^{a}	1.71^{a}	0.02			
Grower	71	2.23^{b}	2.32^{b}	0.01	0.586	<0.001	0.251
Finisher	36	2.85^{c}	2.94^{c}	0.03			
Total	36	2.31^{a}	2.38^{b}	0.02	0.044	NA	NA
Daily lean meat gain [g/day]	175	454	481	2.67	0.068	NA	NA
Lean meat thickness [mm]	175	67.3^{b}	61.7^{a}	0.40	<0.001	NA	NA
Fat thickness [mm]	175	7.42	8.59	0.21	0.147	NA	NA

$^{a-f}$ Values across columns with different superscripts differ significantly at $p < 0.05$ for sire line × phase interaction

$^{x-y}$ values across columns with different superscripts differ significantly at $p < 0.05$ for sire line

S_{growth} = sire line selected for optimal growth, $S_{carcass}$ = sire line selected for optimal carcass quality, NA = not applicable

n = number of observed pens/fattening pigs (for carcass traits)

3.3 Evaluation of terminal sire line on their difference in reaction to heat load

3.3.1 Physiological parameters and behaviour

The RR of S_{growth} pigs did not significantly differ from that of $S_{carcass}$ pigs when THI values increased (interaction term ΔTHI × sire line, $p = 0.461$, Figure 3A). However, it was clearly shown that, regardless of the different terminal sire lines, heavier fattening pigs (100 kg) had a steeper slope in RR with rising THI values compared to lighter fattening pigs (interaction term ΔTHI × weight, $p < 0.001$).

The T_{rectal} of the offspring of the two terminal sire lines was not significantly different when THI values increased (interaction term ΔTHI × sire line, $p = 0.980$, Figure 3B). In addition, regardless of the terminal sire lines, heavier fattening pigs had a lower baseline of T_{rectal} during thermoneutral observations (THI < 75) than lighter fattening pigs. However, T_{rectal} increased more strongly with rising THI as the weight of the fattening pigs increased. For the pigs weighing 35 kg, T_{rectal} even decreased with increasing THI (interaction term ΔTHI × weight, $p < 0.001$).

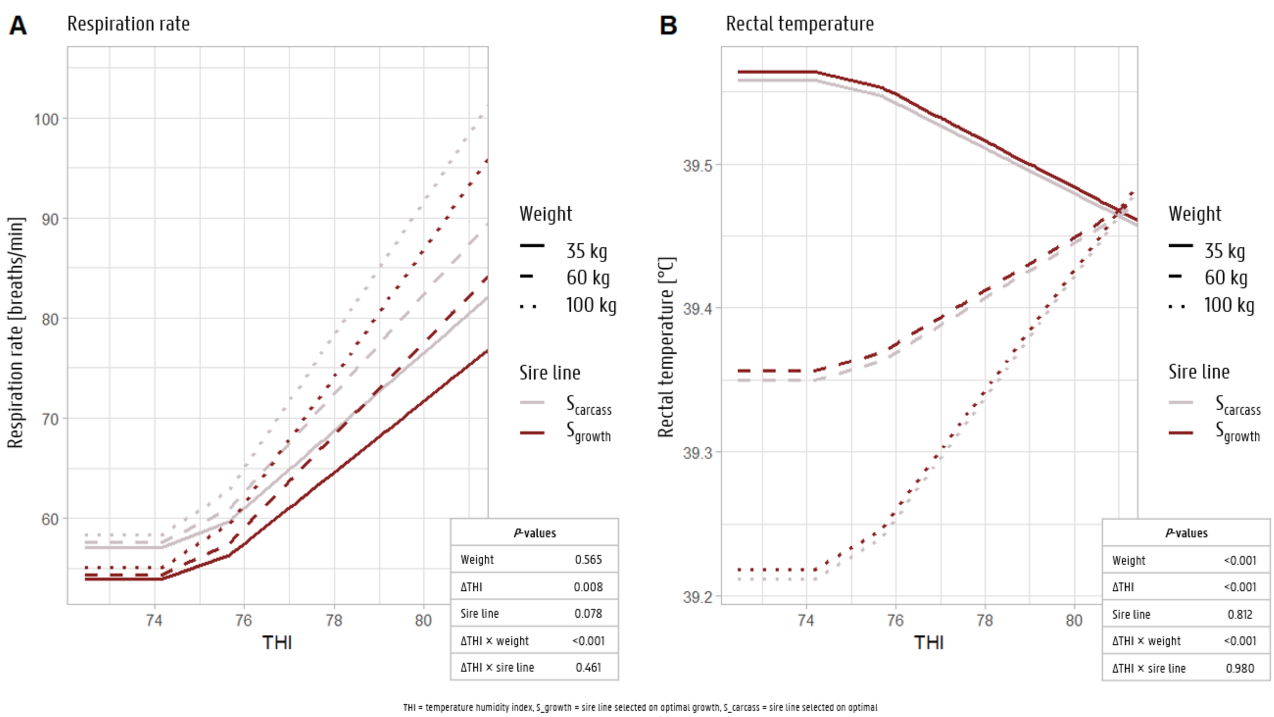


Figure 3: (A) Respiration rate ($R^2_c = 0.34$) and (B) rectal temperature ($R^2_c = 0.50$) of fattening pigs (estimated means) according to the effect of terminal sire line ($S_{carcass}$ and S_{growth}), individual weight (illustrated for 35, 60 and 100 kg) and Temperature-humidity index (ΔTHI)

A post-hoc analysis between the two sire lines within the statistical model for passive behaviour revealed a trend towards a higher passive behaviour rate of S_{growth} pigs compared to $S_{carcass}$ pigs (71% vs. 67%, p = 0.078). However, no behavioural differences were observed in the two terminal sire lines as THI values increased (interaction term ΔTHI × sire line, p = 0.581, Figure 4).

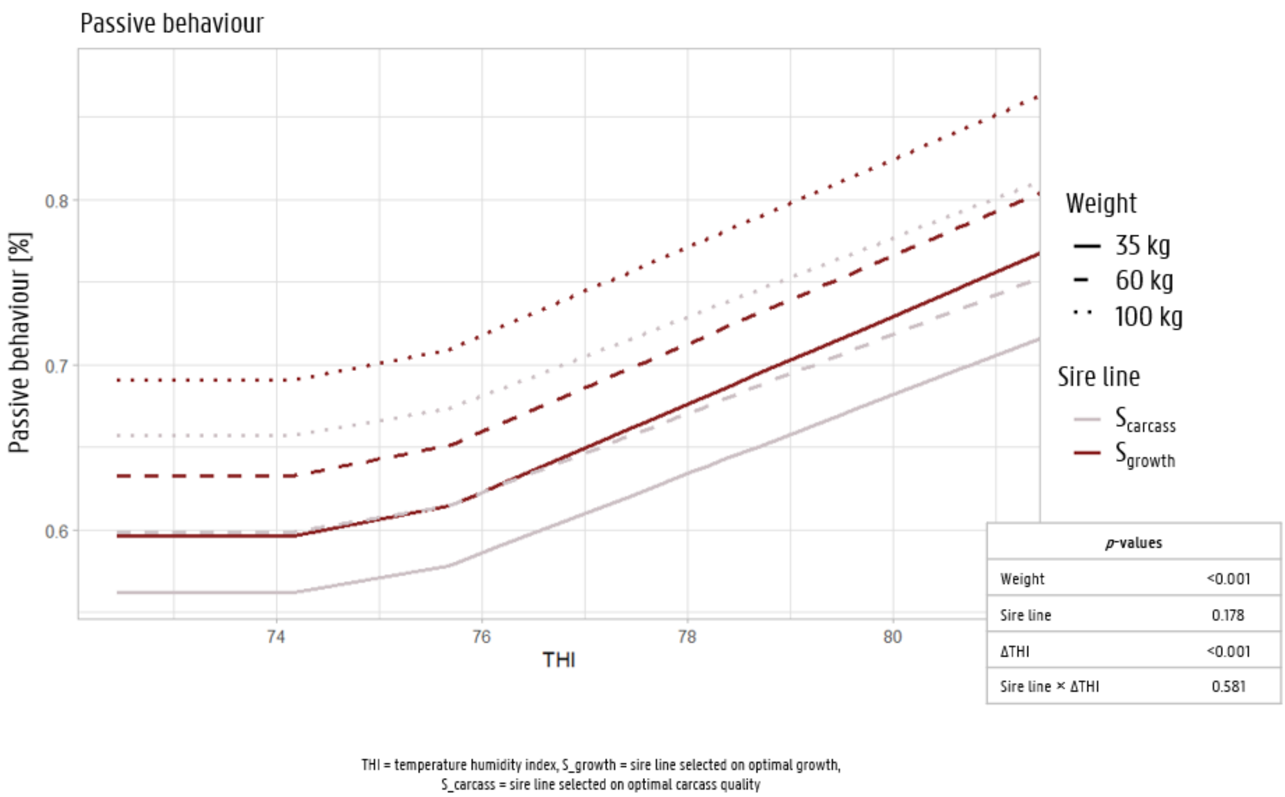


Figure 4: Passive behaviour of fattening pigs (estimated means) according to the effect of terminal sire line ($S_{carcass}$ and S_{growth}), pen weight (illustrated for 35, 60 and 100 kg) and Temperature-humidity index (ΔTHI) (R^2_c = 0.45)

3.3.2 Blood parameters

During thermoneutral days, S_{growth} pigs had significantly lower CO_2 partial pressure blood values compared to $S_{carcass}$ pigs (6.92 vs. 8.06 kPa), whereas no differences were observed during heat days (7.76 vs 7.69 kPa, interaction term sire line × heat load, p = 0.028, Table 4). Similar patterns were observed in haematocrit and haemoglobin levels, where S_{growth} pigs had lower haematocrit and haemoglobin values than $S_{carcass}$ pigs despite showing no differences during heat days (interaction term sire line × heat load, p = 0.012 and p = 0.013, respectively).

Table 4: Blood parameters of fattening pigs (estimated means) according to the effect of terminal sire line ($S_{carcass}$ and S_{growth}) and the heat load (thermoneutral and heat)

Parameter	**n**	**Terminal sire line**		**SEM**	***p*-value**		
		$S_{carcass}$	**S_{growth}**		**Terminal sire line**	**Heat load**	**Sire line x heat load**
pH					0.016	0.502	0.037
Thermoneutral	34	7.36[a]	7.42[a]	0.01			
Heat	35	7.37[a]	7.36[a]	0.01			
pCO_2 [kPa]					0.008	0.327	0.028
Thermoneutral	34	8.06[b]	6.92[a]	0.25			
Heat	35	7.69[ab]	7.76[ab]	0.17			
pO_2 [kPa]					0.902	0.973	0.804
Thermoneutral	34	3.79	3.77	0.08			
Heat	35	3.79	3.71	0.09			
HCO_3					0.909	0.915	0.958
Thermoneutral	34	33.00	33.10	0.33			
Heat	35	33.00	33.10	0.22			
BE					0.200	0.836	0.307
Thermoneutral	34	7.53	8.55	0.46			
Heat	35	7.67	7.72	0.30			
sO_2					0.409	0.884	0.365
Thermoneutral	34	48.60	51.70	1.81			
Heat	35	49.10	47.60	1.79			
TCO_2					0.645	0.559	0.519
Thermoneutral	34	34.90	34.70	0.34			
Heat	35	34.60	34.80	0.22			
Na					0.001	0.075	0.173
Thermoneutral	35	143[b]	141[a]	0.39			
Heat	35	142[ab]	141[a]	0.28			
K					0.027	0.064	0.034
Thermoneutral	35	4.99[a]	4.59[a]	0.09			
Heat	35	4.69[a]	4.77[a]	0.09			
iCa					0.714	0.071	0.747
Thermoneutral	35	1.39	1.40	0.01			
Heat	35	1.43	1.45	0.01			
Glu [mmol/L]					0.525	0.125	0.699
Thermoneutral	35	5.05	5.15	0.09			
Heat	35	5.28	5.30	0.07			
Hct [dL/L]					0.002	0.217	0.012
Thermoneutral	35	35.10[b]	32.40[a]	0.47			
Heat	35	34.40[ab]	33.90[ab]	0.39			
Hb* [g/L]					0.002	0.208	0.013
Thermoneutral	35	120[b]	110[a]	1.61			
Heat	35	117[ab]	115[ab]	1.32			

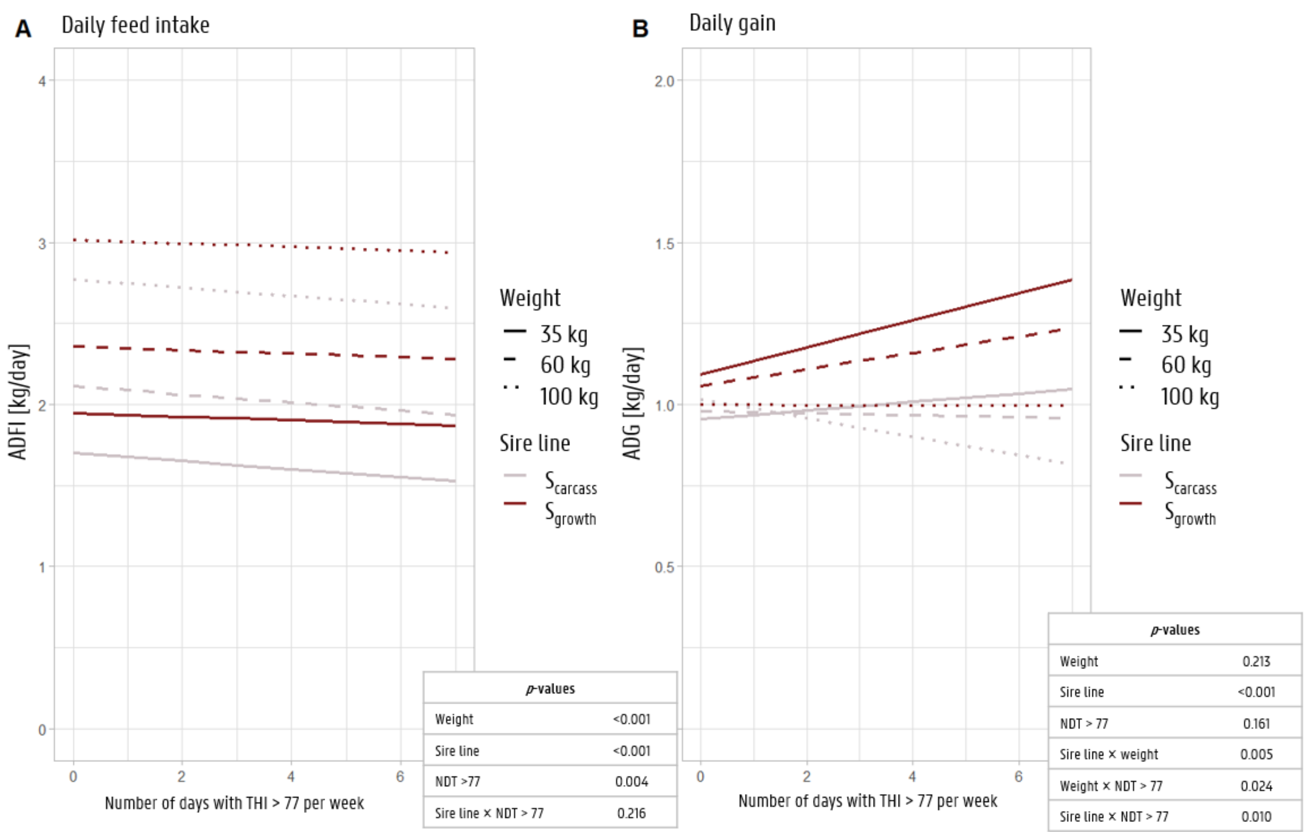
[a-b] Values across columns with different superscripts differ significantly at $p < 0.05$ for sire line × heat load interaction

S_{growth} = sire line selected for optimal growth, $S_{carcass}$ = sire line selected for optimal carcass quality, pCO_2=carbon dioxide partial pressure, pO_2=oxygen partial pressure, BE=base excess, sO_2=oxygen saturation, tCO_2=total carbon dioxide, iCa=ionized calcium, Glu=glucose, Hct=haematocrit, Hb*=haemoglobin, n = number of observed fattening pigs

3.3.3 Performance parameters

Overall, ADFI decreased numerically as the number of days with a THI above 77 (NDT) increased, a typical response to prolonged heat loads (Figure 5A). However, no significant differences in ADFI were observed according to terminal sire line during periods of increasing heat (interaction term sire line × NDT, p = 0.216).

For ADG, the interaction term sire line × NDT (p = 0.010) indicates that the daily gain of $S_{carcass}$ is more suppressed than S_{growth} pigs as the duration of heat loads increased (Figure 5B). Regardless of terminal sire line, increasing live weight corresponded to stronger suppression of ADG during extended heat loads (interaction term weight × NDT, p = 0.024).



THI = temperature humidity index, NDT > 77 = number of days with THI > 77 within a week, S_growth = sire line selected on optimal growth, S_carcass = sire line selected on optimal carcass quality, ADFI = average daily feed intake, ADG = average daily gain

Figure 5: Performance parameters (average daily feed intake (R^2_c = 0.82) and average daily gain (R^2_c = 0.20)) of fattening pigs (estimated means) according to the effect of terminal sire line ($S_{carcass}$ and S_{growth}), and number of days with THI > 77 (NDT) during the grower and finisher phase

4 Discussion

The first question that arises in terms of genetic potential is how to define sensitivity to heat stress and how to evaluate the capacity for coping with or adapting to a high heat load. This is a complex discussion that depends on the parameters being investigated. For instance, should an animal's sensitivity to heat be measured by its reduction in daily feed intake, and to what extent, or should physiological changes be the primary focus rather than performance? In the present study we illustrate the effect of heat stress through an increase in body temperature as proposed by Mount (1979c). When an animal uses more energy to maintain homeothermy than can be compensated by heat loss mechanisms, this results in an uncontrolled rise in body temperature, indicating the presence of heat stress. Increased RR is the first physiological response to environmental heat (Huynh et al., 2005a), thus an initial increase in RR should be viewed as an adaptation rather than a sign of sensitivity to heat. Panting allows pigs to prevent a 3 to 4 °C rise in T_{rectal} and helps them to maintain a constant feed intake when the ambient temperature rises by a few degrees (Huynh et al., 2005a). Problems start to arise when an increase in RR is accompanied by a rise in T_{rectal}. Renaudeau et al. (2010) assert that T_{rectal} is the most relevant parameter for evaluating an animal's heat tolerance, as it indicates the level of homeothermy during heat stress periods. In terms of productivity, heat load becomes problematic when reduced feed intake (an adaptation to excessive heat, as this is the most effective mechanism to reduce internal heat production (Huynh et al., 2005a)) results in lower daily gain. In the present study we consider an excessive heat load to be problematic when it leads to a reduction in feed efficiency (Renaudeau et al., 2012b).

Piétrain pigs are frequently incorporated into breeding programs due to their superior protein deposition efficiency (Jiang et al., 2012; Ropka-Molik et al., 2018). This is indeed reflected in the carcass quality results in this study, with a significantly higher lean meat thickness in $S_{carcass}$ pigs compared to S_{growth} pigs at comparable slaughter weights. However, the deposition of lean tissue is associated with increased metabolic heat production compared to fat deposition (Brown-Brandl et al., 2004), as protein deposition requires 5 ATP for peptide bond synthesis from AA's while lipid deposition only requires 2 ATP (Van Milgen & Noblet, 2003). In the absence of environmental stress, the maintenance requirement is primarily determined by lean mass, with FHP being most accurately predicted by the pig's lean mass. According to correlations by Tess et al. (1984), a 1% increase in lean tissue corresponds to an 8.9% increase in FHP. Thus, pigs of similar bodyweight but with higher fat content/lower lean mass content such as S_{growth} pigs may have a lower FHP than leaner pigs such as $S_{carcass}$ pigs (Tess et al., 1984; van Milgen et al., 1998). Besides muscle gain, FHP is also strongly correlated with weight of bone, blood and viscera, while weight of fat and skin have a lower correlation. It has been shown for example, that very lean pigs (Piétrain) have a lower FHP compared to less lean breeds (Large White) due to a smaller visceral mass (van Milgen et al., 1998). The lower visceral mass may be beneficial for the heat production of $S_{carcass}$ pigs, as they have a higher slaughter yield and significantly lower ADFI, which may contribute to a smaller gastrointestinal package, thus a lower visceral mass content compared to S_{growth} pigs (though organ weights were not directly measured). In addition, the ADFI also influences the animal's heat production. Pigs with high growth potential and significantly higher ADFI, as observed in S_{growth} pigs as compared to the $S_{carcass}$ pigs, may be more affected by a high heat load. The increased feed intake can elevate internal heat production due to the thermic effect of feed (heat increment) (Brown-Brandl et al., 2004; Collin et al., 2001b). Moreover, the numerically higher back fat thickness such as observed in S_{growth} pigs could impair heat loss via radiation, as the thick subcutaneous fat layer hinders efficient heat dissipation during periods of high temperature (Bruce & Clark, 2010). Despite these theoretical considerations, our findings did not reveal a greater sensitivity to heat stress in either $S_{carcass}$ or S_{growth} pigs based on physiological parameters, as no significant interaction between ΔTHI and sire line was observed for RR or T_{rectal}. This suggests that any differences between these sire lines in terms of internal heat production and lean tissue content were likely minimal, or that the factors contributing to increased heat sensitivity may have counterbalanced each other.

In terms of overall performance, S_{growth} pigs demonstrated better ADG during extended heat periods compared to $S_{carcass}$ pigs. Throughout the trial, S_{growth} pigs maintained a higher ADFI, which translated into a significantly higher ADG. Remarkably, this increased growth rate persisted even as heat load increased in duration and ADFI declined, indicating that S_{growth} pigs exhibited greater resilience under heat stress conditions, even in the higher body weight category of 100 kg. Note that the analysis of physiological data provided insight into the pigs' response to increased THI (intensity), while the analysis of performance data focused on the impact of prolonged heat exposure (duration) as measured by NDT. This distinction may explain differences in observed responses to heat regarding carcass composition of the two sire lines. The enhanced performance of S_{growth} could be attributed to the lower FHP in S_{growth} pigs, likely due to their reduced lean mass thickness (Brown-Brandl et al., 2004; Ramirez et al., 2022; Tess et al., 1984; Van Milgen & Noblet, 2003). Lower lean mass may be beneficial during prolonged high heat loads, as reduced internal metabolic heat production minimizes the energy required to maintain homeostasis (Mount, 1979a). However, as discussed above, visceral mass also plays a crucial role, as does the amount of back fat. Pigs experiencing low nutrient intake mobilize adipose tissue as evidenced by an elevated concentration of NEFA in the plasma (Vernon, 1992). This lipid mobilization may offer an adaptive advantage for S_{growth} pigs, with their numerically higher back fat thickness, when ADFI declines due to heat stress as it provides an alternative energy source. However, the mechanisms regulating lipid mobilization in pigs under heat stress conditions remain unclear and warrant further investigation (Qu et al., 2016) to better understand their impact on performance and resilience. Another possible explanation is the tendency of less active behaviour in S_{growth} pigs compared to $S_{carcass}$ pigs. Activity may contribute to extra energy needs for maintenance (Kyriazakis & Emmans, 1995a). In addition, the amount of energy used when sitting or standing depends on the breed and may contribute to additional kJ/h. For instance, the energy cost of activity in Meishan pigs (a breed with a high back fat percentage) was 69% above FHP; in comparison, Piétrain pigs showed a 105% increase (van Milgen et al., 1998). This illustrates that heat production is influenced by both physical activity and breed as well as their interaction. Offspring from S_{growth} tended to be less active, which may be associated with lower maintenance energy requirements, in addition to differences in carcass composition and genetic background as compared to $S_{carcass}$ pigs. However, since we did not measure energy costs for activity, maintenance requirements, or (fasting) heat production, this conclusion remains speculative. Additionally, other factors may contribute to the more consistent daily gain observed in S_{growth} pigs during prolonged heat stress periods. Based on our criteria for heat stress sensitivity for physiological parameters (Mount, 1979c), we cannot definitively conclude that one sire line is better able to cope with heat than the other, as no significant differences in T_{rectal} were observed during high heat loads. However, in terms of performance (Renaudeau et al., 2012b), S_{growth} pigs maintained their ADG, whereas $S_{carcass}$ did not. Therefore, we can conclude that in terms of performance, S_{growth} may exhibit lower sensitivity to heat stress compared to $S_{carcass}$.

The CO_2 partial pressure levels were different between the terminal sire lines during thermoneutral days. However, in observations made during high heat load, no significant differences were observed between both terminal sire lines. In addition, post-hoc tests revealed no within-group differences between thermoneutral and heat days. An increase in blood pH and a decrease in CO_2 partial pressure can be expected during the heat weeks due to elevated RR's, but in this study, no significant CO_2 partial pressure decrease was detected during high heat loads, which may suggest that neither of the sire lines showed signs of respiratory alkalosis. The use of venous blood for CO_2 partial pressure could result in less pronounced differences, as it may result in a different acid-base profile (described by Augustinsson and Forslid (1989) and Patience et al. (2005)). The haematocrit, which represents the volume percentage of red blood cells in the blood, and the haemoglobin levels, which reflect the amount of protein in the red blood cells responsible for oxygen transport, both increase with body weight (Czech et al., 2018). The rapid growth observed in growing pigs is closely associated with an increase in blood volume (Klem et al., 2010) and a decrease in haematocrit and haemoglobin levels (Perri et al., 2016). This is in agreement with our study as S_{growth} pigs had lower haematocrit and haemoglobin levels than $S_{carcass}$ pigs when taking into account that at the same age, S_{growth} pigs have slightly higher body weights. This may indicate that while blood volume increased, the concentration of red blood cells could not match the rapid growth and resulted in lower haematocrit levels. In the present study, haematocrit and haemoglobin levels remained within the reference intervals outlined by Klem et al. (2010) during both thermoneutral and heat days. This implies that the interaction between heat and genetics had a relatively minor impact on these parameters.

Regardless of genetic background, the FHP per unit of live weight (W/kg) decreases as body mass increases in growing-finishing pigs (Brown-Brandl et al., 2004; Kleiber, 1932; Tess et al., 1984). This trend is shown in our study by the lower T_{rectal} observed in heavier fattening pigs during thermoneutral conditions. When ambient temperature exceeds the pig's UCT, heat production increases (Mount, 1979a), leading to heat acclimatization characterized by elevated RR in combination with increased T_{rectal} (Renaudeau et al., 2010; Renaudeau et al., 2007). This is manifested by the increasing slope of both RR and T_{rectal} across all weight categories in our study, except for the smallest weight group of 35 kg. Noticeably, heavier fattening pigs demonstrate faster changes in response to heat, as evidenced by the more rapid increase in RR and T_{rectal} with rising THI. Despite their lower heat production relative to body mass, heavier pigs have a smaller surface area-to-mass ratio, increased insulation (subcutaneous fat layer) (Bruce & Clark, 2010), and higher maintenance requirements compared to lighter pigs (van Milgen et al., 1998). This makes them more vulnerable to high heat loads (Renaudeau et al., 2011). While it is well-established that heavier pigs are more affected by heat in terms of ADFI and ADG as shown in the present and previous studies (Quiniou et al., 2000; Renaudeau et al., 2011), the influence of body weight on thermoregulatory parameters such as RR (latent heat loss) and T_{rectal} (body core temperature) have received less attention. In the present study, a significant interaction between environment (heat load as indicated by THI) × body weight on RR and T_{rectal} was observed, underscoring the observations of older fattening pigs with increased sensitivity to heat stress.

5 Conclusions

This study highlights the differences between terminal sire lines selected for optimal growth rate, S_{growth}, and those selected for carcass quality, $S_{carcass}$, in their responses to heat stress. S_{growth} pigs maintained a more consistent ADG as the duration of the heat load increased, potentially due to their lower carcass lean meat thickness or the trend of reduced activity level, both of which may contribute to decreased internal heat production. No significant differences in physiological or blood parameters were observed between sire lines under high heat load conditions. Additionally, the study highlighted the heightened sensitivity of heavier pigs to heat stress, both in terms of performance and physiological responses. Future research should prioritize exploration of less lean genetic lines to enhance resilience against heat stress. Identifying a terminal sire line that balances the quality of stable performance under high heat loads with optimal performance traits—such as FCR, ADG, and carcass quality—is essential. This trade-off will be critical for sustaining productivity while improving robustness in pigs facing increasing thermal challenges.

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CHAPTER 7 | RE-EVALUATING THERMOREGULATION MODELS IN FATTENING PIGS

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Abstract

Thermoregulation in fattening pigs is commonly described using theoretical models; however, precise lower and upper critical temperature thresholds and the corresponding responses remain poorly defined due to the complexity of influencing factors and variability in the literature. To address this gap, a comprehensive dataset comprising 9,000 individual records from eight trials conducted within the Coolpigs project was analyzed to identify key factors influencing thermoregulation models in fattening pigs. Physiological (rectal temperature, respiration rate, heat stress score) and performance (average daily gain) indicators were linked to the THI and ambient temperature at the time of observation. A total of 165 broken-line models—81 linear and 84 quadratic—were tested. Based on model simplicity, Akaike Information Criterion (AIC), and error metrics, four linear models (physiological responses vs. THI; performance vs. temperature) were selected for interpretation. Body weight and sex emerged as the most influential factors. Heavier pigs (>100 kg) exhibited earlier increases in respiration rate (THI of 71.3 vs. 73.6), suggesting a comfort zone that ends earlier, and showed a more pronounced rise in rectal temperature beyond the upper critical threshold (0.09°C/THI vs 0.03 °C/THI), indicating higher heat stress susceptibility than lighter pigs (60-80 kg). In contrast, gilts started rectal temperature increases later than barrows (THI of 76.8 vs 75.9), exhibited lower heat stress scores (-1.1 vs +2.2 mm per THI), and had improved growth performance after the breakpoint (+0.02 vs -0.02 kg/day per °C), suggesting greater thermal resilience. Furthermore, rectal temperature increased gradually with rising THI, even within the presumed comfort and thermoneutral zones, across all sexes and weight categories. These findings underscore the importance of accounting for weight and sex in predictive models of pig thermoregulation.

Keywords

Fattening pigs, Broken line models, Temperature-Humidity Index, Sex, Weight

1 Introduction

The thermoregulation of pigs is described by a theoretical model developed by Mount (1979a). While this model remains valid, it does not define precise lower and upper critical temperature points or the physiological reactions before and beyond these thresholds, due to the complexity of influencing parameters, as discussed in Chapter 2. Many studies have attempted to analyse the reactions of heat stress-related parameters during high heat loads (Brown-Brandl et al., 2001; Huynh et al., 2005a; Renaudeau et al., 2007), but results vary depending on the heat stress-related parameter, pig and farm characteristics, climate parameters and methodology.

The T_{rectal} is considered the most representative heat stress-related parameter for assessing the level of homeothermy during heat stress periods (Renaudeau et al., 2010), and is therefore useful for defining the thermoneutral zone and reactions beyond. However, indicators like RR and ADG are also valuable, as they can be more easily monitored by visual assessment or automated systems (electronic feeders with RFID tags), as described in Chapter 4A. In addition, a better understanding of the possible relationship between T_{rectal} and RR may contribute to a more practical assessment of heat stress. Additionally, various pig, farm and experiment-related characteristics influence thermoregulation (Chapter 2). For instance, heavier fattening pigs may reach their T_{rectal} breakpoint at lower THI or temperature values, or their T_{rectal} increase beyond the breakpoint may be more intense compared to lighter pigs. Identifying the most influential characteristics is essential for optimizing the description models and improving practical heat stress management.

The choice of the climate (independent) parameter significantly impacts the reliability of regression models describing heat stress responses. While ambient temperature alone may serve as a basic descriptor of T_{rectal}, incorporating THI could improve model reliability, as RH also plays a crucial role in heat stress (Granier et al., 1998; Huynh et al., 2005a).

Broken-line regression analysis is a common method for identifying critical temperature thresholds, and reactions before and beyond these thresholds. In such a model, the relationship between the dependent (e.g. T_{rectal}) and independent variables (e.g. THI) shifts abruptly at a certain point, known as the breakpoint. This marks a transition from one linear trend to another, potentially indicating a critical threshold or limit as there is a deviation from the 'normal' situation. For example, T_{rectal} remains constant within the thermoneutral zone (Brown-Brandl et al., 2001), but beyond a certain ambient temperature (breakpoint), a sudden rise in T_{rectal} occurs, signalling a response to a high heat load. This deviation from normal equilibrium can serve as a critical point for thermal discomfort in warm-blooded animals.

To address all these complex interactions, a comprehensive dataset was compiled from all experiments conducted in fattening pigs during this PhD research. This dataset included approximately 9,000 individual observations under varying farm conditions, integrating previously discussed studies (Chapters 3-6) and other studies conducted within the COOLPIGS project. The most interesting and relevant characteristics were selected based on literature and the thesis outline. Three pig-related characteristics and three farm/experiment characteristics were chosen, as shown in Figure 1:

The three pig characteristics included are body weight of the pig, terminal sire and sex:

- Body weight: Heavier fattening pigs are more susceptible to high heat loads and typically have a lower UCT, as discussed in Chapter 2 and observed in Chapter 6. Therefore, the body weight at the moment of observation may significantly affect thermoregulation thresholds.
- Terminal sire: The genetics, primarily the lean tissue gain and visceral mass, as highlighted in Chapter 2 and observed in Chapter 6, also has an important impact on thermoregulation.
- Sex: Barrows and gilts exhibit differences in feeding characteristics, carcass composition, behaviour and energy requirements, as outlined in Chapter 2. These differences may influence the responses to heat load. However, the impact of sex on heat load is not well-studied, making it an interesting variable to evaluate in the thermoregulation models.

Also three farm or experiment specific characteristics are included:

- Type of heat load: Although this parameter might not be directly relevant for practical use, it may influence the response to the heat load. In many experiments, heat load was generated by natural conditions, artificial heating (e.g. Chapter 3), or a combination of both. This may account for a possible impact.
- Space allowance: As discussed in Chapter 2 and observed in Chapter 5, space allowance may influence thermoregulation.
- Floor type: Floor type (concrete, fully slatted, partially slatted, etc.) impacts the heat transfer of the pig, as outlined in Chapter 2, which may affect thermoregulation models.

By re-evaluating thermoregulation models using this broad dataset generated under diverse conditions in Belgium, we aim to answer the following questions (Figure 1):

1. What is the impact of relative humidity on top of temperature on heat stress-related parameters?
2. Which pig and farm characteristic has the greatest effect on heat stress-related parameters and what is the effect during increasing heat loads?
3. What is the relationship between respiration rate and rectal temperature?

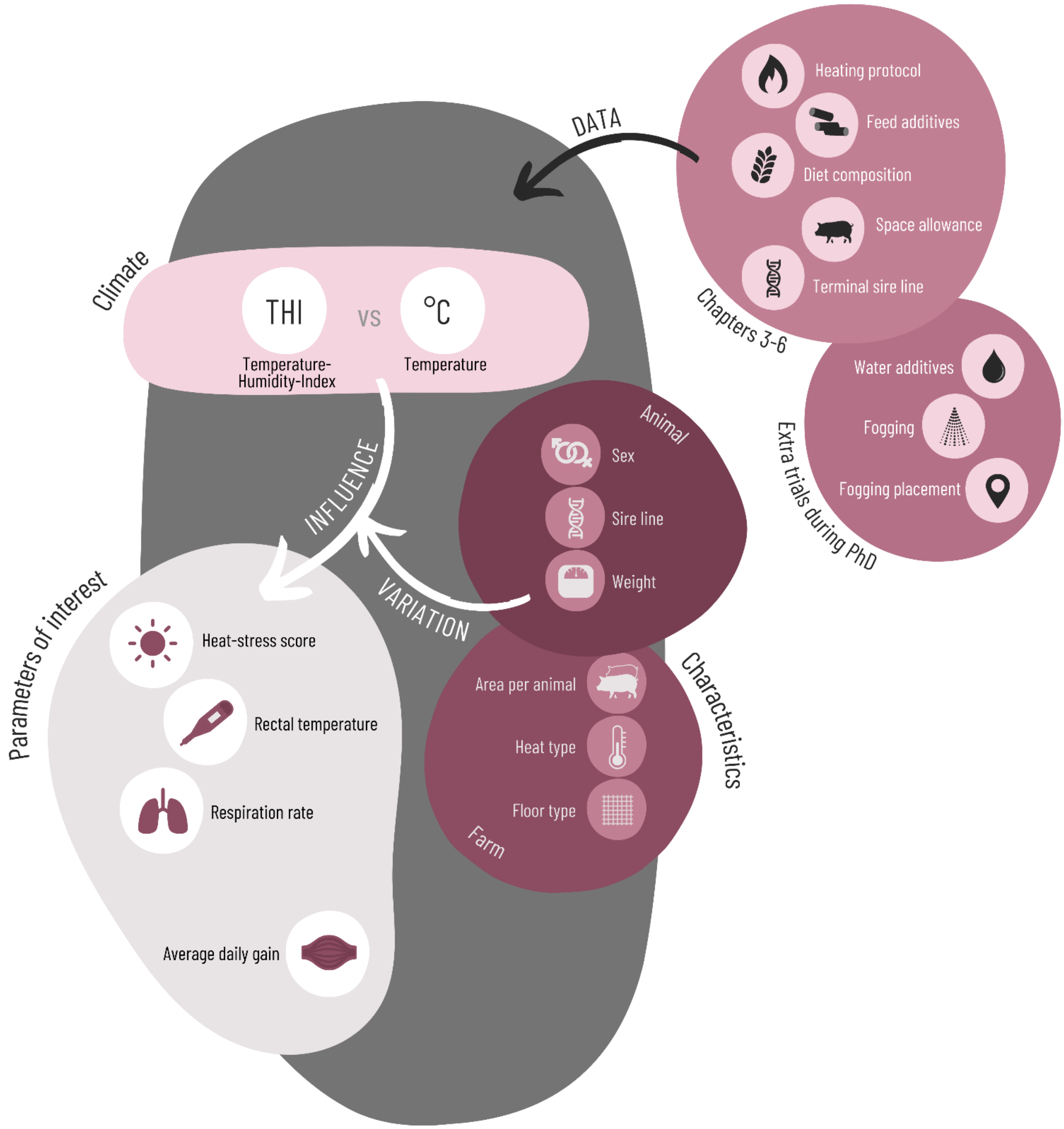


Figure 1: Schematic overview of the approach used for model building. With the data of all the experiments within this thesis and extra trials during the PhD project, 3 main questions were answered: 1) What is the impact of relative humidity on top of temperature on heat stress-related parameters (THI vs temperature)? 2) Which pig and farm characteristic has the greatest effect on heat stress-related parameters and what is the effect during increasing heat loads? (variation of the animal and farm characteristics on the association between the climate and the parameters of interest)? 3) What is the relationship between respiration rate and rectal temperature?

2 Material and Methods

2.1 Experimental trials

From all the data collected on fattening pigs during the PhD research, individual observations were selected and compiled into a single dataset. All experiments were conducted with specific treatments to test heat stress mitigation strategies, and therefore, many variables were included. In total, eight different experiments with fattening pigs were conducted, with data from five trials as discussed in previous chapters. These included data from the artificial heating protocol (no treatment), feed additives (control vs. enriched diet), nutritional composition (control vs. energy-dense diet), space allowance (0.8 vs. 1.0 vs. 1.3 m²/pig), and terminal sire line (terminal sire line selected on optimal carcass vs. optimal growth). The three extra trials also involved fattening pigs for assessing different control measures against heat stress.

The first extra trial tested drinking water additives (control vs. enriched). This drinking water was supplemented with a natural product based on concentrated essential oils, including mint oil, eucalyptus, star anise, oregano, selenium, and vitamin E. During the summer of 2024, three rooms with fattening pigs, each room with ten pens of three pigs (1.0 m²/pig), were evaluated. Within each room, one side (five pens) received drinking water supplemented with 50 ml additive per 1000 L water, while the other side received regular drinking water, the control treatment. The drinking water was supplemented from 16 weeks of age until slaughter. Rectal temperature, RR and HSS were observed once per week, on the day with the highest predicted outdoor temperature of the week. Furthermore, ADG was calculated based on the individual weight observations at the beginning and end of the trial.

The second extra trial assessed the efficacy of a high-pressure fogging system operating at 70 bar, installed within the pig rooms (control vs. fogging). This system was activated when the barn temperature exceeded 27.5 °C and deactivated when relative humidity surpassed 75%. The system's pulse duration increased incrementally with rising barn temperatures, ranging from a minimum of 45 seconds to a maximum of 240 seconds. It was a cross-over trial, with two identical rooms per production batch: one with the fogging system active and one without. Every three weeks, the active and inactive rooms were alternated, resulting in three switches. In total, three consecutive pig batches were conducted. The experiment started when the pigs were 16 weeks old and continued until slaughter. The same parameters as in the previous experiment were monitored, with observations conducted twice per week during both thermoneutral and warm days in the summer of 2023.

The third extra trial assessed the impact of fogging system placement (fogging inside the room vs. fogging at the air inlet). One room utilized the same fogging system as described in the previous experiment, while another identical room had the system installed in the air inlet (down in the underground air channel, representing the facility's ventilation system).

Additionally, axial fans were installed in the room, and were activated when the temperature exceeded 23°C. Two consecutive pig batches were evaluated during the summer of 2024. The same parameters were observed concurrently with the drinking water additive trial.

2.2 Chosen heat stress-related parameters

Four different heat stress-related parameters were chosen to observe their relationship with temperature or THI: RR, T_{rectal}, HSS and ADG. For each trial, the number of individual observations, the mean value, the standard deviation and the range [minimum; maximum] is represented for each heat stress-related parameter (Table 1). The T_{rectal}, RR ad HSS are further referred to as physiological heat stress-related parameters, while ADG is referred to as performance heat stress-related parameter.

The HSS was scored in almost every trial, however not yet included in previous chapters, except in Chapter 4A. This scoring system was consistently applied by a group of observers who were trained during the PhD research studies from 2021 to 2024.

Due to the large number of observations and the presence of various experimental designs, certain decisions or assumptions were made to ensure the uniformity of the dataset while minimizing errors. In most trials, individual weight was measured weekly. Average daily gain was then calculated using the formula: (weight at timepoint b – weight at timepoint a) / (number of days between the two weight measurements, typically 7 days). Observations of RR, T_{rectal} and HSS were linked to the weight measured at the previous weight measurement timepoint and the calculated ADG between the previous and following weight measurement timepoints. For the experiments involving drinking water additives and fogging at the air inlet, weight measurements were taken only at two or three timepoints. The weight at the time of observation was therefore adjusted by: weight from the previous measurement + ADG * number of days since the previous weight assessment. As a result, observations of RR, T_{rectal} and HSS conducted within the same week for an individual animal had the same ADG and weight values but different climate data.

Table 1: Overview of the number of observations (n), mean value, standard deviation (St. Dev.) and range of each heat stress-related parameter for each experimental trial

		Heat stress-related parameters			
Trial	**Descriptives**	**Respiration Rate (Breaths/min)**	**Rectal temperature (°C)**	**Heat Stress Score**	**Average Daily Gain (kg/day)**
Heating Protocol (Chapter 3)	Observations (n)	224	217	n.a.	n.a.
	Mean	53	39.1		
	St. Dev.	30	0.4		
	[Min;Max]	[14;166]	[37.1;40.9]		
Feed additives (Chapter 4A)	Observations (n)	448	448	167	448
	Mean	64	39.3	3	0.91
	St. Dev.	22	0.3	2	0.29
	[Min;Max]	[18;132]	[38.5;40.5]	[0;7]	[-1.2;1.8]
Feed additives and composition (Chapter 4B)	Observations (n)	1657	1652	1635	1654
	Mean	54	39.2	30	0.82
	St. Dev.	25	0.3	22	0.41
	[Min;Max]	[12;202]	[37.9;40.8]	[0;117]	[-1.1;2.7]
Space allowance (Chapter 5)	Observations (n)	2726	2726	n.a.	2726
	Mean	57	39.4		0.94
	St. Dev.	19	0.3		0.29
	[Min;Max]	[18;152]	[38.3;41.4]		[-0.6;1.9]
Terminal sire line (Chapter 6)	Observations (n)	1776	1776	1758	1750
	Mean	71	39.3	34	0.97
	St. Dev.	25	0.3	17	0.26
	[Min;Max]	[18;186]	[38.5,40.8]	[4;115]	[-0.2;2.4]
Water additives (Extra)	Observations (n)	257	256	257	257
	Mean	67	39.3	35	0.95
	St. Dev.	20	0.3	14	0.11
	[Min;Max]	[22;132]	[38.6;40.1]	[14;111]	[0.7;1.2]
Fogging (Extra)	Observations (n)	1589	1589	1586	1589
	Mean	64	39.2	27	0.91
	St. Dev.	21	0.3	13	0.28
	[Min;Max]	[16;168]	[38;41.4]	[0;102]	[-0.1;2.7]
Fogging placement (Extra)	Observations (n)	613	613	613	613
	Mean	79	39.4	36	0.92
	St. Dev.	26	0.4	16	0.14
	[Min;Max]	[28;200]	[38.3;40.6]	[0;113]	[0.3;1.2]
Total observations		9290	9277	6016	9037
Total observations after correction*		**8990**	**8990**	**6016**	**8990**

*The original dataset contained 9,290 individual pig observations weighing 60 kg or more. Additionally, observations with missing values for one or more heat stress-related parameters (except for HSS) on the same observation date for the same pig were excluded. As a result, the dataset was reduced to 8,990 observations, n.a. = not applicable

2.3 Link between individual observations, climate data and pig, farm and experiment-related characteristics

Climate data was linked to the observation dates of the individual observations. The mean room temperature, RH and THI (Lucas et al., 2000; NWSCR, 1976) per hour for the specific observation date were collected between 13:00 and 17:00, as the observations were consistently conducted during this period . The mean values for this time range were then calculated for each observation.

Pig, farm and experiment related characteristics were linked to each individual pig (ID), observation moment, climate parameters and heat stress-related parameters: weight at the moment of observation (23-152 kg), sex (gilt or barrow), terminal sire (31 different sires), terminal sire line (optimal carcass or optimal growth), sow line (TN70 or RASE), and carcass characteristics at slaughter like warm carcass weight (68.2-116.4 kg), lean meat content (56.0-69.8%), fat thickness (3.34-18.74 mm) and slaughter date (12/10/2021-10/09/2024), experiment number (1-8), experimental farm (1 or 2), batch number (1-3), room number (1-20), pen (1-59), water treatment (control or enriched), feed type (control, enriched, energy-dense, low- or high-lysine), floor type (partially slatted or fully slatted), number of animals per pen (3-10), floor area (3.60-19.76 m^2), space allowance (0.8 - 1.3 m^2/pig), fogging status at the time of observation (no or yes), forced air speed with axial fan at the time of observation (no or yes) and type of heat load (natural or artificial heating).

The dataset contained numerous variables, but it is not feasible to test the effect of all these variables on the heat stress-related parameters within a single statistical model. Therefore, three relevant pig-related characteristics and three farm / experiment characteristics were finally chosen, as represented in Table 2. These characteristics were chosen based on factors that showed the most promising or relevant results in the context of heat stress and our objectives, such as space allowance and terminal sire line (Chapter 5 and 6). Body weight was also included due to its well-established influence on heat stress response in literature. The effect of sex, less explored in literature, was considered particularly interesting because the dataset was well balanced for this variable. Additionally, floor type was examined as a management factor, as this was not yet extensively studied in relation to heat stress but has potential practical implications. Given that the heat load conditions varied across trials, the type of heat load (natural vs. artificial) was also included to account for these differences. Conversely, feed treatments were excluded as they showed limited effects in our trials. Similarly, carcass traits were not analyzed in depth, since all animals were exposed to both thermoneutral and heat load conditions, making it difficult to attribute specific outcomes. Sow line was also excluded, as most pigs came from TN70 sows, resulting in an unbalanced dataset. Other treatments like misting or axial fans were unevenly distributed and their potential effects might already be reflected indirectly through climate data. Lastly, water treatments showed no significant effects and were therefore not pursued further.

Table 2: The chosen pig, farm and experiment characteristics used for evaluating their effects on heat stress-related parameters, along with the number of observations per characteristic per category.

Characteristics		Category	Observations (n)
Pig	Weight (60-152 kg)[1]	Light (60-80 kg)	2626
		Medium (80-100 kg)	4017
		Heavy (>100 kg)	2347
	Terminal sire	Sire name	31 sires
	Sex	Barrow	4676
		Gilts	4314
Farm / Experiment	Type of heat load	Natural	4293
		Artificial	4697
	Floor type	Fully slatted (concrete)	3332
		Partially slatted (concrete)	5658
	Space allowance[2]	0.8 m²/pig	2206
		1.0 m²/pig	4895
		1.2 m²/pig	1889

[1]The pigs' weight was categorized into three relevant groups for practical implementation, [2]To achieve a more equal distribution of observations, the densities were categorized into three groups: 0.8 m²/pig (observations of 0.81 m²/pig), 1.0 m²/pig (observations of 0.98 m²/pig), and 1.2 m²/pig (observations of 1.20, 1.22, and 1.32 m²/pig).

2.4 Statistical analysis

Statistics were performed in R® software (version 4.4.2). QQ-plots and histograms of the residuals of the models for the physiological and performance heat stress-related parameters were evaluated to check the normality of the residuals. No relevant deviations from normality were observed. Differences were considered significant if $p \leq 0.05$, if not mentioned specifically.

2.4.1 Which pig and farm characteristic has the greatest effect on heat stress-related parameters and what is the effect during increasing heat loads?

2.4.1.1 How big is the impact of characteristics on heat stress parameters?

The characteristic with the greatest effect on the different heat stress-related parameters was evaluated using a model with either mean room THI or temperature values. A linear mixed model was fitted to determine the effect of climate (room THI or temperature), animal-related characteristics, and farm/experiment characteristics as follows:

$$Y = C \times \beta_C + Sex \times \beta_{Sex} + TS \times \beta_{TS} + BW \times \beta_{BW} + FT \times \beta_{Floor} + SA \times \beta_{SA} + H \times \beta_H + Z \times \mu + \varepsilon$$

Where:

- Dependent variables (heat stress-related parameters)
    - Y = (RR, T_{rectal}, HSS or ADG)
- Independent variables
    - Climate
        - C= Climate parameter (continuous variable), which was mean room THI or temperature
    - Animal (characteristics)
        - Sex = Sex
        - TS = Terminal sire
        - BW = Body weight at moment of observation
    - Farm/experiment (characteristics)
        - FT = Floor type
        - SA = Space allowance
        - H= Heat type
- β = vector of the fixed effects
- Z = design matrix of random effects
    - Individual animal
    - Experiment number
- μ = vector of the random effects
- ε = vector of random errors.

For each heat stress-related parameter (dependent variable), characteristics (independent variables) were tested for their effect (F-value: the greater the F-value, the greater the impact of the predictor) and associated significance in the linear mixed regression model. The characteristic with the lowest effect (F-value) and a non-significant *p*-value ($p > 0.01$) (given the large dataset, high significance levels were expected) was excluded from the model. The model was then recalculated, and the remaining characteristics were re-evaluated for significant effects. This process was repeated until all included characteristics in the model had a significant effect ($p \leq 0.01$) on the regression between the heat stress-related parameter and the climate parameter. These final characteristics were used for the calculation of the intercept of the heat stress-related parameter in relation to the climate parameters, further referred to as the baseline. These final models were then further used as a starting model in the analysis of the broken-line models.

2.4.1.2 How do characteristics affect heat stress parameters: What is the impact on the breakpoints and beyond?

Broken-line models were developed to evaluate the effect of different pig- and farm/experiment characteristics on the breakpoints and slopes (also referred to as reaction (increase or decrease) before or beyond the breakpoint) of the heat stress related parameters (RR, T_{rectal}, HSS and ADG) with increasing climate parameters (THI or temperature):

$$Y_1 = I + C_1 \times x \text{ for } x < BP$$

$$Y_2 = Y_1 + C_2 \times (BP - x) \text{ for } x > BP$$

Where:

- Y = dependent variable (RR, T_{rectal}, HSS and ADG)
- X = independent variable (THI or Temperature)
- I = intercept (baseline)
- C = regression coefficients (reaction before (1) or beyond (2) the breakpoint)
- BP = breakpoint

Random variables were included in every model within a nested structure: individual animal within terminal sire. The characteristic 'terminal sire' had a significant effect ($p \leq 0.01$) on every heat stress-related parameter. However, the effect was small (low F-value), and therefore, it was included as a random variable. Additional random variables included individual animal ID within a room within an experiment.

Several different broken-line models were calculated using a stepwise procedure, where characteristics (with significant effects in previous analysis) were alternately included and excluded as effects on the baseline, the breakpoint, and the reaction beyond the breakpoint in various combinations. Ultimately, the best fit was chosen based on the lowest Akaike Information Criterion (AIC) values among models that included the same climate and heat stress-related parameters, with only the characteristics differing.

Bodyweight of the pig was the characteristic with the highest effect on the baselines of the heat stress-related parameters. Therefore, two approaches were conducted:

- In the first approach, the stepwise model calculation was performed with weight categorized into three groups (light, medium, and heavy).

- In the second approach, all models were tested using weight as a continuous variable from 60 kg onwards, in a quadratic equation (weight + $weight^2$).

In total, 81 models were tested using categorized weight groups, and 84 models were tested using weight as a continuous quadratic variable. This resulted in 16 final models (based on AIC), one for each heat stress-related parameter (T_{rectal}, RR, HSS, and ADG) in relation to two different climate parameters (mean THI or temperature), using two approaches regarding body weight (category or continuous). However, dividing weight into categories makes the results easier to interpret and more practical for on-farm application. Farmers typically adjust management strategies based on weight categories, rather than per kilogram of growth. Also, both models differed only slightly from each other. Therefore, only models that included weight categories were selected, resulting in 8 final models. These models were then used to determine the importance of RH during high heat loads, which is discussed in the next section.

2.4.2 What is the impact of the relative humidity on top of temperature on heat stress-related parameters?

For each heat stress-related parameter, the mean absolute errors and mean squared errors of the models in relation to temperature or THI were calculated and compared. This allowed for the selection of the best-fitting model (temperature vs. THI) to assess the impact of RH during heat load. The best-fitting model (with the smallest errors) for the physiological heat stress-related parameters was in relation to THI, while for the performance heat stress-related parameters, it was in relation to temperature. This resulted in four final broken-line models, which were further used to discuss the reliability of the thermoregulation models used in the literature.

2.4.3 What is the relationship between respiration rate and rectal temperature?

The same broken-line models were used to analyse the relationship between RR and T_{rectal}, starting with following model:

$$T_{rectal} = RR \times \beta_{RR} + BW \times \beta_{BW} + Sex \times \beta_{sex} + + Z \times \mu + \varepsilon$$

Where:

- Dependent variable:
    - T_{rectal} = Rectal temperature
- Independent variables
    - RR = Respiration rate
    - BW = Body weight at moment of observation

    - Sex = Sex
- β = vector of the fixed effects
- Z = design matrix of random effects
    - Individual animal within terminal sire
    - Individual animal within a compartment within an experiment
- μ = vector of the random effects
- ε = vector of random errors.

Several different broken-line models were calculated, including and excluding weight category and sex as variables that could determine the breakpoint and/or the reaction before or beyond the breakpoint. In total 15 different models were tested. The best fit was chosen based on the lowest AIC. The pseudo marginal (R^2_m) and conditional (R^2_c) R^2 values of the final model were calculated to evaluate the proportion of variance explained by the independent variables (weight category and sex) and by both the independent and random variables, respectively (Nakagawa et al., 2017).

3 Results and discussion

3.1 What is the impact of relative humidity on top of temperature on heat stress-related parameters?

Based on comparing the mean absolute and squared errors of the models, physiological heat stress-related parameters (RR, T_{rectal}, and HSS) were best described by THI, while the performance heat stress-related parameter (ADG) was better described by temperature (Table 3). The differences in errors were however very small.

Table 3: Mean absolute and squared errors for every heat stress-related parameter in relation to Temperature-Humidity Index (THI) or temperature. The model with the lowest errors was chosen as final model.

Model	RR		T_{rectal}		HSS		ADG	
	THI	Temp	THI	Temp	THI	Temp	THI	Temp
Mean absolute error	16	16	0.225	0.226	11	11	0.212	0.210
Mean squared error	20	21	0.296	0.297	15	15	0.294	0.292

RR = Respiration Rate (breaths/min), T_{rectal} = Rectal temperature (°C), HSS = Heat Stress Score (scale 0-150), ADG = Average Daily Gain (kg/day), THI = Temperature-humidity index (mean value between 13:00-17:00), Temp = Temperature (mean value between 13:00-17:00) (°C)

For RR and HSS, this result makes sense because RH affects evaporative cooling (Huynh et al., 2005a). Higher RH reduces the efficiency of heat dissipation through breathing, making it harder for pigs to cool down. Since RR is influenced by RH, it follows that T_{rectal} would also be better described by THI: as the pig struggles to lose heat efficiently by evaporation, T_{rectal} inevitably rises. Huynh et al. (2005a) also observed the influence of RH on physiological parameters, reporting that only the breakpoints of T_{rectal} and RR were significantly affected, while performance remained unchanged. Similarly, another study found that RR and T_{rectal} correlated more strongly with 'effective temperature' than with temperature alone (Brandt & Bjerg, 2024). The 'effective temperature' accounts for the combined effects of temperature, RH, and air velocity based on a specific formula (Bjerg et al., 2018). The THI values used in this thesis do not include air velocity and follows a different formula for the relationship between RH and temperature. Nevertheless, these findings suggest that temperature alone describes physiological responses to heat load with slightly less reliability. However, the error differences between THI and temperature models were very small. This could be attributed to several hypothetical factors:

1) A low variation of RH in the dataset
   - In our dataset there were only a few data points where RH was >80%, however the datapoints of RH were within the range of 40-80% and evenly distributed. As a result, extreme RH data points are underrepresented in the dataset
2) An existing relationship between temperature and RH, whereby the effect of both follows the same direction
   - In our dataset this was not the case, as the correlation coefficient between temperature and RH was -0.45, indicating a moderate negative correlation. This suggests that as ambient temperature increases, RH tends to decrease
3) The effect of RH is rather marginal for the temperature and linked RH values present during the observations
   - In our dataset, this may be true, as the previous hypothesis regarding a positive relation between temperature and RH is rejected. However, the small differences observed between the THI and temperature models could also be attributed to the underrepresentation of extreme RH values, or potentially a combination of both factors.

The ADG was best described by temperature rather than THI, suggesting that RH has even less impact on pig performance under a high heat load within the temperature and RH combinations present during our study. This is in contrast with the findings of Huynh et al. (2005a), who observed that pigs kept at a RH of 50% grew faster than pigs kept at 65% or 80%. However, they also found that RH had no effect on ADFI. Feed intake reduction, which directly influences ADG, is a strategy to lower heat increment by digestion and absorption (Renaudeau et al., 2011; Secor, 2009). Since feed intake is more of an internal metabolic process rather than an external heat dissipation mechanism, RH may have less impact on this kind of adaptation to lower THP, compared to RR.

It should be noted that these descriptive models are based on a single THI formula, used by Lucas et al. (2000). Using different THI formulas may lead to slightly different outcomes, potentially altering the conclusions. Therefore, the findings cannot be fully generalized across all THI formulas, and further research using alternative formulas would be necessary to validate these results. However, since THI formulas are widely used in the literature and their fundamental principles remain consistent, major deviations in outcomes are not expected.

In conclusion, the physiological reaction of pigs to higher heat loads is best described by THI. However, based on the artificial and natural heat days that occurred during the experiments, the impact of RH on heat stress-related parameters was relatively small. Therefore, temperature can be used for practical implementation, as it is easier for farmers to monitor and manage. In research, the use of THI for describing physiological heat stress-related parameters is more reliable.

3.2 Which pig and farm characteristic has the greatest effect on heat stress-related parameters and what is the effect during increasing heat loads?

As extensively discussed in Chapter 2, numerous characteristics influence thermoregulation. Many studies have utilized the thermoregulation model of Mount (1979a), or adapted versions. However, this diagram is not based on actual measurements of heat production or heat loss. Mount (1979a) clearly stated that this is a generalized illustration, with scales that are dependent on species, age, nutrition, acclimation history, and environmental factors. Numerous controlled studies in the literature have been conducted on each of these characteristics (Pedersen & Ravn, 2008; Renaudeau et al., 2011; Rudolph et al., 2024a), with some receiving more attention than others, all contributing to the significance of one specific characteristic. Nevertheless, the question arises: which of these characteristics has the greatest effect on the parameters that are most important to evaluate heat stress. Therefore, a total of 165 models with various characteristics and heat stress-related parameters were tested in multiple combinations.

First the effect size (F-value) and significance of the pig, farm or experiment characteristics on heat stress-related parameters in relation to THI for physiological parameters and temperature for performance is discussed. The real effects and the extent to which these characteristics affect the breakpoints and the response before and beyond the breakpoints is discussed further point-by-point for every heat stress-related parameter. The baseline and breakpoints for RR apply to the comfort zone, while the baseline and breakpoints for T_{rectal} cover the whole thermoneutral zone, as explained in Chapter 2.

3.2.1 How big is the impact of characteristics on heat stress parameters?

Within the range of tested characteristics in our specific dataset, the body weight of the pig had the greatest impact on the heat stress-related parameters, except for HSS (Figure 2). Many studies that evaluated thermoregulation models of fattening pigs, did not implement weight as co-factor (Brown-Brandl et al., 2001), or only observed pigs of one specific weight class (Huynh et al., 2005a). However, we can assert that, in comparison with terminal sire lines commonly used in Belgium, sex (barrows and gilts), floor type (partially or fully slatted) and space allowance, the weight of growing-fattening pigs is the most influential parameter regarding heat stress-related parameters, especially in terms of T_{rectal}.

The second most influential characteristic was sex, which had a significant impact on all heat stress-related parameters (except for RR) (Figure 2). The size of this effect on heat stress-related parameters underscores the importance of considering sex in heat stress research. Terminal sire had a significant but small effect on all heat stress-related parameters (Figure 2). Therefore, genetic selection may lead to gradual but slow improvements in heat stress experience within the studied population. Our dataset primarily included commonly used terminal sire lines in Belgium, such as the Belgian Piétrain or hybrids. A broader selection of sire lines might reveal greater variability, helping to identify breeds with a wider thermoneutral range. The type of heat load of the trial, artificial or natural, had a significant impact on the physiological heat stress-related parameters (Figure 2). Artificial heating conditions increased all baselines of RR, T_{rectal} and HSS. This is logical, since the artificial heating conditions included controlled heat waves around 31°C for an entire week (De Prekel et al., 2024b), whereas the natural heat waves were more moderate with colder nights during which the pigs were able to recover, and therefore had less impact compared to the artificial heat waves. Floor type and space allowance had no significant impact on any of the heat stress-related parameters (Figure 2).

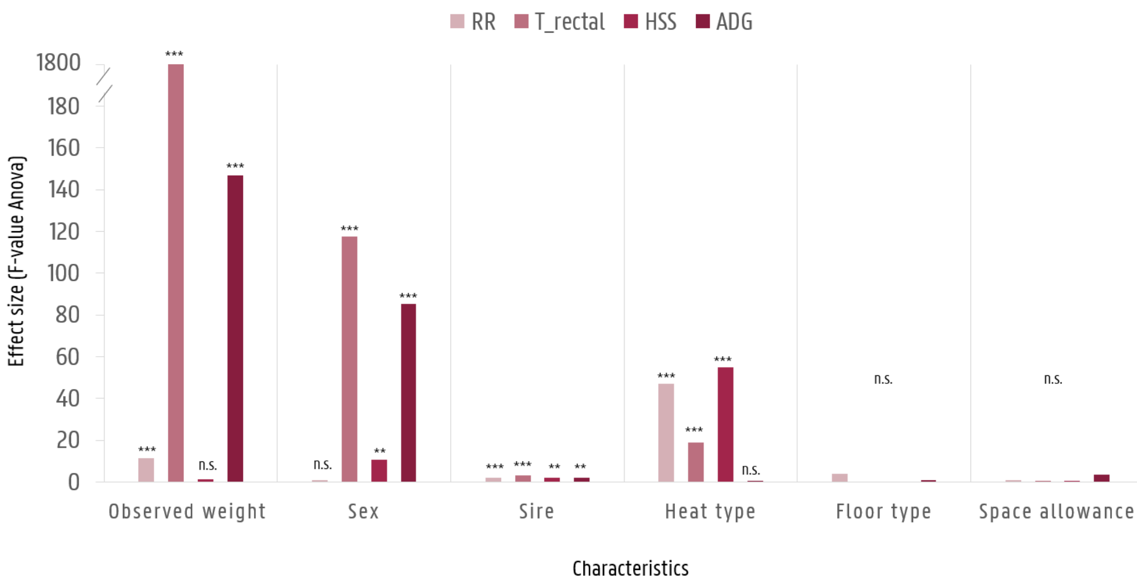


Figure 2: The effect size (F-value of Anova) and their *p*-value (n.s. = non-significant ($p > 0.01$), ** = $p \leq 0.01$, *** = $p \leq 0.001$) of different animal- and farm-related characteristics on heat stress related parameters in relation to Temperature-humidity index (THI) for RR, T_{rectal} and HSS or temperature for ADG. The greater the F-value, the greater the effect of the characteristics on the heat stress-related parameters. The effect of the climate parameters on the heat stress-related parameter was very high and all significant $p < 0.001$.

RR = Respiration Rate, T_{rectal} = Rectal temperature, HSS = Heat Stress Score, ADG = Average Daily Gain

3.2.2 How do characteristics affect heat stress parameters: What is the impact on the breakpoints and beyond?

Table 4 presents the final best-fitting models of the heat stress-related parameters for THI (RR, T_{rectal} and HSS) and temperature (ADG). These models are also represented in Figure 3. For each heat stress-related parameter, boxplots of the real data (left graph) and the model estimations (right graph) in relation to the chosen climate parameter are shown. The variation in the real data is much larger than the variation in the estimated data, as only a selection of different characteristics and random variables was made. The general baseline and breakpoints (barrows in the light weight group under natural heat conditions) is indicated by a vertical, red-dotted line, but can differ based on the implemented characteristics, which is discussed further. Overall, the model estimations provide a relatively good representation of the real observed data.

Table 4: The baseline at a THI of 63 or an ambient temperature of 19°C, the reactions before and after the breakpoint, and the breakpoints for different weight categories and sexes of the heat stress-related parameters in relation to THI or temperature.

Heat stress parameter	Characteristic	Category	Baseline (at THI =63)	Reaction before breakpoint	Breakpoint [THI]	Reaction after breakpoint
Respiration rate	Weight group	Light	28 breaths/min	+0.0 breaths/min per THI	73.6	+4.1 breaths/min per THI
		Medium			72.7	
		Heavy	24 breaths/min		71.3	
	Sex	Barrows	28 breaths/min		73.6	
		Gilts				
Rectal temperature	Weight group	Light	39.2 °C	+0.01 °C per THI unit	75.9	+0.03 °C per THI
		Medium	39.1 °C		73.2	
		Heavy	38.9 °C		75.9	+0.09 °C per THI
	Sex	Barrows	39.2 °C		75.9	+0.03 °C per THI
		Gilts	39.1 °C		76.8	
Heat stress score	Weight group	Light	-15	+3.7 per THI unit	77.7	+2.2 per THI
		Medium				
		Heavy				
	Sex	Barrows	-15			
		Gilts	-16			-1.1 per THI
Heat stress parameter	**Characteristic**	**Category**	**Baseline (at Temp = 19°C)**	**Reaction before breakpoint**	**Breakpoint [°C]**	**Reaction after breakpoint**
Average daily gain	Weight group	Light	1.19 kg/day	-0.03 kg/day per °C	26.1	-0.02 kg/day per °C
		Medium				-0.04 kg/day per °C
		Heavy	1.12 kg/day			-0.02 kg/day per °C
	Sex	Barrows	1.19 kg/day		26.1	-0.02 kg/day per °C
		Gilts	1.07 kg/day		29.2	+0.02 kg/day per °C

THI = Temperature-humidity index (mean value between 13:00-17:00), Temp = Temperature (mean value between 13:00-17:00) (°C)

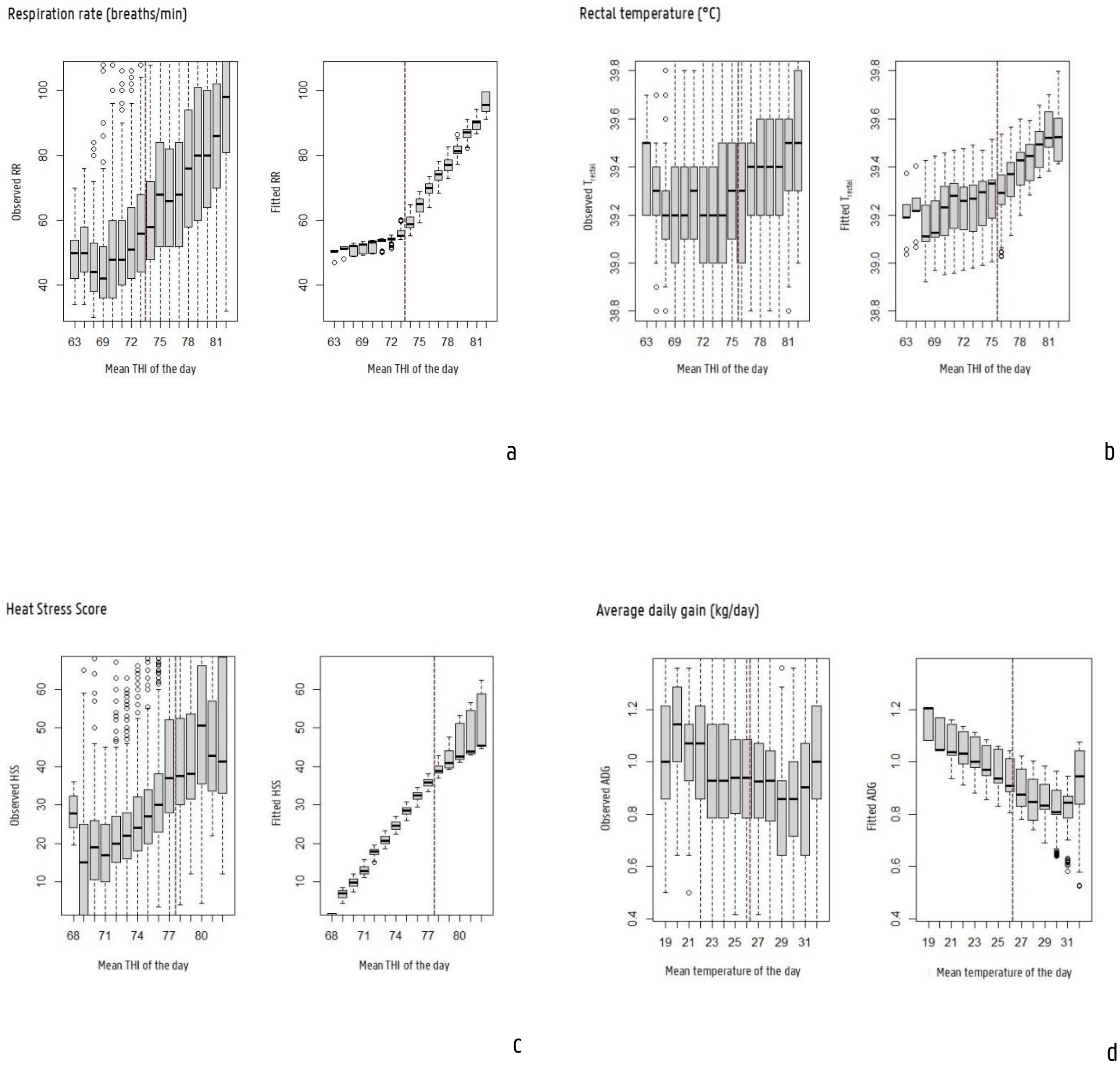


Figure 3: Representation of the final models of the heat stress related parameters according to the effects described in Table 4. For a) respiration rate, b) rectal temperature, c) heat stress score (scale 0-150) and d) average daily gain, boxplots of the real data (left graph) and the fitted values of the calculated model (right graph) in function of the chosen climate parameter is given. The general (of a barrow within the light weight group under natural heating conditions) baseline and breakpoint are given by the vertical red-dotted line.

3.2.2.1 Respiration rate

Before the breakpoint (at a THI of 63), the baseline of RR of the heaviest pigs was lower than the light-medium pigs (24 vs. 28 breaths/min) (Table 4). The RR of the lowest weight category began to increase more intensively at a THI of 73.6 (Figure 4), equivalent to a temperature of 25.6°C (calculated with a RH of 61%, which is the average value within our stables during the summer). However, as pigs become heavier, this increase occurs earlier: at a THI of 72.7 and 71.3 for the medium and heavy weight groups, respectively. This corresponds to temperatures of 24.6°C and 23.9°C at a relative humidity of 61%, respectively. Since these breakpoints occur before the T_{rectal} breakpoint, these represent the ECT, marking the upper end of the pig's thermal comfort zone (Huynh, 2005). This implies that the comfort zone of heavier pigs ends earlier than lighter ones. After these breakpoints, RR increased much faster than before, at a rate of 4.1 breaths/min per unit THI. This increase was consistent across all weight groups. There were no differences in RR response between barrows and gilts with increasing heat load.

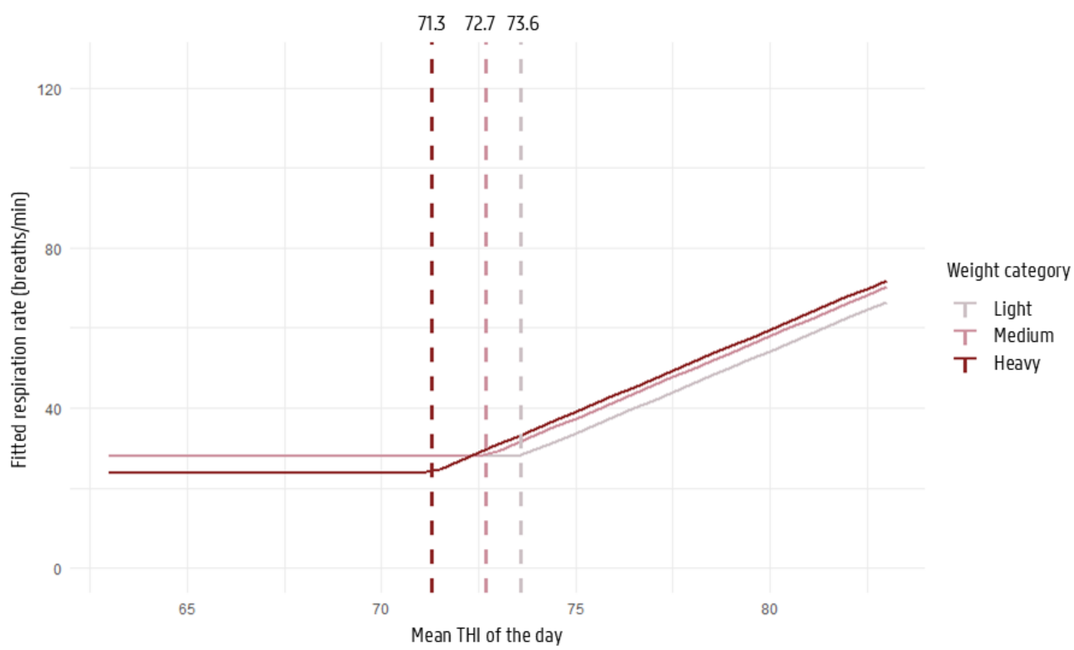


Figure 4: The final simplified broken-line model of respiration rate (RR) in relation to the Temperature-humidity index (THI) for categorized weight groups (light, medium and heavy). The vertical dotted line represents the breakpoint of each weight category, if it was significant.

3.2.2.2 Rectal temperature

The baseline of T_{rectal} decreases with increasing pig weight (39.2 vs. 39.1 vs. 38.9 °C, for light, medium and heavy pigs, respectively), as also discussed in Chapter 6 (Table 4). The T_{rectal} of the lightest and heaviest weight group barrows began to increase more intensively at a THI of 75.9 (Figure 5a), equivalent to a temperature of 27.3°C at 61% RH. For the medium weight group barrows, this increase started earlier at a THI of 73.2. After these breakpoints, T_{rectal} increased three times faster than before, at a rate of 0.03°C per unit THI. In the heaviest weight group, this increase was even tripled compared to the light-medium weight groups, at 0.09°C per unit THI, which confirms that heavier pigs react more intensively to heat. These steep increases, especially for the heavy weight group, indicate that RR is no longer sufficient to dissipate excess heat and maintain a 'stable' T_{rectal}, which implies the upper end of the thermoneutral zone. The RR-breakpoint of the heavy weight group was at a THI of 71.3 (end of the comfort zone), while the T_{rectal}-breakpoint of the same group was 75.9 (end of the thermoneutral zone). This suggests that heavy pigs can sustain their T_{rectal} for 4.6 THI units (75.9-71.3) by increasing their RR, which corresponds to 3.4°C at 61% RH. In the study of Huynh et al. (2005a), pigs around 70 kg at 65% RH maintained a stable T_{rectal} over a temperature increase of 4.6°C by increasing their RR, with RR and T_{rectal}-breakpoints at 22.5°C and 27.1°C, respectively. In our study, using the same weight category of 70 kg (light weight group), T_{rectal} remained stable for 2.3 THI units (1.7°C at 65% RH), showing a r thermoneutral zone that ends earlier.

Before the breakpoint, light weight gilts started at lower T_{rectal} than barrows (39.1 vs. 39.2 °C), which aligns with previous findings (Rudolph et al., 2024a). This could be due to the hormone oestrogen, which has been showed to reduce body temperature in mice and humans (Charkoudian & Stachenfeld, 2016; Chen & Yu, 2018). Oestrogens should play a role in modulating the regulation of skin blood flow (and sweating). Additionally, oestrogens appear to enhance vasodilation through direct effects on peripheral vasculature (Charkoudian & Stachenfeld, 2016). Another explanation of the lower T_{rectal} of gilts is the lower feed intake and growth compared to barrows, which contribute to internal heat production. However this can be counterbalanced by the higher lean muscle gain of gilts (Cromwell et al., 1993).Light weight barrows began to increase their T_{rectal} at a THI of 75.9, while this increase started later for gilts at a THI of 76.8 (Figure 5b), equivalent to 28°C at 61% RH. Beyond the breakpoint, there were no differences in T_{rectal} response between barrows and gilts. The breakpoint difference may indicate that gilts can maintain their T_{rectal} within a stable range for a longer time, and therefore have a thermoneutral zone that ends later.

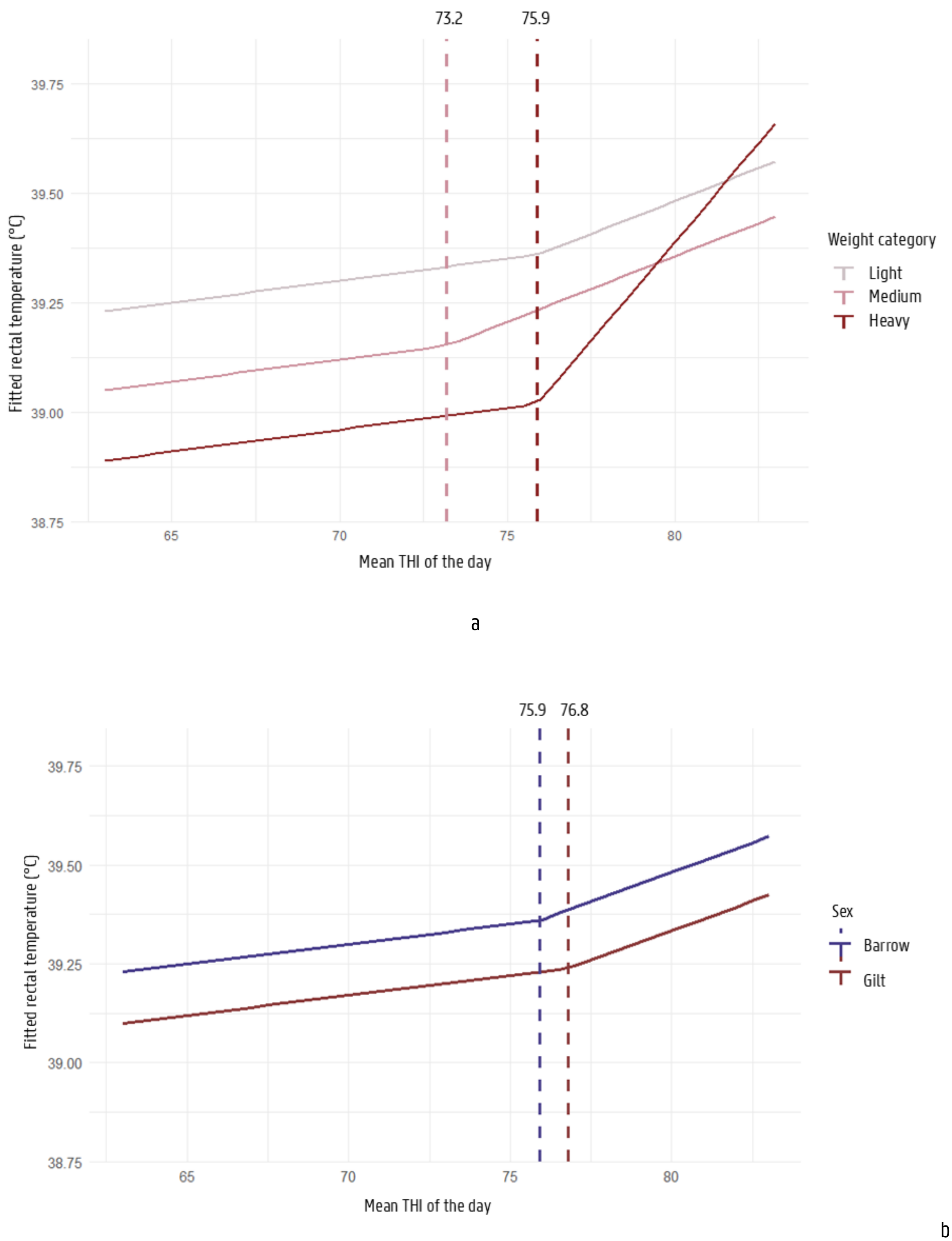


Figure 5: The final simplified broken-line model of rectal temperature (T_{rectal}) in relation to the Temperature-humidity index (THI) for a) categorized weight groups (light, medium and heavy) and b) sex (barrows and gilts). The vertical dotted line represents the breakpoint of each weight category/sex, if it was significant.

The sex difference may explain the large difference between our results and those of Huynh et al. (2005a), who only used gilts. Adjusting for this sex difference, our estimated temperature range becomes 2.4°C, bringing it closer to their findings. This further emphasizes the important role of sex and weight in thermoregulation. Additionally, the study of Huynh et al. (2005a) had only nine replicates, while our dataset is significantly larger, providing a more robust analysis. In practice, implementing sex-specific heat stress management strategies is challenging, as separating or treating barrows and gilts differently is not always feasible. However, in some farms, female and male pigs are housed in different rooms e.g. to optimize nutrition strategies and/or to facilitate vaccination against boar taint, which offers possibilities for sex-focussed strategies.

Another interesting observation is that T_{rectal} already increased before the breakpoint at a rate of 0.01°C per unit THI. In contrast, the response of RR did not show a significant increase until the breakpoint. This may imply that pigs don't increase breathing frequency to lose excess heat until the breakpoint, which refers to the upper end of the comfort zone. Traditional thermoregulation models representing evaporative heat exchange by a horizontal baseline to the end of the comfort zone, such as Mount (1979a), corroborate with our data. However, T_{rectal} behaves differently and starts to rise slightly even before the breakpoint. This suggests that pigs experience a gradual T_{rectal} increase with THI, rather than maintaining a constant body temperature within the thermoneutral zone. This challenges traditional thermoregulation models, which often depict T_{rectal} as perfectly constant/horizontal within the thermoneutral zone and not being affected by ambient temperature (Brown-Brandl et al., 2001; Huynh et al., 2005a; Mount, 1979a). However, it should be noted that all fattening pigs in this study were fed ad libitum, and T_{rectal} was measured within the pen while pigs were active. As a result, the pigs were not in a resting or fasting state during data collection. This likely led to elevated baseline T_{rectal} values, since both physical activity and feed intake contribute to increased metabolic heat production (National Research Council, 2012; Pearce et al., 2013a). Nevertheless, while this may have influenced the absolute baseline levels, it does not necessarily affect the relative change in T_{rectal} in response to increasing THI levels. Our findings suggest that, under our specific study conditions, a truly "stable" T_{rectal} does not exist. Instead, pigs gradually increase body temperature with rising ambient temperature, starting as early as THI 63 (lowest THI in our dataset).

3.2.2.3 Heat stress score

The HSS across all weight categories began to change at a THI of 77.7, equivalent to a temperature of 28.6°C at 61% RH. After this breakpoint, HSS increased more consistently across all weight categories.

A lower HSS (-16 vs. -15) was observed at the baseline for gilts, compared to barrows (Table 4). Both barrows and gilts reached their HSS breakpoint at a THI of 77.7 (Figure 6). Beyond this threshold, the HSS of barrows increased at a slower rate, whereas the HSS of gilts showed a slight decline, reinforcing the idea that gilts and barrows experience heat stress differently. Typically, HSS is expected to rise with increasing THI. The observed decline in gilts may be due to fewer recorded HSS observations at higher THI values.

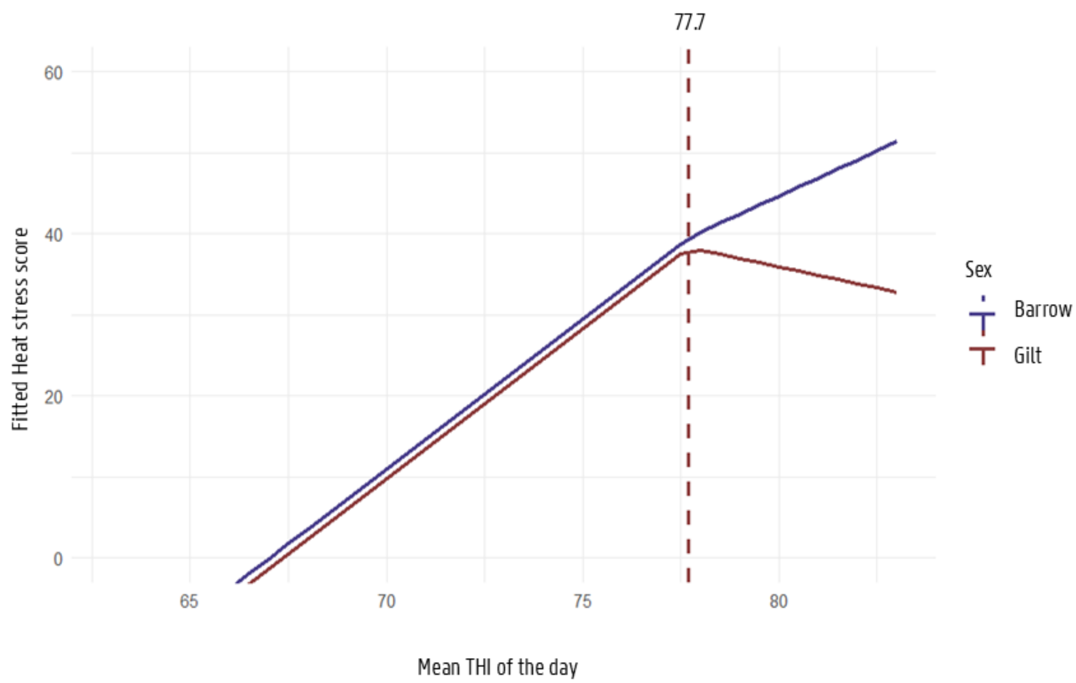


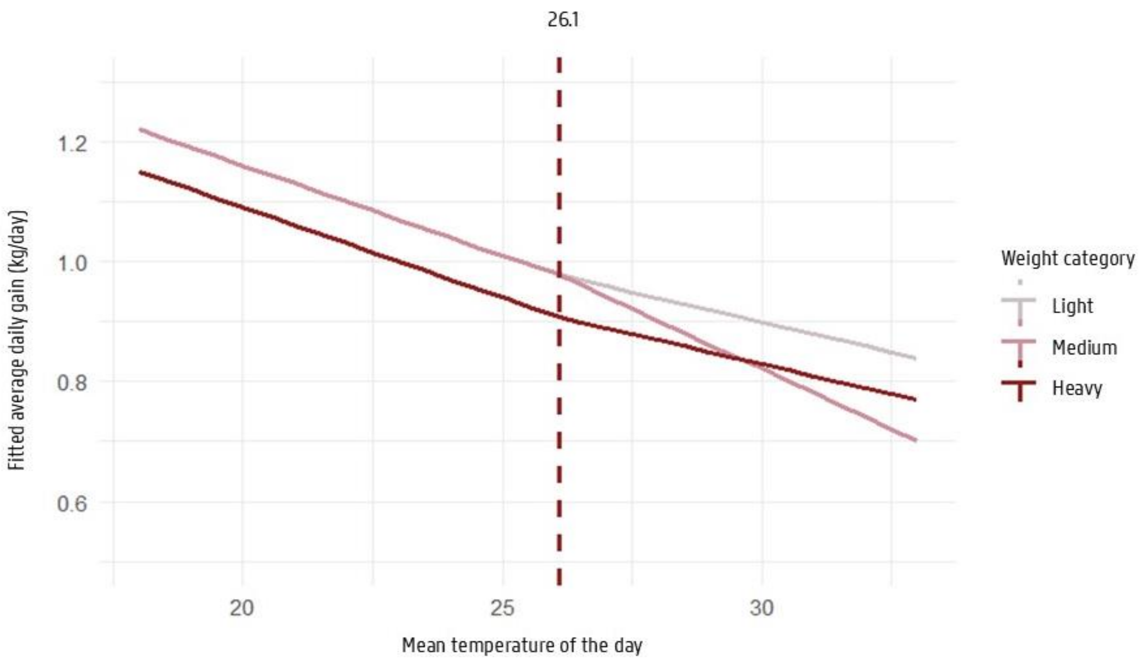
Figure 6: The final simplified broken-line model of heat stress score (HSS, scale 0-150) in relation to the Temperature-humidity index (THI) for sex (barrows and gilts). The vertical dotted line represents the breakpoint of each sex, if it was significant.

3.2.2.4 Average daily gain

Heavier pigs started with a lower ADG than light-medium pigs at the baseline (1.12 vs. 1.19 kg/day) (Table 4). Across all weight groups, ADG of barrows already started to decrease around 20°C and changed slightly at a temperature of 26.1°C (THI = 74.3) (Figure 7a). After this breakpoint, the ADG of the lightest and heaviest weight categories decreased less intensively than before, at a rate of -0.02 kg/day per °C, while in the medium weight category, the ADG decrease was more pronounced namely -0.04 kg/day per °C. Despite this, the overall response of all weight groups beyond the breakpoint was less pronounced compared to the physiological heat stress-related parameters. This observation may call into question the validity of using a breakpoint in the ADG-temperature model.

A lower ADG (1.07 vs. 1.19 kg/day) was observed at the baseline for gilts, compared to barrows (Table 4). The ADG of barrows changed at 26.1°C, whereas this change started much later for gilts at 29.2°C (THI = 78.5) (Figure 7b). Moreover, after these breakpoints, barrows continued to experience a decrease in ADG, while gilts showed an increase. This may imply that gilts are less affected by high heat loads in terms of growth performance as also observed for the physiological parameters. This trend was also comparable with the study of Rudolph et al. (2024a), who found that gilts experienced a smaller decrease in ADG compared to barrows during high heat loads. These differences may be linked to hormonal variations, feed intake patterns, and metabolic differences, as discussed in Chapter 2. However, the potential physiological and hormonal mechanisms regarding sex differences are underexplored. Future research is needed to explain these discrepancies.

In conclusion, the body weight of the pig, followed by sex, had the greatest impact on heat stress-related parameters. Heavier fattening pigs had a comfort zone that ends earlier. These findings suggest that the ECT is primarily influenced by body weight, although some studies have proposed that it is the UCT that is predominantly affected by weight (Quiniou et al., 2000; Renaudeau et al., 2011). In addition, we observed a more intensive increase in T_{rectal} in heavier fattening pigs beyond the UCT. Gilts had a thermoneutral zone that ends later and although they grew more slowly in the thermoneutral zone, their ADG was less negatively impacted by increasing THI than that of barrows.



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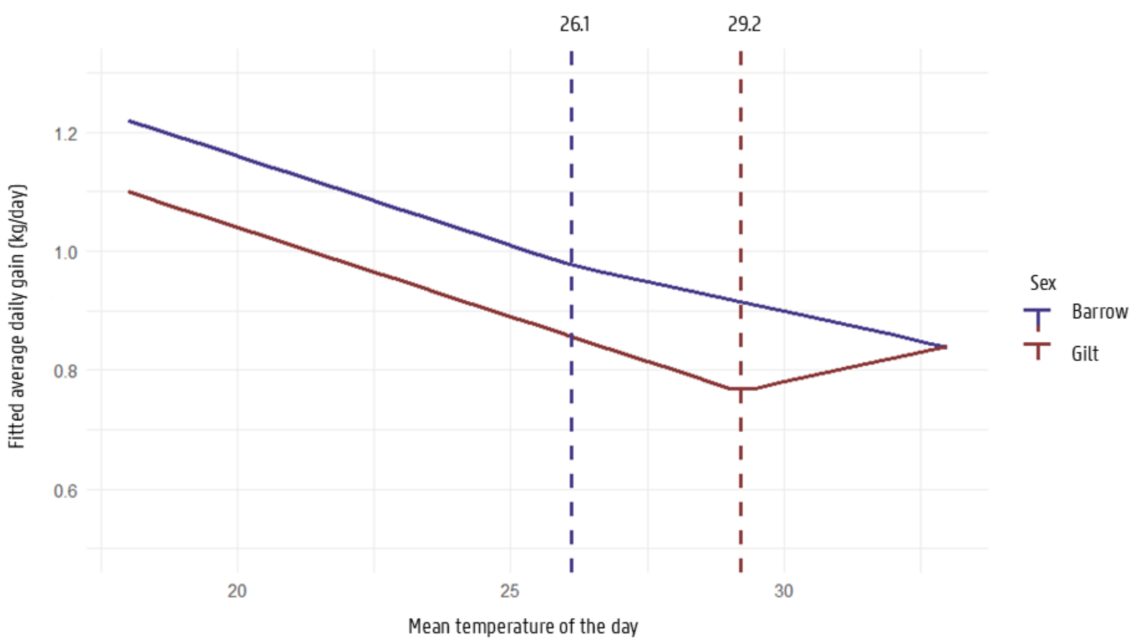

b

Figure 7: The final simplified broken-line model of average daily gain (ADG) in relation to temperature for a) categorized weight groups (light, medium and heavy) and b) sex (barrows and gilts). The vertical dotted line represents the breakpoint of each weight category/sex, if it was significant.

3.3 What is the relationship between respiration rate and rectal temperature?

Exploring the relationship between RR and T_{rectal} allows to determine when an increased RR is no longer sufficient to dissipate excess heat, leading to a rise in T_{rectal}. This information is crucial for farmers, as RR is easier to measure than T_{rectal}. Rectal temperature remains a more reliable indicator of thermoregulation, which makes the relationship between RR and T_{rectal}, and particularly their breakpoint, highly interesting for monitoring and managing heat stress.

The final model is represented in Figure 8. Boxplots of the real data (Figure 8, left graph) and the model estimations (Figure 8, right graph) of T_{rectal} in relation to RR are shown. The variation in the real data is much larger than the variation in the model, as only a selection of different characteristics and random variables was included. The model errors were also relatively large, with a variation of 0.230°C in T_{rectal}. Since a similar error was observed in the THI-T_{rectal} model, this level of error may still be acceptable for descriptive purposes.

The key question is whether RR and T_{rectal} are directly related. The R^2_m was 0.153, indicating that 15.3% of the variance in T_{rectal} can be explained by RR, weight category, and sex, suggesting that these variables are not strong predictors of T_{rectal}. The R^2_c was 0.411, meaning that 41.1% of the variance can be explained by the whole model, including the random effects of individual animal, terminal sire, compartment, and experiment number. This might be acceptable for descriptive analysis.

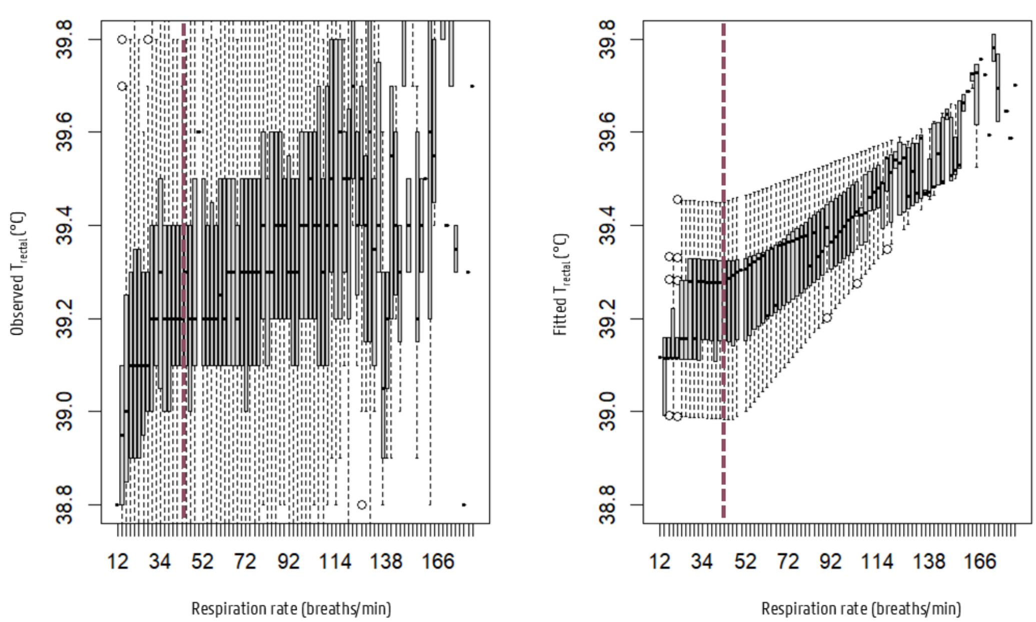

Figure 8: Representation of the final model of rectal temperature (T_{rectal}) in relation to respiration rate (RR). Boxplots of the real data (left graph) and the fitted values of the calculated model (right graph) of T_{rectal} in function of RR is given. The calculated breakpoint is given by the vertical, red-dotted line.

Before the breakpoint, the baseline of T_{rectal} decreased with increasing weight category (Figure 9), which was already observed in the T_{rectal}-THI model (Figure 5a). Furthermore, gilts also showed a lower T_{rectal} in this model, as previously observed (not included in Figure 9). Rectal temperature began to increase when the RR was 43 breaths/min, which occurred simultaneously across all weight groups and sexes. Comparing this with the RR-THI model, a RR of 43 breaths/min was found around a THI of 76 (Figure 4), aligning with the breakpoint of T_{rectal} in the T_{rectal}-THI model (Figure 5a). This reinforces the T_{rectal}-RR model. This implies that at a RR of around > 40 breaths/min, pigs can no longer maintain stable T_{rectal}, which can serve as a practical indicator for farmers. However, due to the large variation in RR among pigs, this may be challenging. In addition, the reaction after the breakpoint differed, with T_{rectal} increasing more intensively with increasing weight, which is consistent with previous findings. No sex differences were observed in the reaction after the breakpoint.

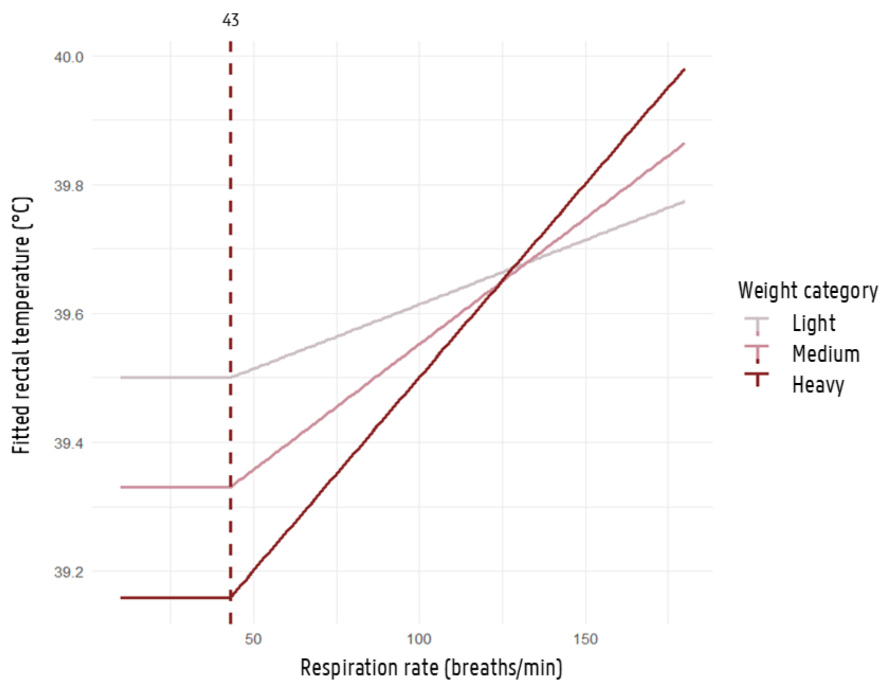

Figure 9: The final simplified broken-line model of rectal temperature (T_{rectal}) in relation to respiration rate (RR). The vertical dotted line represents the breakpoint.

In conclusion, the association between T_{rectal} and RR alone is rather moderate, implying that predicting these variables based on the reaction of only one is not possible. However, within our calculated model (with random variables), describing this association is possible. When pigs have an RR higher than ±40 breaths/minute, T_{rectal} starts to increase. This increase is more pronounced in heavier pigs compared to lighter ones.

4 Conclusions

The THI is more accurate than ambient temperature and therefore should be used for research purposes. As the impact of relative humidity is small, temperature (instead of THI) may be suitable for use under practical on-farm conditions based on the observed climate conditions in Flanders.

The effect size of weight and sex on heat stress-related parameters is important and should be considered in future heat stress studies. Heavier pigs showed changes in respiration rate earlier, implying a comfort zone that ends earlier. In addition, T_{rectal} increased much faster in heavier pigs than in lighter pigs, highlighting a higher susceptibility of heavier fattening pigs when surpassing the UCT. Gilts showed signs of a thermoneutral zone that ends later, as they start to increase T_{rectal} later than barrows and are less susceptible to heat stress due to their decrease in heat stress score and increase in average daily gain after the breakpoint. In addition, across all weight categories and sexes the T_{rectal} of fattening pigs, was not constant within the comfort and thermoneutral zones, but increased slightly with increasing THI.

The transition out of the thermoneutral zone (steep increase in T_{rectal}) may be indicated by a RR exceeding 40 breaths per minute, providing a practical on-farm tool for assessing heat stress in pigs. This threshold could help farmers identify when intervention is needed to mitigate the negative effects of heat stress.

5 Acknowledgments

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CHAPTER 8 | GENERAL DISCUSSION

1 Introduction

Climate adaptation is essential to address the existing challenges posed by rising global temperatures (IPCC, 2024) (Chapter 1). In animal production, one of the challenges is heat stress in farm animals. This can compromise both animal comfort, welfare and productivity (Campos et al., 2017) and result in economic losses (St-Pierre et al., 2003) and increased workload for the farmer. Pigs are particularly vulnerable to heat stress due the widely used indoor housing systems, the genetic selection for high performance and lean meat (Brown-Brandl et al., 2004; Ramirez et al., 2022), the lack of functional sweat glands, and the small surface area-to-volume ratio (Bruce & Clark, 2010), among other factors (Chapter 2). The combination of climate change (IPCC, 2023a) and the genetic selection of high-performance pig breeds (Ramirez et al., 2022; Renaudeau et al., 2011) in temperate environments, such as Belgium, challenges the sustainability of modern commercial pig production.

The main objective of this thesis, as introduced in Chapter 1, was to mitigate heat stress in fattening pigs by:

a) Assessing the impact of heat load on fattening pigs and investigate the variations in pig and farm-related characteristics (**revisiting thermoregulation models and threshold values in fattening pigs**);
b) Developing and validating a heating protocol that enables rapid investigation of heat stress mitigation measures (**optimizing heat stress research**);
c) Investigating the effects of different management strategies on the physiological and performance parameters of fattening pigs under heat stress (**implementing management-strategies as heat stress mitigation: from research to farm**).

2 Revisiting thermoregulation models and threshold values in fattening pigs

In the first place, to effectively mitigate heat stress, it is essential to first identify when pigs experience discomfort. This involves analysing the effects of high heat loads on physiological parameters and determining the thresholds at which discomfort becomes detrimental, such as a rise in T_{rectal}. While previous models (Brown-Brandl et al., 2001; Huynh, 2005) have attempted to define these limits, they often lack key pig-specific characteristics that influence thermoregulation. Based on our findings in Chapter 7, where we found that weight and sex are critical parameters for thermoregulation models, a part of Mount (1979a) original thermoregulation model (Figure 1) was adapted to incorporate these new insights. Based on this new model, THI thresholds used in literature (NWSCR, 1976) could be verified or challenged (Figure 2).

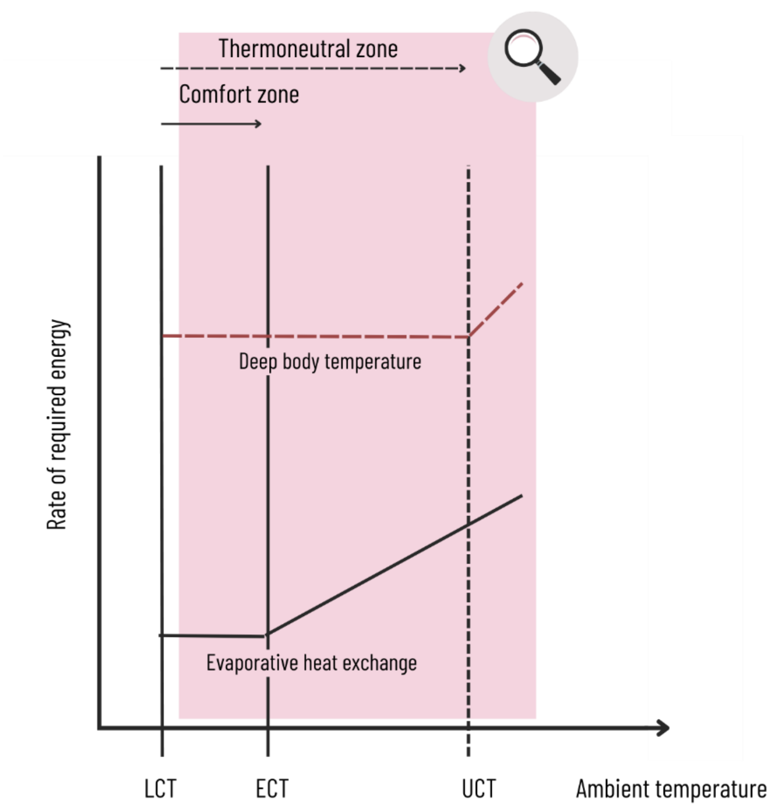

Figure 1: Original thermoregulation model by Mount (1979a), with adaptations made to the red area, based on the findings from Chapter 7. LCT = Lower critical temperature, ECT = Evaporative critical temperature, UCT = Upper critical temperature

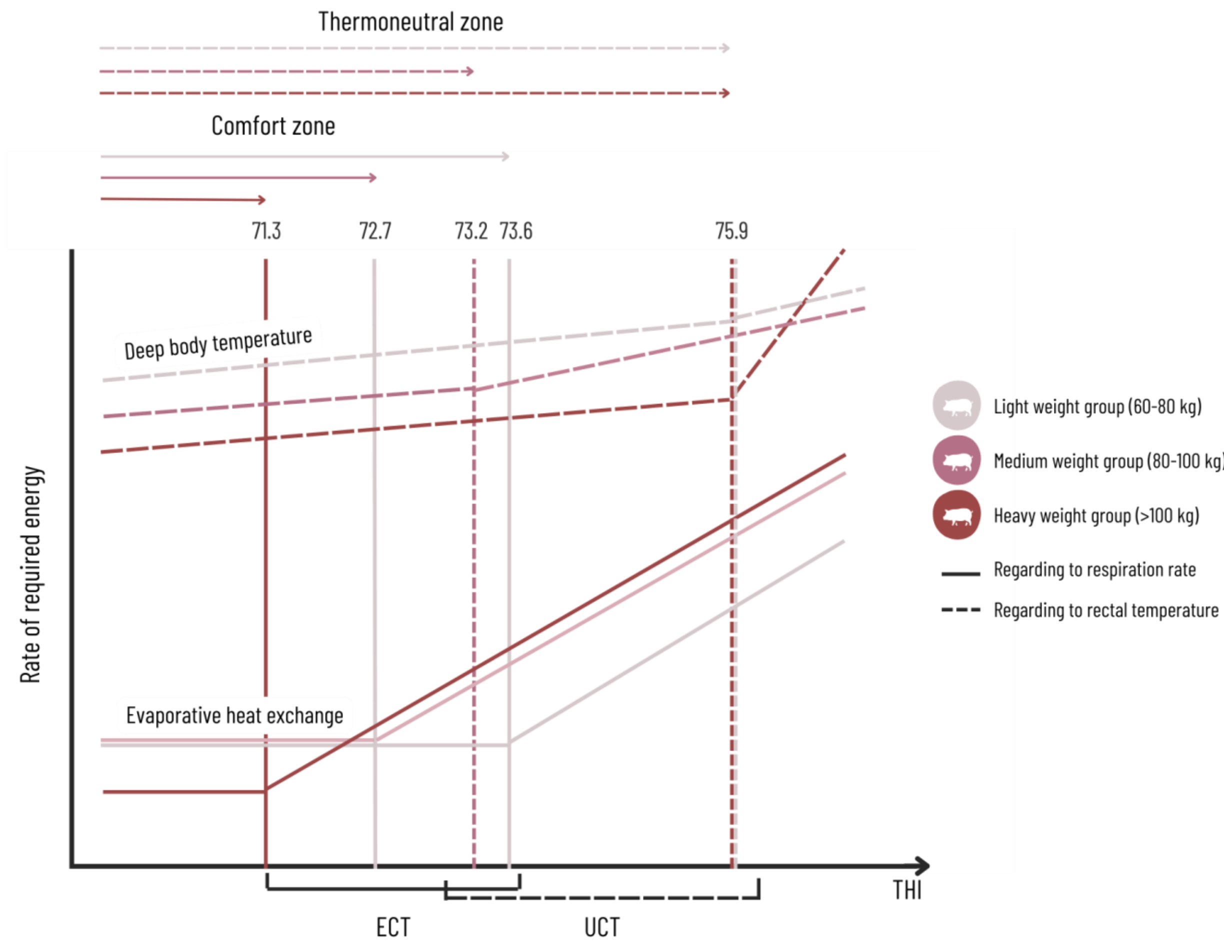

Figure 2: Adapted thermoregulation model for pigs of different weight categories, based on our findings from Chapter 7. This model illustrates the reactions of respiration rate (evaporative heat exchange) and rectal temperature (deep body temperature) to the Temperature-humidity index (THI) in fattening pigs from different weight groups (placement of breakpoints and slopes are not plotted to scale but are for illustrative purposes). LCT = Lower critical temperature, ECT = Evaporative critical temperature, UCT = Upper critical temperature

Given that weight is the most significant characteristic among all the tested variables, the new thermoregulation figure is solely dependent on weight. The other important characteristic, sex, was not incorporated into the same figure to avoid complexity (Figure 2). Including sex would result in differences in deep body temperature, with lower baselines and breakpoints at higher THI values for gilts.

The three key findings from Chapter 7, are easy to visualize with this new model:

1. the T_{rectal} of fattening pigs, an indicator of core body temperature, increases slightly with increasing THI, even within the thermoneutral zone,
2. the comfort zone for heavier fattening pigs (>100 kg) ends earlier compared to fattening pigs with a body weight <100 kg and,
3. the T_{rectal} of heavier fattening pigs (>100 kg and above) increases three times faster than that of pigs with a body weight <100 kg for temperature higher than the UCT.

From these findings, it can be concluded that fattening pigs weighing 100 kg and above, especially barrows, should be closely monitored, and heat stress mitigation strategies should be specifically applied to this group. Considering this, breeding sows, which generally weigh more than 100 kg, may have a similar reaction to a high heat load as older fattening pigs. The experience during high heat load may be even more intense for sows, as lactation and gestation increase their metabolic rate (Eissen et al., 2000) and therefore their total heat production. On the other hand, the growth is minimal, which also affects heat production. Since the physiology and maintenance requirements of lactating or gestating sows differ from that of female fattening pigs (National Research Council, 2012), the different reactions of gilts to a high heat load cannot be extended as such to the thermoregulation model for sows. Therefore, future research could explore the impact of sow parity and weight on heat stress responses and how these characteristics influence thermoregulation, similar to how weight and sex was incorporated into our model.

This new thermoregulation model includes also one new insight: the boundaries of the used THI-formula to prevent heat stress in fattening pigs occur at slightly lower values than previously reported in the literature. Current THI-thresholds and temperature recommendations are widely used to assess the impact of heat stress and to control stable climate management. However, these thresholds may not fully incorporate the complexity of heat stress responses regarding to different animal or farm-related characteristics. Evaluating breakpoints of thermoregulatory parameters for different characteristics with accurate and enough data points is crucial, as its outcomes can provide valuable insights for farmers, veterinarians, climate experts, system developers, researchers, and other manufacturers. The existing limits of NWSCR (1976) for the formula used by Lucas et al. (2000) corroborate closely with our recommendations, yet they can be refined (Table 1).

Table 1: Recommendations and their meaning of Temperature-humidity index (THI) and temperature limits according the formula used by Lucas et al. (2000)

Warning level for the effects of heat stress	**THI range**	**Temperature range**[1]	**Upper limit (beyond the…)**	**Meaning**
Early warning	71 ≤ THI < 76	24 ≤ °C < 27	Comfort zone	The range wherein all weight groups, and all sexes started to increase their RR
Warning	76 ≤ THI < 77	27 ≤ °C < 28	Thermoneutral zone of the most sensitive group	The moment where the heavy weight groups started to increase their T_{rectal} extensively
Danger	THI ≥ 77	°C ≥ 28	Thermoneutral zone of the less sensitive group	After this breakpoint, gilts, who experience the heat load less intense, showed also a rapid increase in T_{rectal}

[1]For more practical on-farm use, THI limits are translated into temperature limits, according to a RH of ±60% (corresponding to the average RH within the stable during the PhD research)

The new thermoregulation model provides a general overview but remains incomplete. Sex was assumed to have an additional effect on top of the influence of weight (light weight group). Consequently, the combined effect (interaction) of these two characteristics on the breakpoint and the response before and beyond the breakpoint may differ slightly. For example, medium-weight gilts may exhibit a slightly different reaction compared to heavy-weight gilts, which is not really anticipated. Future research should also explore the other end of the spectrum, cold stress, while incorporating various pig and farm characteristics. Younger pigs are particularly vulnerable to cold conditions due to their high surface-area-to-volume ratio (Bruce & Clark, 2010), low subcutaneous fat levels for insulation (Bruce & Clark, 2010; Mount, 1979b), and less developed immunity. As a result, weight may likely be a significant factor when temperatures drop below the LCT. Furthermore, our model does not yet account for the progression of non-evaporative heat exchange, as it was not evaluated extensively. A more comprehensive understanding of the combined effect of conductive, convective, and radiative heat loss, which presents a significant research challenge, could help refine the model. Ultimately, integrating both evaporative and non-evaporative heat exchange into the model could provide a more complete picture of adaptive thermogenesis in pigs. However, including all these variables into the model will make the figure more complex and more difficult to use in practice.

3 Optimizing heat stress research

A second goal was to develop and validate a heating protocol that enables rapid investigation of heat stress mitigation measures for research purposes and for use in practice. In the context of climate change, the urgency of testing strategies to mitigate heat stress in pigs is increasingly important. Artificial heat stress trials offer a controlled approach to accelerating research on potential solutions. We developed and validated an artificial heating protocol (Chapter 3) and consistently applied it across different trials in this thesis (Chapters 4–6). The protocol effectively induced heat stress, which was observed by significant changes in heat stress related physiological parameters.

In Chapter 7, it was shown that the type of heat load (artificial vs. natural) significantly influenced physiological responses, raising baseline values across all data. However, ADG was not significantly affected by the type of heat load across the entire dataset in Chapter 7. This discrepancy likely comes from the differing time scales of physiological versus performance parameters. Respiration rate and T_{rectal} are measured as snapshots at specific moments. Under artificial heat stress, these physiological measurements were taken when the heat load remained consistently high (~31°C) for several consecutive days without cooler intervals. In contrast, natural heat stress conditions fluctuate, with intermittent periods of lower temperatures. As a result, the impact of artificial heat load was more pronounced on RR and T_{rectal} than the impact of natural heat load, which is a logical outcome.

In contrast, ADG represents a longer-term response to heat stress, as it is measured over weeks rather than days. However, this does not mean that the artificial heat waves in our study had no significant impact on performance. In fact, Chapters 4–6 demonstrate clear effects of the heating protocol on ADG (and ADFI). The key message is that the artificial heating protocol did not influence ADG more than naturally occurring heat waves. To enhance research efficiency and accelerate the identification of effective mitigation strategies, refining the heating protocol to create a more pronounced impact on ADG could be beneficial. This could be achieved by extending the duration of heat exposure or increasing ambient temperatures more drastically. Since performance is crucial for farmers from an economic perspective, future research should explore a more precise relationship between ADG and increasing heat loads, as linking ADG to climate parameters may sometimes be challenging, as described in Chapter 7 (Materials & Methods).

To improve the artificial heating protocol, it is crucial to test artificial heat waves on heavier pigs, particularly those higher than 100 kg. Additionally, barrows should be specifically included in the development and evaluation of the protocol, as they represent the most heat-sensitive group. Targeting heat stress mitigation strategies for heavy barrows (like in Chapter 3, but with even heavier fattening pigs) is essential, given their heightened susceptibility. In addition, an optimal balance must be found between applying a sufficiently severe heat load, one that significantly impacts growth and allows for effective evaluation of mitigation strategies, while minimizing the duration and intensity of heat stress to protect animal welfare. Achieving this balance is challenging but necessary to ensure both ethical and scientifically robust research. To ensure optimal evaluation of performance-related outcomes under heat stress, it is recommended that the duration of the applied heat load last at least seven consecutive days. This recommendation is based on observations from the initial heating protocol of Chapter 3, which lasted only three days and did not result in a significant reduction in ADFI, while in the other chapters a prolonged heating protocol resulted in altered performances. In my view, the most detrimental form of heat stress occurs when pigs are not provided with sufficient opportunity to recover during cooler periods, such as at night. This concern is especially relevant when daytime heat load reaches extreme levels. From this perspective, and to enable testing of the intervention's efficacy under worst-case conditions, it is advisable to maintain a constant elevated temperature throughout both day and night. This approach ensures that any observed benefits of the tested measure are applicable even in scenarios where animals experience prolonged and uninterrupted heat stress. If a treatment demonstrates promising effects under these controlled and continuous heat load conditions, it might be appropriate to evaluate its effectiveness under practical farm conditions, particularly during naturally occurring heat waves. However, conducting such trials poses greater challenges due to the unpredictability of natural heat waves and the increased complexity of on-farm experimental setups. Furthermore, sex and weight should be consistently incorporated into the statistical analysis of experimental trials, as these factors significantly influence thermoregulatory responses. This consideration was not always accounted for in Chapters 4 and 5, where effects were often analysed based on the "average" animal by combining data from barrows and gilts to draw general conclusions. While this approach provides valuable insights, it is slightly less precise, particularly when evaluating T_{rectal}, which is affected by sex and weight differences. However, research must balance complexity with feasibility, requiring certain decisions to streamline analysis while maintaining accuracy.

4 Implementing management-strategies as heat stress mitigation: from research to farm

The last goal was to investigate management-strategies to mitigate heat stress. Three management strategies were extensively studied in this thesis: the effects of feeding strategies (Chapter 4), space allowance (Chapter 5), and currently used terminal sire lines (Chapter 6).

4.1 Low/high investments vs. flexible/fixed strategies

Farmers have the option to choose between various strategies for mitigating heat stress. The choice depends on several factors, including the severity of the heat stress problem on the farm, the construction of the building, the financial situation, the geographical location, the efficacy of the strategy, and the farmer's willingness. Often, a distinction is made between short-term and long-term strategies. Short-term strategies can be defined as immediate and autonomous measures that require only low investments and can be adjusted from year to year (Schauberger et al., 2020). Long-term strategies, on the other hand, often require structural changes to the farm, involve financial considerations, need technical guidance, and require strategic planning (Mitter et al., 2018). However, these definitions may be too broad, and the decision-making process is more complex than simply choosing between short- and long-term adaptations. The most important factors in decision-making for farmers may be the investment costs, the flexibility and ease of implementation, and the efficacy of the adaptations. The first two aspects are combined in Table 2, for a wide set of strategies, including all evaluated management strategies discussed in Chapter 2. The efficacy of the latter is discussed in the next section.

In Table 2, a distinction is made between different levels of financial investment for the farmer. No-investment measures imply no additional costs but are often more labour-intensive, such as shifting feeding times, which can also be considered a form of cost. Low-investment measures are relatively easy to consider financially, while high-investment strategies require a financial plan. Alongside financial considerations, it is important to evaluate the ease and flexibility of the measure: can it be easily implemented, reversed, repurposed, or does it offer a win-win strategy? Conversely, fixed strategies necessitate structural changes and are more difficult to reverse or undo.

Table 2: Decision making model for heat stress-mitigating strategies that can be implemented by farmers

Financial investment for the farmer	Heat stress-mitigating strategy	
	Flexible / reversible	**Fixed**
No-investment	○ Shifting feeding time	
Low-investment	○ Using feed additives (Chapter 4A) ○ Changing diet composition (Chapter 4B) ○ Using drinking water additives ○ Increasing space allowance (Chapter 5) ○ Increasing summer ventilation rate	○ Shifting genetic background (Chapter 6) ○ Improving air ventilation ○ Using forced air ventilation ○ Adding green vegetation or other shading in general ○ Adding green vegetation or other shading at the air inlet ○ Changing inlet design
High-investment	○ Using heat exchanger (cooling and heating) ○ Using fogging system within room (with soaking options)	○ Using fogging system at air inlet ○ Using floor cooling ○ Using roof irrigation ○ Using green roof ○ Using pad cooling

All strategies discussed in this thesis require a relatively low financial investment for farmers compared to most climate-technical measures.

Farmers can implement diet treatments from the beginning of the fattening period. If they have the necessary infrastructure, such as additional silos and feeding lines, it is also possible to switch to different diets during the fattening phase. This allows them to incorporate feed additives or adjust diet composition specifically during periods of high heat stress. Given the flexibility to modify feed at any time (provided that the infrastructure or labour force allows it) or when refilling feed silos, this approach represents a reversible, adaptable, and low-investment management strategy.

Another flexible and low-investment management measure is adjusting space allowance. Practically, this can only be done at the beginning of the fattening period. Increasing space allowance mid-period is feasible only if there are extra or larger pens available. However, moving heavy fattening pigs to different pens is labour-intensive and can disrupt dominance and hierarchy among the animals. An alternative strategy during the summer or periods of high heat load is to send the heaviest pig(s) in a pen to the slaughterhouse earlier. This approach increases the space available to the remaining pigs for the rest of the fattening period.

Lastly, implementing more heat-tolerant sire lines can be done using sperm from boars already present in artificial insemination centres, e.g. by anticipating that these will be present at higher slaughter weight during summer. Once inseminated, the farmer cannot change the genetic background of the fattening pigs, making this a fixed, low-investment strategy. However, in the next batch, other terminal sire lines can be chosen. Artificial insemination centres can also reflect on the sires that are present and potentially enhance genetic selection for more heat-tolerant breeds. Selecting pigs based on more different traits requires more time.

4.2 Efficacy, challenges and trade-offs of practical implementation of the management measures

In addition to deciding between flexible, fixed, low, or high-investment strategies for reducing heat stress in fattening pigs, farmers must consider the efficacy, challenges, and trade-offs of heat stress mitigation measures. These factors complicate the decision-making process.

The efficacy of the tested strategies is summarized in Figure 3. Schauberger et al. (2020) estimated efficacy based on expert opinions in agricultural engineering and veterinary medicine or model calculations. This estimation is based on growth parameters compared to a control group. We calculated the efficacy of the management strategies observed in the trials within this thesis according following formula:

$$\text{Efficacy (100\%)} = (1 - (\Delta T/\Delta C)) \cdot 100\%$$

Where ΔT = ADG-decrease between thermoneutral and heat weeks in the treatment group, and ΔC = ADG-decrease between thermoneutral and heat weeks in the control group.

Growth of the animal is important for economic purposes, however, other performance parameters like FCR or mortality may also affect efficacy. Furthermore, productivity alone should not be the only consideration when addressing heat stress, as productivity is not necessarily linked to animal welfare (Fraser, 2008; Jensen et al., 2012). Therefore, the efficacy based on the increase in T_{rectal} was also presented from data of the PhD. This provides a more accurate assessment of animal comfort, as a steep increase in T_{rectal} indicates the end of the thermoneutral zone.

The efficacy of a heat stress mitigation strategy depends largely on the specific parameter being assessed, as illustrated in Figure 3, and the reference conditions. Obviously, the intensity of heat stress also has a significant impact. During the PhD project (2021–2024), no official natural heat waves were recorded in the summers of 2021 and 2024 (KMI, 2025). This absence of natural heat waves may have influenced the observed effectiveness of various strategies tested under natural summer conditions (Chapters 4A and 6). Given this variability, future research should include a sensitivity analysis to evaluate how different strategies perform under varying levels of heat stress.

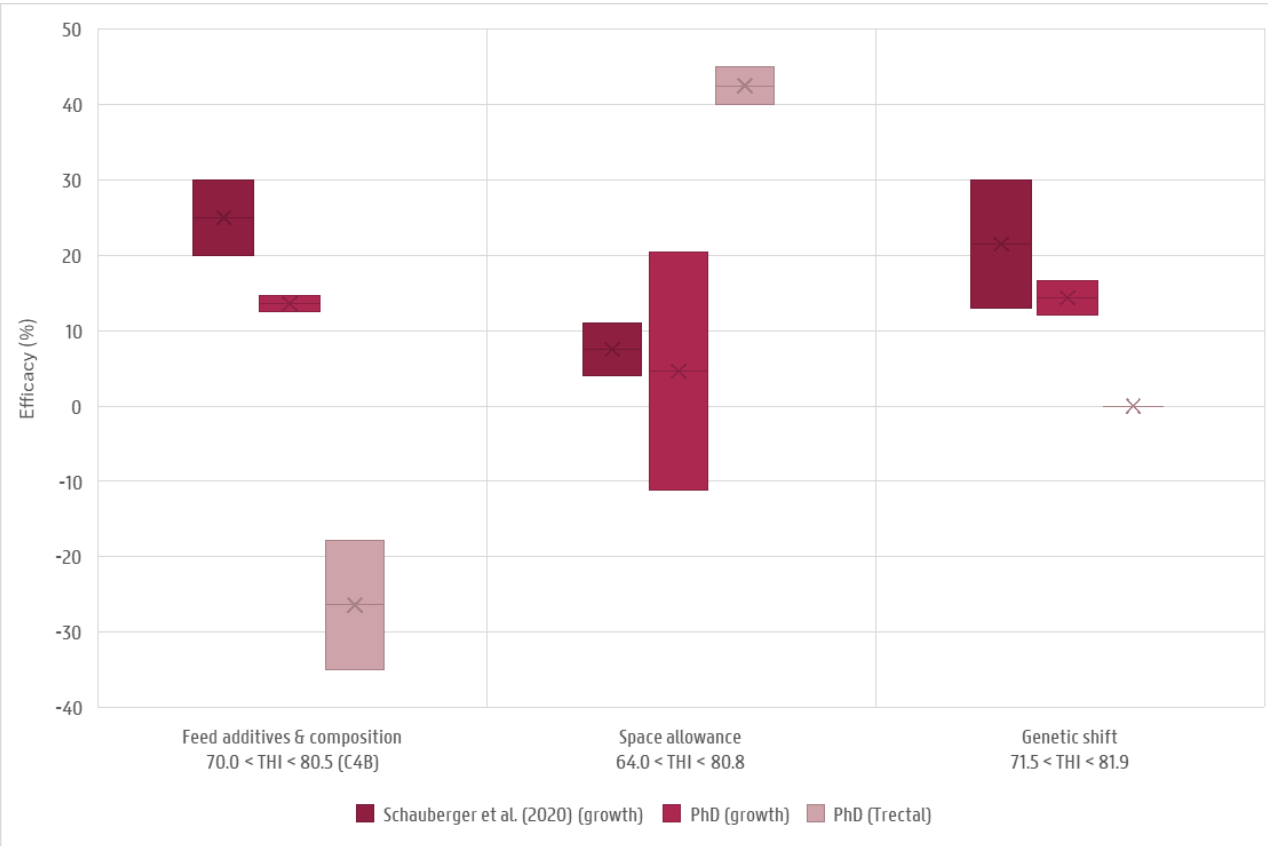

Figure 3: Efficacy of heat stress-mitigating strategies, as estimated (range) based on growth parameters by Schauberger et al. (2020), and observed (range) on growth and rectal temperature during the PhD research with results of feed additives from Chapter 4B, space allowance of Chapter 5 and genetic background of Chapter 6.

4.2.1 Feed additives and composition

According to Schauberger et al. (2020), feed adaptations can reduce heat stress by 20-30%. However, in Chapter 4B (Figure 3), the observed growth improvements were numerically smaller. Additionally, the effect on T_{rectal} was negative, as pigs in the enriched and energy-dense diet groups had a greater increase in T_{rectal} during a heat wave compared to the control group. This can likely be attributed to their lower T_{rectal} baseline. Furthermore, the efficacy of dietary adaptations depends on the composition of the basal diet. In our trials, the control diets were formulated to meet the pigs' productivity needs and were likely already optimized for their health, which may have influenced the relative impact of dietary modifications.

In our study, we tested a limited number of additives and one diet with an altered composition. However, the literature on feed strategies for mitigating heat stress in growing-finishing pigs is extensive. Various studies have investigated different additives, such as betaine, selenium, and vitamin E (Chapter 4), as well as chromium, cinnamon, and glutamine (Cottrell et al., 2015). Additionally, research has explored varying diet compositions, adjusting crude protein, fat, and fibre levels, with mixed results. Determining the optimal combination of feed additives, while ensuring compliance with legal limits, maintaining high-quality ingredients, and formulating a nutritionally balanced diet, remains a challenge. The goal is to provide sufficient essential amino acids, protein, and energy without increasing heat production or compromising productivity. However, achieving this balance is complicated by contradictory study findings, differences in experimental setups, variations in legally permitted dosages (e.g., US vs. Europe), and differences between organic and inorganic additive forms. Given these challenges, developing a universally effective diet remains nearly impossible, highlighting the need for more standardized and consistent research.

The effectiveness of implementing feeding strategies in practice during high heat loads may be greater when the basal diet is suboptimal, compared to situations where optimal health and productivity are already prioritized. The additional cost of various feed additives may not be justified if the standard diet already meets the pigs' nutritional needs. However, certain additives may offer added value beyond heat stress mitigation, for example for consumer health, as demonstrated in Chapter 4B. This added benefit could help justify the additional cost. The application of nutritional strategies to mitigate heat stress may be partly justified in production systems where environmental or structural interventions are limited or impractical. In settings where cooling systems such as mechanical ventilation or evaporative cooling are unavailable or insufficient, dietary interventions (especially optimizing nutritional needs for the used genetic line) represent one of the few feasible approaches to reduce heat load and maintain animal performance under elevated temperatures. Moreover, the effectiveness of dietary additives is influenced not only by their inclusion but also by their formulation and dosage. The chemical form of the additive can affect its physiological impact. For example, pure betaine has shown better performance compared to betaine hydrochloride (HCl) in certain contexts, potentially due to its influence on gut pH and microbial balance. Consequently, the efficacy of an additive depends on factors such as its formulation, stability, and bioavailability. Legal constraints regarding the permitted inclusion levels of certain additives may also influence observed outcomes. In cases where higher dosages of non-toxic additives are permissible, more pronounced effects may be achieved, which could, in turn, improve the cost-effectiveness and economic justification for their use in commercial settings.

4.2.2 Increasing space allowance

The efficacy of increasing space allowance by 20% or 40% during high heat loads has been modelled to improve growth by 4% and 11% (Schauberger et al., 2019), while the range observed in our study was much broader (from -11.2 to 20.4% (Figure 3). When evaluating the efficacy of increased space allowance using T_{rectal}, a 20% ($SD_{1.0}$ vs. $SD_{0.8}$) or a 40% increase ($SD_{1.3}$ vs. $SD_{0.8}$) resulted in an efficacy of approximately 42%, as ΔT_{rectal} doubled during high heat load conditions in $SD_{0.8}$ compared to $SD_{1.0}$ or $SD_{1.3}$. This suggests a substantial improvement in animal comfort.

This strategy presents several challenges and trade-offs, particularly for farrow-to-finish farms. Firstly, implementing a higher space allowance requires additional available space, which depends on the number of weaned piglets, a factor that is largely beyond the farmer's control. Additionally, increasing space allowance means fewer fattening pigs overall, potentially impacting profit margins. As demonstrated in Chapter 5, pigs with greater space allowance generally exhibit higher ADG. This, combined with improved heat stress mitigation during heat waves, suggests possible benefits. Faster-growing pigs reach slaughter weight more quickly, and with fewer pigs overall, feed costs may decrease if the FCR is favourable. As a result, increasing space allowance during summer may positively affect profitability. However, the cost-benefit analysis remains complex, influenced by factors such as feed prices, piglet and slaughter pig prices, and the number of piglets per sow (Van den Broeke & Aluwé, 2024). Another key consideration is emissions. Reducing the emitting surface per animal is generally associated with lower pollutant gas emissions, such as NH_3 (Philippe & Nicks, 2015). Increasing space allowance could increase the emitting surface per pig. The emission factor increases with 1 kg NH_3 per animal place per year when the space allowance is above 0.8 m²/pig (Van Gansbeke & Van den Bogaert, 2020), which presents a potential drawback. However, previous studies indicate that heavier pigs in half slatted pens foul their solid resting areas more due to insufficient space (Aarnink et al., 2006). Expanding space allowance in half slatted floors may help mitigate this issue, potentially preventing an overall increase in emissions (Philippe et al., 2011). Furthermore, during periods of high heat load, pigs shift their lying behaviour to slatted floors, as they feel colder. This change leads to increased excretion on solid floors, further contributing to unwanted emissions (Huynh et al., 2005b). In such cases, providing more space in pens with half slatted floors could counteract the negative effects of a larger emitting surface per pig by promoting cleaner resting areas and improving overall hygiene.

Implementing more space allowance during the summer presents more benefits than drawbacks in the context of this thesis. The primary advantages include improved pig comfort by preventing excessive increases in T_{rectal} and enhanced average daily gain, possibly due to reduced competition at the feeder. While an increase in emissions is a potential concern, its impact is likely minimal and depends on the type of flooring used. Additionally, previous studies have emphasized the positive effects of increased space on health, welfare, and overall performance (Dewulf et al., 2007). As this strategy requires low investment and offers flexibility (Table 2), it provides a modest yet valuable contribution to mitigating heat stress in pigs.

4.2.3 Shifting genetic background

The efficacy of shifting genetics on growth, as estimated by experts, ranges from 13% to 30% (Schauberger et al., 2020). Our findings indicate an efficacy of 13% to 17% based on growth parameters, aligning with the estimations of Schauberger et al. (2020). However, no differences between the tested terminal sire lines were observed in terms of T_{rectal}. The comparison was limited to two commonly used terminal sire lines in Belgium, both already optimized for production traits (Van den Broeke, 2023). Despite a significant difference in lean meat thickness, the overall lean meat percentage varied by only 0.8% (not mentioned in Chapter 6). Therefore, a higher efficacy may be achievable if lean meat percentage is further reduced.

Shifting genetics presents several challenges. In Western Europe, farmers are given financial incentives by slaughterhouses based on carcass quality, primarily focusing on lean meat percentage and pig weight. A higher lean meat percentage results in higher payments, reducing the motivation for farmers to adopt fewer lean breeds unless the payment structure changes. However, small changes in lean meat percentage, like those observed in our trial (a difference of only 0.8%), may lead to small variations in financial returns. For example, a carcass with 66% lean meat qualifies for a €0.10 per kg quality supplement, whereas one with 64% receives only €0.07 (Van den Broeke & Aluwé, 2024). The financial impact of this difference also depends on FCR, which generally increases with decreasing lean meat percentage. Therefore, the choice of terminal sire line is important and the balance between a slightly lower lean meat percentage while still enhancing a good FCR could have potential benefits.

Implementing breeds with a slightly lower lean meat content as a heat stress mitigation strategy requires a significant shift in socio-economic perspectives. However, achieving this transition is challenging. Currently, small reductions in lean meat percentage may be justifiable if the FCR remains favourable, as they do not drastically impact farm profitability. Furthermore, it is highly possible that, even within high-performance sire lines, individual sires differ in their capacity to cope with heat stress, analogous to the variation in heat tolerance observed among individual humans. In Chapter 7, rather than evaluating differences at the sire line level, we examined the effects of individual terminal sires. The analysis revealed that sires had a statistically significant effect on baseline physiological and performance parameters, including respiration rate, rectal temperature, and average daily gain. Although these effects were relatively small, they were present prior to the onset of heat stress, indicating variation among sires in physiological traits that may influence their response to elevated temperatures. These findings suggest that the identification of heat-tolerant sires within existing sire lines could offer valuable insights for future research. However, from a practical standpoint, the usefulness of this approach may be limited, particularly if only a small proportion of sires within a sire line have meaningful heat tolerance. Therefore, more robust and sustainable strategies are needed to enhance resilience to heat stress in commercial pig production. This would likely involve broader genetic selection for heat-tolerant traits across sire lines towards an energy-efficient breed with a low fasting heat production, reduced feed intake, and maintained protein accumulation without compromising animal welfare, a concept that has been recognized for decades (van Milgen et al., 1998). As climate change continues to increase the frequency and severity of heat stress events, such long-term genetic approaches will become critical for ensuring animal welfare and maintaining productivity.

4.3 The general future of heat stress-mitigating management strategies

The effectiveness of heat stress mitigation strategies varies depending on the heat stress-related parameters, the reference system, and the heat load conditions. Among these, the reference system is likely the most crucial factor. In experimental trials, a suboptimal control group, such as pigs receiving an imbalanced diet lacking essential protein or amino acids, or those housed with limited space (e.g., 0.65 m^2/pig), can make a given strategy appear more effective than it would be in an already optimized system. The same applies to commercial farms with suboptimal management, where standard improvements could yield greater benefits. Therefore, fundamental strategies, such as providing good drinking facilities, ensuring well-insulated roofs, applying appropriate ventilation systems and settings, providing nutritionally balanced diets, ensuring adequate space, and selecting more heat-resilient breeds, should be prioritized before implementing additional measures. However, farms that already maintain high management standards may see limited benefits from further optimizations.

Management strategies are primarily low-investment and flexible adaptations. However, compared to climate-technical strategies, management strategies demonstrated lower efficacy (Schauberger et al., 2020). This is expected, as management strategies do not alter the ambient temperature or relative humidity, meaning that pigs remain exposed to the same environmental stressors. In contrast, climate-technical strategies, such as air treatment systems, actively modify the environment, resulting in a greater impact on animal comfort. Therefore, these measures should be considered as a next step once standard management practices have been fully optimized.

Despite the low number of heat waves during this PhD project, the general frequency, duration, impact, and intensity of heat waves have increased in Belgium during the past 20 years (Vlaamse Milieumaatschappij, 2025). Furthermore, global temperature is rising (IPCC, 2024) and increasing at a faster rate than climate models predicted (van Oldenborgh et al., 2009; Vautard et al., 2023). This indicates negative future expectations and an urgent need for heat stress adaptation in pigs, in addition to climate mitigation. As the impact of global warming continues to rise, there is an even greater necessity to enhance management strategies and ensure that farms optimize their standard management procedure to handle rising temperatures.

5 Considerations for further research

1. **Focus on genetic selection for more heat-tolerance**

   The importance of genetic selection should be emphasized, while simultaneously ensuring that breeds maintain the highest possible productivity levels. Therefore, research on selection within a terminal sire line (and sow line) could also be a valuable approach for identifying and enhancing heat stress tolerance within a specific sire line.

2. **Promote the importance of animal-related characteristics in heat stress studies**

   Pig weight and sex should be incorporated in future heat stress studies and different models should be re-assessed to account for these impacts. Research efforts on heavier barrows should be intensified, as they are the most vulnerable group to the effects of high heat loads. In addition, future research could also explore the impact of sow parity and weight on heat stress responses and how these characteristics influence thermoregulation, similar to how weight and sex was incorporated into our models.

3. **Enhance average daily gain measurements during increasing heat loads**

   Since performance is crucial for farmers from an economic perspective, future research should focus on establishing a more accurate relationship between ADG and increasing heat loads, as associating ADG with climate parameters can sometimes be complex and less precise.

4. **Enhance sensitivity analysis of the efficacy of heat stress-mitigating strategies**

   There is a lot of variation in efficacy of different heat stress reducing strategies. A sensitivity analysis to evaluate how different strategies perform under varying levels of heat stress could help with the decision-making process for choosing the right adaptation.

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SUMMARY

Summary

Climate change is becoming increasingly evident worldwide, bringing extreme weather events such as floods and forest fires, as well as a general rise in global temperatures. While climate mitigation remains a priority, adaptation is also necessary to address the emerging challenges caused by global warming. One such challenge is heat stress in livestock, particularly in pigs, due to their biological characteristics, the increasing lean breed selection, and the widely used indoor housing conditions. The thesis *"Mitigating Heat Stress in Fattening Pigs"* explores the effects of heat stress on fattening pigs and evaluates different strategies to mitigate these effects. The primary objectives, outlined in **Chapter 1**, include assessing the impact of heat load and affecting characteristics on fattening pigs, developing an artificial heating protocol, and investigating feeding strategies, space allowance, and genetic selection.

In **Chapter 2**, the thesis focused on the thermoregulation mechanisms of pigs, which are highly complex and influenced by multiple environmental and animal-related characteristics. A general overview is provided on heat production, normal thermoregulation models, and the key characteristics affecting thermoregulation. The consequences of heat stress on various parameters, such as respiration rate, feed intake, rectal temperature, and daily gain, are examined in detail. Additionally, existing research on heat thresholds for indoor-raised fattening pigs is reviewed, and various management and climate-based mitigation strategies were discussed.

To enhance heat stress research and accelerate the evaluation of different mitigation strategies, **Chapter 3** introduces and validates an artificial heating protocol. This protocol was tested using 80 barrows divided into two weight groups within a range of 70–100 kg. Results demonstrated significant increases in physiological parameters during heat exposure, confirming the protocol's effectiveness. This standardized heating protocol was subsequently used in all other experiments throughout the thesis to assess different management interventions.

One of the first management strategies evaluated was feed adaptation, discussed in **Chapter 4**. Dietary supplementation with antioxidants and osmolytes could enhance oxidative stress induced by a high heat load and may provide positive effects for the intestinal health and nutrient digestibility. Therefore, **Chapter 4A and 4B** examined the impact of supplementing the diet with betaine, selenomethionine, and vitamins E and C. These supplements were found to reduce the need for increased water intake during heat waves. Also, the general average daily gain tended to ameliorate in pigs supplemented with those additives. Additionally, selenium and vitamin E supplementation enriched meat quality, providing potential added value for consumers. In **Chapter 4B**, a high-energy diet with lower crude protein content and increased fat was tested to determine if it could reduce internal metabolic heat production by reduced heat increment (digestion). However, no significant improvements in heat stress parameters were observed.

Secondly, in **Chapter 5**, the study explored the impact of space allowance on fattening pigs during periods of high heat load. Providing pigs with more space allows for better heat dissipation through radiation and convective cooling, as pigs can avoid direct contact with pen mates and position themselves on cooler floor surfaces. The study found that increasing space allowance significantly prevented a high rise in rectal temperature during heat waves. Furthermore, pigs housed with more space tended to show improved daily gain, likely due to reduced competition at the feeder. These findings suggest that increasing space allowance provides a modest yet valuable contribution to mitigating heat stress in pigs.

Another important strategy that may ameliorate the pigs' response to heat stress is genetics, which was explored in **Chapter 6**. Pigs selected for high lean growth efficiency cope less well with heat stress due to the higher total heat production. Therefore, the study compared the effects of different terminal sire lines selected either for optimal growth (lower lean meat thickness) or carcass quality (higher lean meat thickness). Results indicated that heavier pigs, particularly those reaching 100 kg, experience a more rapid increase in rectal temperature and respiration rate when exposed to heat stress. Furthermore, pigs selected for growth efficiency were better able to maintain daily gain during prolonged heat waves, whereas those bred for carcass quality were more negatively affected. These findings highlight the trade-offs between genetic selection for lean meat production and resilience to heat stress.

Building on the findings from previous chapters, **Chapter 7** reassessed thermoregulation models for heat stress in fattening pigs, using data collected from eight trials conducted during the PhD project. Heat stress reactions for heat stress related physiological and performance parameters, including respiration rate, rectal temperature, heat stress score, and average daily gain, were determined. The study identified significant influences of body weight, sex, and the type of heat load experienced.

Finally, **Chapter 8** provided a general discussion of the overall findings and revisited thermoregulation thresholds in fattening pigs. Based on these findings, new Temperature-humidity index limits were proposed, suggesting that heat stress warnings should be issued earlier than previously recommended to improve early intervention. Furthermore, a discussion about optimizing heat stress research and evaluating the challenges and trade-offs associated with different heat stress mitigation strategies was assessed. The thesis emphasized that management strategies can have a modest impact on mitigating heat stress, particularly when baseline conditions are suboptimal.

SAMENVATTING

Samenvatting

Klimaatverandering wordt wereldwijd steeds duidelijker zichtbaar en brengt extreme weersomstandigheden met zich mee, zoals overstromingen en bosbranden, evenals een algemene stijging van de globale temperaturen. Hoewel klimaatmitigatie een prioriteit blijft, is ook aanpassing noodzakelijk om de uitdagingen als gevolg van de opwarming van de aarde aan te pakken. Een van deze uitdagingen is hittestress bij landbouwhuisdieren, en in het bijzonder varkens, vanwege hun biologische kenmerken, de toenemende selectie op magere rassen en de veelgebruikte binnenhuisvesting. Deze doctoraatsthesis met als titel *"Mitigating Heat Stress in Fattening Pigs"* onderzoekt de effecten van hittestress bij vleesvarkens en evalueert verschillende strategieën om deze effecten te verminderen. De belangrijkste doelstellingen, zoals samengevat in **Hoofdstuk 1**, omvatten het beoordelen van de impact van hittestress en beïnvloedende kenmerken bij vleesvarkens, het ontwikkelen van een model om hittestress te simuleren en het onderzoeken van de effecten van voedingsstrategieën, ruimtevoorziening en genetische selectie.

Hoofdstuk 2 richt zich op de thermoregulatiemechanismen van varkens, die uiterst complex zijn en worden beïnvloed door meerdere omgevings- en dier-gerelateerde karakteristieken. Er wordt een algemeen overzicht gegeven van warmteproductie, normale thermoregulatiemodellen en de belangrijkste kenmerken die de thermoregulatie beïnvloeden. De gevolgen van een hoge hittebelasting op verschillende parameters, zoals ademhalingsfrequentie, voederopname, rectale temperatuur en dagelijkse groei, worden in detail geanalyseerd. Daarnaast wordt bestaande literatuur naar temperatuurlimieten voor binnengehuisveste vleesvarkens besproken en worden verschillende management- en klimaatmaatregelen voor het reduceren van hittestress geëvalueerd.

Om hittestress-onderzoek te verbeteren en de evaluatie van verschillende mitigatiestrategieën te versnellen, introduceert en valideert **Hoofdstuk 3** een kunstmatig verwarmingsprotocol. Dit protocol werd getest op 80 bargen, verdeeld in twee gewichtsgroepen binnen een gewichtstraject van 70–100 kg. De resultaten toonden significante stijgingen aan in fysiologische parameters tijdens hittegolven, wat de effectiviteit van het protocol bevestigde. Dit gestandaardiseerde verwarmingsprotocol werd vervolgens in alle andere experimenten binnen de thesis gebruikt om verschillende managementmaatregelen te beoordelen.

Een van de eerste onderzochte managementstrategieën was de aanpassing van het voeder, zoals besproken in **Hoofdstuk 4**. Voeder supplementen met antioxidanten en osmolyten kunnen oxidatieve stress, veroorzaakt door een hoge hittebelasting, verminderen en mogelijks positieve effecten hebben op de darmgezondheid en nutriëntenvertering. Daarom werd in **Hoofdstuk 4A en 4B** de impact onderzocht van voederadditieven in het voeder, zoals betaine, selenomethionine en de vitamines E en C. Deze supplementen verminderden de verhoogde wateropname tijdens hittegolven en leidden tot een numerieke verbetering van de algemene dagelijkse groei. Bovendien werd de vleeskwaliteit verbeterd door een hogere beschikbaarheid van selenium en vitamine E, wat een potentiële meerwaarde biedt voor de consument. In **Hoofdstuk 4B** werd een energierijk dieet met een lager ruw eiwitgehalte en verhoogd vetgehalte getest om te bepalen of het de interne metabole warmteproductie van de vertering kon verminderen. Er werden echter geen significante verbeteringen in hittestressparameters waargenomen.

Vervolgens onderzocht **Hoofdstuk 5** de impact van ruimtevoorziening voor vleesvarkens tijdens periodes van hoge hittebelasting. Meer ruimte per varken kan zorgen voor een betere warmte uitwisseling via straling en convectie, doordat varkens direct contact met hokgenoten kunnen vermijden en meer op koelere vloeroppervlakken kunnen verblijven. De studie toonde aan dat een grotere ruimtevoorziening een sterke stijging van de rectale temperatuur tijdens hittegolven kon voorkomen. Daarnaast vertoonden varkens met meer ruimte een numerieke verbeterde dagelijkse groei, mogelijks door een verminderde competitie aan de voerbak. Een grotere ruimtevoorziening per dier kan dus een bescheiden maar waardevolle bijdrage leveren aan het verminderen van hittestress bij vleesvarkens.

De invloed van de genetica van de varkens op hittestress wordt besproken in **Hoofdstuk 6**. Varkens geselecteerd voor een hoger mager vleesaandeel, kunnen minder goed omgaan met hitte vanwege hun hogere totale warmteproductie. Daarom werden de effecten vergeleken van verschillende eindberenlijnen die waren geselecteerd op optimale groei (minder spekdikte) of karkaskwaliteit (meer spekdikte). De algemene resultaten lieten zien dat zwaardere varkens, rond de 100 kg, een snellere stijging van de rectale temperatuur en ademhalingsfrequentie vertoonden bij blootstelling aan hitte. Bovendien konden varkens geselecteerd op optimale groei hun dagelijkse groei beter behouden tijdens langdurige hittegolven, terwijl varkens geselecteerd op karkaskwaliteit dit minder goed konden. Deze bevindingen illustreren het belang van genetica en de verschillen in veerkracht tegen hittestress .

Voortbouwend op de bevindingen uit de vorige hoofdstukken, werd in **Hoofdstuk 7** het thermoregulatiemodel voor hittestress bij vleesvarkens herbekeken, aan de hand van gegevens uit acht proeven die tijdens het doctoraat werden uitgevoerd. De reacties op hittestress, kunstmatig of natuurlijk geïnduceerd, werden in kaart gebracht op basis van fysiologische en prestatieparameters, waaronder ademhalingsfrequentie, rectale temperatuur, hittestress-score en gemiddelde dagelijkse groei . Uit het onderzoek bleek dat lichaamsgewicht, geslacht en het type hittestress (kunstmatig of natuurlijk) een significante invloed hadden op deze parameters.

Tot slot wordt in **Hoofdstuk 8** een algemene bespreking van de belangrijkste bevindingen gedaan en worden de drempelwaarden voor thermoregulatie bij vleesvarkens opnieuw geëvalueerd. Op basis van deze resultaten werden nieuwe grenzen voor de THI voorgesteld, waarbij werd aangetoond dat de waarschuwingswaarden voor hittestress al vroeger optreden dan tot nu toe werd aanbevolen. Het optimaliseren van hittestress onderzoek en het evalueren van verschillende strategieën om hittestress te beperken, werden ook besproken in dit hoofdstuk. Dit doctoraatsonderzoek toonde aan dat managementstrategieën een bescheiden, maar waardevolle impact kunnen hebben op het beperken van hittestress, vooral wanneer de basisomstandigheden in het bedrijf suboptimaal zijn.

CURRICULUM VITAE

Curriculum Vitae

Lotte De Prekel was born in Beveren on 20th of October 1997. She graduated magna cum laude in 2020 with a Master of Science degree in Agriculture and Horticulture, majoring in Plant and Animal Production. In 2020, she began working as a PhD student on the LA VLAIO project 'Coolpigs' in collaboration with Ghent University, Faculty of Veterinary Science, Department of Internal Medicine, Reproductive and Population Medicine, and the Institute for Agricultural, Fisheries and Food Research (ILVO) in Merelbeke-Melle. Her PhD research focused on the effects of heat stress in fattening pigs and strategies to mitigate these effects. Additionally, she collaborated on other experiments, data analysis, and field studies during the project.

Lotte is the first author of several studies published in international peer-reviewed journals and has presented her work at numerous conferences and study days. Furthermore, she is the author or co-author of 28 magazine articles for the sector. She also contributed to the 'Hitteplan voor Vlaanderen'.

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Dankwoord

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