



Dynamic responses of growing pigs to heat stress modulated by prenatal life and feeding practices

Aira Maye Serviento

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Aira Maye Serviento. Dynamic responses of growing pigs to heat stress modulated by prenatal life and feeding practices. Agricultural sciences. Agrocampus Ouest, 2022. English. NNT : 2022NSARB360 . tel-04061173

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THESE DE DOCTORAT DE

L'INSTITUT NATIONAL D'ENSEIGNEMENT SUPERIEUR POUR L'AGRICULTURE, L'ALIMENTATION ET
L'ENVIRONNEMENT
ECOLE INTERNE L'Institut Agro Rennes-Angers

ECOLE DOCTORALE N° 600
Ecole doctorale Ecologie, Géosciences, Agronomie et Alimentation
Spécialité : « *Biologie et Physiologie animale* »

Par

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**Réponses dynamiques des porcs en croissance au stress thermique
modulées par la vie prénatale et les pratiques d'alimentation**

**English title: Dynamic responses of growing pigs to heat stress modulated by prenatal life and
feeding practices**

Thèse présentée et soutenue à Rennes, le 02 septembre 2022
Unité de recherche : PEGASE, INRAE, Institut Agro, 35590, Saint-Gilles, France
Thèse N° : B-360 ; 2022-18

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September 2022

Acknowledgements

This thesis project would not have been possible without the help of everyone who graciously contributed their time, effort, and ideas.

I would like to thank Jeremy Cottrell and Bruno Silva for accepting to review my thesis dissertation and for their appreciation of the work we have conducted. Je remercie également Lucile Montagne, Sophie Tesseraud, and Sophie Blat d'avoir accepté d'évaluer mon travail et de faire partie de mon jury. Un grand merci à mon comité de thèse Olivier Zemb, Anne Collin, et Annick Bernalier. Merci d'avoir pris le temps de suivre mon travail et merci pour vos précieux conseils pendant ma thèse.

Je remercie Jaap van Milgen et Florence Gondret qui m'ont accueilli dans l'unité PEGASE pendant mon master et pendant ma thèse. Je tiens également à remercier Serge Dubois et Eloise Delamaire pour leur aide dans toutes mes essais à l'UEPR/UE3P. Un grand merci également à Lallemand SAS et ANRT pour le financement de ce projet CIFRE.

Le plus grand MERCI à mes encadrants, David Renaudeau, Etienne Labussière, et Mathieu Castex pour lancer ce projet de thèse et pour votre confiance. Mathieu, thank you for giving me the opportunity to be part of this project. Your appreciation and confidence in our project were very encouraging and meant a lot. David, thank you for the trust and all the enormous opportunities you have given me from the day we worked together beginning from my Master internship and all throughout my PhD. You have opened so many doors for me and I will always be grateful. Etienne, thank you for the guidance during the thesis and for always reminding me to take time to breathe and enjoy my PhD life. More especially, thank you for the boost of confidence I sometimes need when things are not going so well. To David and Etienne, thank you for the day-to-day, for your close participation in the experiments (sometimes even during the weekends), for the exchange of ideas, the training, and for the words of encouragement most especially in the last 2 days before my deadline. I really am very proud of the work we have done in this project... and I am 110% sure it would not have been possible without all of you steering me in the right direction.

This thesis consisted of four experiments that would not be possible without all who helped from UEPR/UE3P and INRAE PEGASE. Dans l'unité expérimentale, un merci ne serait pas suffisant pour décrire ce que l'équipe métabolisme-physiologie a fait pendant ces quatre (!!!!) essais : Francis Le-Gouevic, Julien Georges, Alain Chauvin, Guillaume Poupeau, Mickaël Genissel, Fabien Guerin, Amandine Cauchi, et Julien Fortin. Deux mois pour l'essai

1, sept mois pour l'essai 2, huit mois pour l'essai 3, et encore deux mois pour l'essai 4 ! 28-34°C n'est certainement pas l'environnement de travail idéal à gérer au quotidien, en particulier les jours où on a dû rester dans des salles chaudes pendant de nombreuses heures. Merci pour le suivi quotidien des expérimentations ! Francis, merci pour ta gentillesse et pour tes nombreuses histoires qui ont fait les longues journées des essais 1 et 2 plus faciles et plus agréables. Julien, Alain, et Francis, merci en particulier pour les implantations de pilules, les poses de cathéters, les mesures des températures chez les porcs, et pour beaucoup d'autres choses que vous avez faites pour les essais dans le bâtiment 32. Guillaume, merci pour l'aide dans l'essai 3 (suivi, broyage, lyophilisation, collecte, etc.) et pour suivre fidèlement le planning sur la chaîne de papier collée sur la porte que je mettais à jour semaine après semaine. À l'équipe : Ce n'était pas toujours facile avec la rigueur et les longues périodes des essais, notamment avec le nombre de mesures dans les protocoles donc merci à vous tous pour votre énorme contribution à ce projet ! Je voudrais aussi dire merci à Herve Demay et à Josselin Delamarre qui étaient toujours présents dans tous les essais en partageant leur expertise dans tous les prélèvements de sang que ce soit en conditions thermoneutres ou chaudes, et, avec Renan Deleunay et Georges Guillemos, pour les suivis des essais en garde et/ou les mutations. Merci aussi à l'équipe abattage (Jérôme Liger, Maurice Alix, et Jean-François Rouaud) qui ont participé dans les deux premiers essais particulièrement pour les longues journées d'abattage avec les milliers de prélèvements prévus. Je ne peux pas parler de prélèvements (et des analyses subséquents) sans dire merci aux gens de l'unité PEGASE dans l'équipe Alinut (Cécile Perrier, Colette Mustière, Agathe Costentin, Xenia Arondel-Piekarz, Philippe Ganier, Gwennola Robin, Gwenola Le-Roy, Maryline Lemarchand) à qui nous demandons souvent de l'aide (parfois de manière très urgente) dans tous les essais. Je voudrais remercier aussi Jean-Yves Dourmad pour les conseils et les idées surtout quand j'étais déjà en train d'écrire ma discussion.

L'étude sur le stress thermique prénatal (essai 1) a été un grand projet et je n'ai joué qu'un petit rôle. Je voudrais remercier tous ceux qui en faisaient partie, en particulier ceux avec qui je travaillais directement : l'équipe maternité et post-sevrage (Daniel Boutin, Yannick Surel, Fabien Guerin, Patrick Touanel) pour le suivi des expérimentations avec les truies et les porcelets, et Agathe Costentin et Christine Trefeu pour les analyses de laboratoire. Merci aussi à Benedicte Lebreton pour son aide et ses idées en tant que l'un de nos co-auteurs dans l'un des articles de la thèse et à Elodie Merlot, Armelle Prunier, Helene Quesnel, Isabelle Louveau pour la mise en place de cette étude. Je voudrais aussi dire merci à Olivier Zemb et à Mathilde Le

Sciellour pour leur aide et leurs conseils dans les analyses statistiques du microbiote de cet essai.

Pour l'essai 2, merci à Eric Bobillier pour son expertise en particulier dans le système de barrière infrarouge. Merci à Sophie Daré et à Raphaël Comte pour ses aides dans les analyses de labo dans cette expérience. Un merci particulier à Colette Mustière pour m'avoir toujours aidé à préparer les veilles des jours d'abattage dans les quatre répétitions, et bien sûr aux techniciens du labo Alinut et à Hauteclaire Furbeyre pour leur coup de main dans ces grosses journées. La quantité d'échantillons et de résultats que nous avons obtenus dans cet essai n'aurait pas été possible sans vous.

Pour l'essai 3, je voudrais dire un grand merci à Cécile Perrier pour sa grande aide dans l'analyse des milliers d'échantillons de cette étude et pour avoir passé plusieurs mois avec moi devant le Koné, le leco, la plaque chauffante, l'ICP, le calorimètre, etc. ! Je voudrais remercier aussi Jean-François Rouaud et Simon Duteil pour la fabrication des régimes expérimentaux, et Xenia Arondel-Piekarz et Raphaël Comte pour leur aide dans les analyses de labo.

Cette partie s'agit peut-être de remerciements pendant de ma thèse mais je tiens également à remercier les personnes avec qui j'ai travaillé pendant mon master car ils font aussi une grande partie de mon séjour à l'INRAE. Ils ont été patients non seulement pour travailler avec moi mais aussi pour m'apprendre le français (la pêche, la patate, et la banane) car je ne parlais pas du tout français quand je suis arrivée à l'institut : merci à l'équipe engraissement (Bruno Duteil, Josselin Delamarre, Patrice Roger), et à Ludovic Brossard.

I would also want to thank friends and colleagues I met in INRAE during my master and PhD: Hieu N., Ngan L.T, Alessandra M., Uffe K., Kirstine P.K, and Mohsen D., who became like family in Rennes to me and Francis even sometimes with only a few months spent together, Alicia Z.F. for all the laughs (and tears?) and for the shared experience of being international grad students in France during a pandemic, to Chloe V.B. and Mariana L.M. for regularly passing by my office to check up on me especially in the last weeks of my deadline, to Anastasia A., Amandine C., Emma G., Emmanuel P., Francine d.Q., Alain V., Eric B. pour les pauses-café et surtout quand je faisais mes essais 3 et 4. Merci aussi à Elise D., Maëva D., Clément G., Jean-Charles A., Angélique C. pour les pauses-café. Je voudrais remercier aussi Nathalie L.F., Aleida S., Jaap V.M., Jean-Claude B., et Francoise T. pour leur gentillesse envers Francis puis envers moi. Special thanks to Demi E., Marcel V., Victor V., Pierre F., and Victor R. for the friendship before/during the PhD, and to my Erasmus girls, Daniela V.S. (thanks for travelling all the way for my defense!) and Nga N. for the long-distance support!

I am forever grateful to my family for the endless support and the video calls that really uplifted the spirit especially during lockdown. Thanks Tay, Mother, mga lolo at mga lola, Angle (special shoutout for making most of the illustrations in this manuscript!), Arielle, ate Karen, Thirdy, Albert, and the whole family! To Francis's family (and now mine too), thank you for your continuous support for the both of us.

And to my ex-boyfriend/husband, Francis Amann, there really is no word that can describe how much I appreciate you. Doing a PhD is difficult enough but doing it alongside your partner can be incredibly challenging at times but, *ça y est*, we did it! You have been my rock and my partner through it all. You also unofficially served as my driver and the quality control of all the cakes that helped me improve my baking skills while doing the PhD lol. Thanks for everything. I love you.

Abstract

Heat stress remains to be one of the major limiting factors in swine production in many countries and this concern will rise in the future especially with increasing global temperature and more frequent heat waves due to warming climate. While decreased growth is mainly related to a reduction in feed intake, other aspects of heat stress in growing pigs have emerged in the recent decades. The thesis thus aims to contribute to the current knowledge with the following objectives: 1) to investigate effect of a chronic prenatal heat challenge on pig's heat tolerance, 2) to confirm direct and indirect effects of heat exposure on pig performance, 3) to evaluate feeding practices that can help pigs cope to a heat challenge, and 4) to explore the idea of maintaining thermal balance as a dynamic process that varies throughout the day. This thesis project has demonstrated that an imbalance between heat production and loss (heat load) not only results in reduced growth but also in altered energy metabolism, physiological responses, and body composition. This later effect is exacerbated in pigs exposed to a chronic prenatal heat challenge. Improved heat tolerance in pigs was found to depend on their ability to maintain dynamic equilibrium between heat production and losses within a day, especially during and after a meal. Our results also confirmed the importance of water intake in improving evaporative heat losses in the ability of pigs to cope with heat stress. Nutritional management practices linked to modified feeding behavior were evaluated. Live yeast supplementation improved insulin sensitivity, increased heat loss efficiency under heat stress, and modified feeding behavior with positive effects on energy efficiency and protein deposition during a heat challenge.

Keywords : heat stress, pigs, energy metabolism, thermoregulation feeding behaviour, live yeast

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Chapter 4

Table

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List of abbreviations

ADFI: average daily feed intake
 ADG: average daily gain
 AHP: activity heat production
 AL: *ad libitum*
 ATP: adenosine triphosphate
 BMR: basal metabolic rate
 BW: body weight
 ECT: evaporative critical temperature
 EM: entire male
 F: female
 FCR: feed conversion ratio
 FHP: fasting heat production
 FW: feeding window
 HL: heat loss
 HP: heat production
 HPA axis: hypothalamic–pituitary–adrenal axis
 HS: heat stress / heat stressed
 LCT: lower critical temperature
 LD: lipid deposition
 LTHA: long-term heat acclimation
 LY: live yeast
 ME: metabolizable energy
 MHP: minimal heat production
 NEFA: non esterified fatty acids
 PCA: principal component analysis
 PD: protein deposition
 PDmax: maximum protein deposition
 PF : pair-fed
 PF2: pair-fed with 2 feeding provisions
 PF8: pair-fed with 8 feeding provisions
 RFI: residual feed intake
 RH: relative humidity
 RHP: resting heat production
 RMR: resting metabolic rate
 STHA: short-term heat acclimation
 TEF: thermic effect of feeding
 TN: thermoneutral
 TNZ: thermoneutral zone
 UCT: upper critical temperature

List of journal publications

1. Serviento, A.M., B. Lebret, and D. Renaudeau. 2020. Chronic prenatal heat stress alters growth, carcass composition, and physiological response of growing pigs subjected to postnatal heat stress. *Journal of Animal Science*. doi:10.1093/jas/skaa161.
2. Serviento, A. M., E. Labussière, M. Castex, and D. Renaudeau. 2020. Effect of heat stress and feeding management on growth performance and physiological responses of finishing pigs. *Journal of Animal Science*. doi: 10.1093/jas/skaa387.
3. Serviento, A. M., M. Castex, D. Renaudeau, and E. Labussière. 2022. Effect of live yeast supplementation and feeding frequency in male finishing pigs subjected to heat stress. *British Journal of Nutrition*. doi:10.1017/S0007114522002513.

List of scientific communications

1. Serviento, A. M., E. Merlot, A. Prunier, H. Quesnel, I. Louveau, B. Lebreton, and D. Renaudeau. 2019. Heat stress in pregnant sows: effects on growth performance and carcass composition of the offspring. In: Energy and protein metabolism and nutrition Vol. 138. Presented at 6. International Symposium on Energy and Protein Metabolism (ISEP), Belo Horizonte, Brazil, September 9-12, 2019. (Oral presentation)
2. Serviento, A.M., E. Labussière, M. Castex, and D. Renaudeau. 2020. Effect of heat stress and of feed provision modification on growth and thermoregulation in pigs. In: Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science. Presented at the EAAP 2020 Virtual meeting, December 1 - 4, 2020. (Oral presentation)
3. Serviento, A.M., D. Renaudeau, and O. Zemb. 2021. Effect of heat stress on fecal microbiota composition of pregnant sows and of their female offspring. In: Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science. Presented at the EAAP 2021 Davos, Switzerland, August 30 – September 3, 2021. (Oral presentation)
4. Serviento, A. M., M. Castex, D. Renaudeau, and E. Labussière. 2022. O164 Effect of live yeast supplementation and feeding frequency in heat stressed pigs. In: Animal - science proceedings Volume 13, Issue 3. Presented at 7. International Symposium on Energy and Protein Metabolism (ISEP), Granada, Spain, September 12-15, 2022. (Oral presentation)

General Introduction

General introduction

Climate change remains to be one of the major challenges of the 21st century especially with more frequent heat waves and with global temperature expected to increase by at least 1.5°C between 2030 and 2050 (IPCC, 2018). This poses a threat to livestock production in many countries due to its consequences on animal health and welfare and on the quantity and quality of resources such as crops used for animal feeding (Rojas-Downing et al., 2017; Lacetera, 2019; Renaudeau and Dourmad, 2021). This is especially true for pig production due to its increasing demand and growth in subtropical and tropical counties (OECD-FAO, 2021).

Like most livestock animals, pigs are homeothermic animals that regulate their core body temperature within a narrow range by maintaining a balance between heat production and heat loss. Pigs are particularly susceptible to heat stress not only because of their inability to sweat (Ingram, 1967) but also because selection for higher feed intake and faster lean growth resulted to higher metabolic heat production in modern pigs (Brown-Brandl et al., 2014). In hot conditions, maintenance of body temperature through physiological, and/or behavioral adjustments usually reduce economically important traits related to reproduction and production (DeShazer et al., 2009; Renaudeau et al., 2012). This makes heat stress one of the main limiting factor in pig production especially with associated economic losses related to reduced growth, feed efficiency, and carcass quality in growing pigs and reduced reproduction performance in sows, among others (Mayorga et al., 2019; Liu et al., 2021). While the most widely reported effect of heat stress in growing pigs is the reduction in feed intake that translates to reduced growth (Renaudeau et al., 2011), studies in the past decade have demonstrated that there are still other consequences to be considered such as direct effects of heat exposure and even prenatal thermal environment of the pigs (Ross et al., 2017; Johnson and Baumgard, 2019; Liu et al., 2021). Indeed, in order to develop nutritional and management strategies to mitigate its stress effects, mechanisms by which pigs maintain heat balance and by which heat stress deteriorates animal performance must be further understood.

Therefore, the general objective of this thesis project is to contribute to the growing body of knowledge on heat stress in pigs. Thus, the thesis aims to determine how a prenatal heat challenge can influence a pig's response to a postnatal heat challenge and to distinguish direct effects of heat exposure (i.e., those that are unrelated to both feed intake and behavior). Moreover, the project also aims to understand how pigs maintain heat balance during the day especially that which related to the feeding behavior. Finally, feeding strategies (i.e., feeding

frequency modification and live yeast supplementation) are evaluated that aims to mitigate heat stress effects in pigs.

This thesis dissertation is divided into six sections. After this **general introduction**, the **literature review** presents how the pigs balances heat production and loss to maintain body temperature, and how failing to maintain this heat balance can compromise pig performance. The main objectives of the thesis are then presented in the conclusion of the literature review. Afterwards, the **general experimental strategies** section discusses the experimental methods employed in the thesis project and the rationale behind them. The **results** are then presented in four chapters according to the four experiments conducted in the context of the thesis. The **general discussion** then takes into account the highlights of the thesis and considers the possible implications of these findings in future research of heat stress in pigs. **Conclusions** and **perspectives** are then presented in the final part of the manuscript.

Literature Review

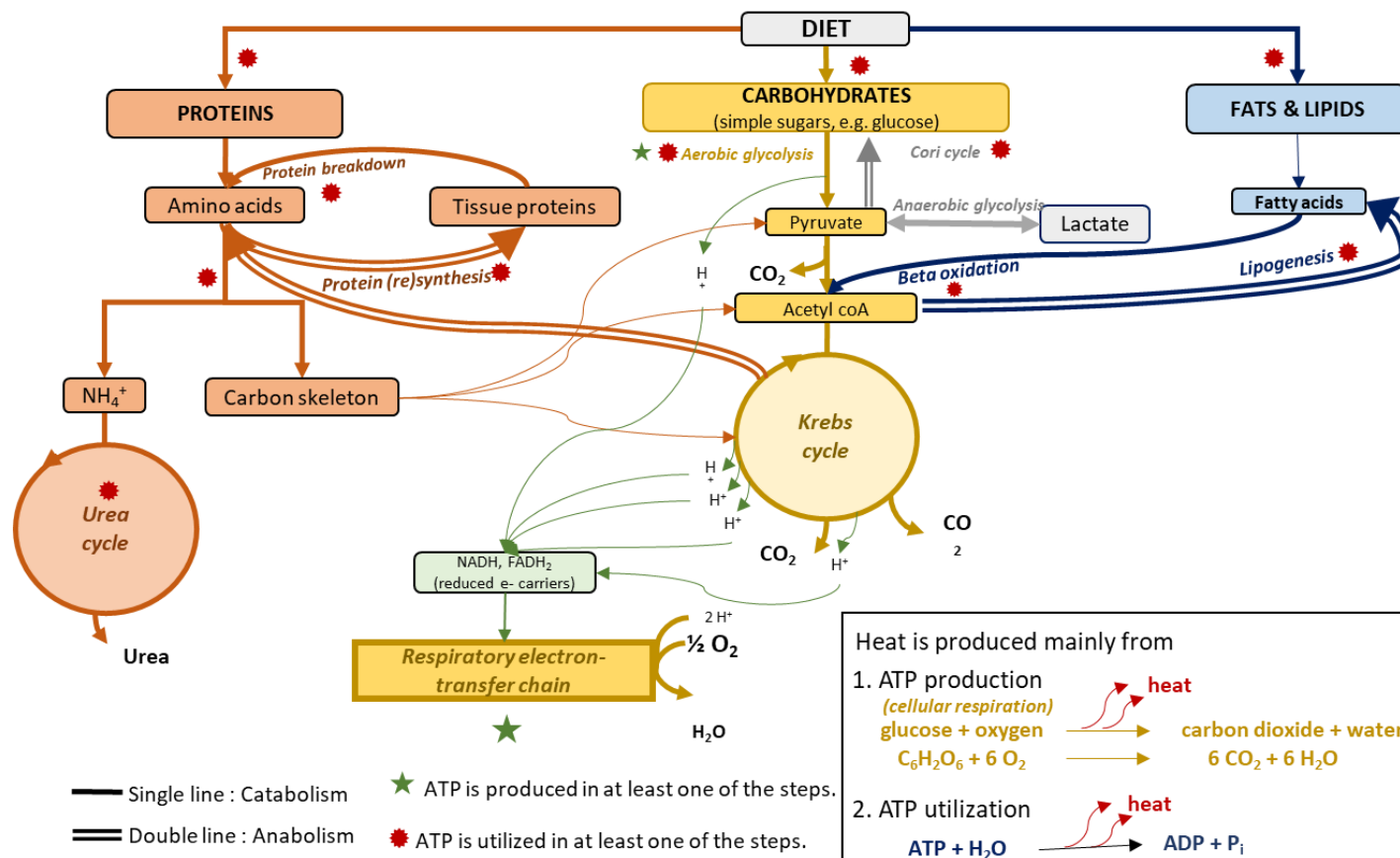


Figure 1. Simplified representation of where heat is generated in animal cells: ATP production and ATP utilization. ATP is the chemical link between catabolism and anabolism and is the energy currency of the living cell. (Adapted from Lehninger et al., 2005)

ATP production in the three steps of cellular respiration (in yellow). 1) Oxidation of fatty acids, glucose, and some amino acids yields acetyl-CoA. 2) Oxidation of acetyl groups in the Krebs cycle includes four steps in which H⁺ ions are abstracted. Stage 3: H⁺ ions carried by NADH and FADH₂ are funneled into a chain of mitochondrial electron carriers—the respiratory chain—ultimately reducing O₂ to H₂O. This electron flow drives the production of ATP. For simplicity's sake, H⁺ ions shown are only those involved in the cellular respiration although these are also generated from other reactions.

ATP utilization or the exergonic conversion of ATP to ADP and Pi, or to AMP and PPi, is coupled to many endergonic reactions and processes.

Literature Review

The pig and its environment

As all living organisms, animals follow the law of thermodynamics and are in constant energy exchange with their environment (Wiegert, 1968; Ingram and Mount, 1975). This section discusses heat production (origin in the animal's body and the different factors affecting it) and exchanges with the environment (with particular focus on heat loss pathways), and how the pig balances them in order to maintain a thermal equilibrium and a core body temperature in a stable range.

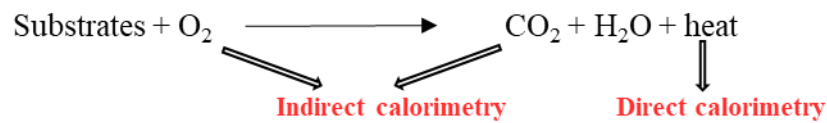
Heat production

In order to grow and reproduce, maintain their structures, and respond to their environment, living organisms need energy provided mostly in the form of adenosine triphosphate (ATP; 30.5 kJ/mole) but also in reduced electron carriers¹. In animals, this energy comes from catabolism of different substrates (Lehninger et al., 2005), e.g. oxidation of nutrients such as carbohydrates, lipids, or proteins. ATP is mostly generated from cellular respiration processes occurring in the mitochondria in the presence of oxygen particularly from oxidative phosphorylation in the electronic transport chain (**Figure 1**). However, this process is only ~40% efficient (Nath, 2016) and the rest of the energy is thus released as heat. A certain amount of heat is therefore generated as a by-product from the inefficiency of energy transformation from oxidation of consumed nutrients to the cellular energy needed by the animals (Lehninger et al., 2005). Catabolism of different substrates for ATP production can also have different thermogenic costs which is particularly higher when protein is used as energy source compared to carbohydrates and lipid. This is not only because of the additional ATP required for amino acid deamination but also for the subsequent urea synthesis and excretion (Bender, 2012; van Milgen, 2021).

Aside from ATP production, heat is also produced when ATP is hydrolyzed to provide energy for vital functions such as muscle contraction (Barclay, 2015). Deposition of nutrients needed for production and reproduction purposes also requires energy. For example, lipid deposition requires the expenditure of one mole of ATP and two moles of NADPH from the

¹ Nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), and flavin adenine dinucleotide (FADH₂)

Text box 1. Principle of the indirect calorimetry technique based on the Brouwer formula (1965)



In direct calorimetry, heat released by an organism is directly quantified from temperature changes; while in indirect calorimetry, heat is calculated from gaseous exchanges. In livestock animals, indirect calorimetry studies uses the Brouwer formula (1965) that considers measurements of the volumes of O_2 , CO_2 , and heat released from oxidation of substrates as summarized in subsequent table:

Substance	O_2 consumed, L/g	CO_2 produced, L/g	Heat produced, kJ/g	RQ
Starch	0.829	0.829	17.57	1.00
Fat	2.013	1.431	39.75	0.71
Protein	0.957	0.774	18.41	0.81

RQ: respiratory quotient ($\text{V CO}_2 / \text{V O}_2$)

Heat production (HP, kJ), and oxidation of carbohydrates and fat were then expressed as a function of the VO_2 (L), VCO_2 (L), and the oxidized protein (P, g; nitrogen in urine $\times 6.25$) resulting to the following equations:

- 1) $\text{HP} = 16.175 \text{ VO}_2 + 5.021 \text{ VCO}_2 - 0.958 \text{ P}$
- 2) Carbohydrate oxidation (g) = $-2.965 \text{ VO}_2 + 4.170 \text{ VCO}_2 - 0.390 \text{ P}$
- 3) Fat oxidation (g) = $1.718 \text{ VO}_2 + 1.718 \text{ VCO}_2 - 0.315 \text{ P}$

In order to correct for the heat lost from anaerobic fermentation, a correction factor proportional to the volume of methane (L) produced in equation 1 was included in the final equation:

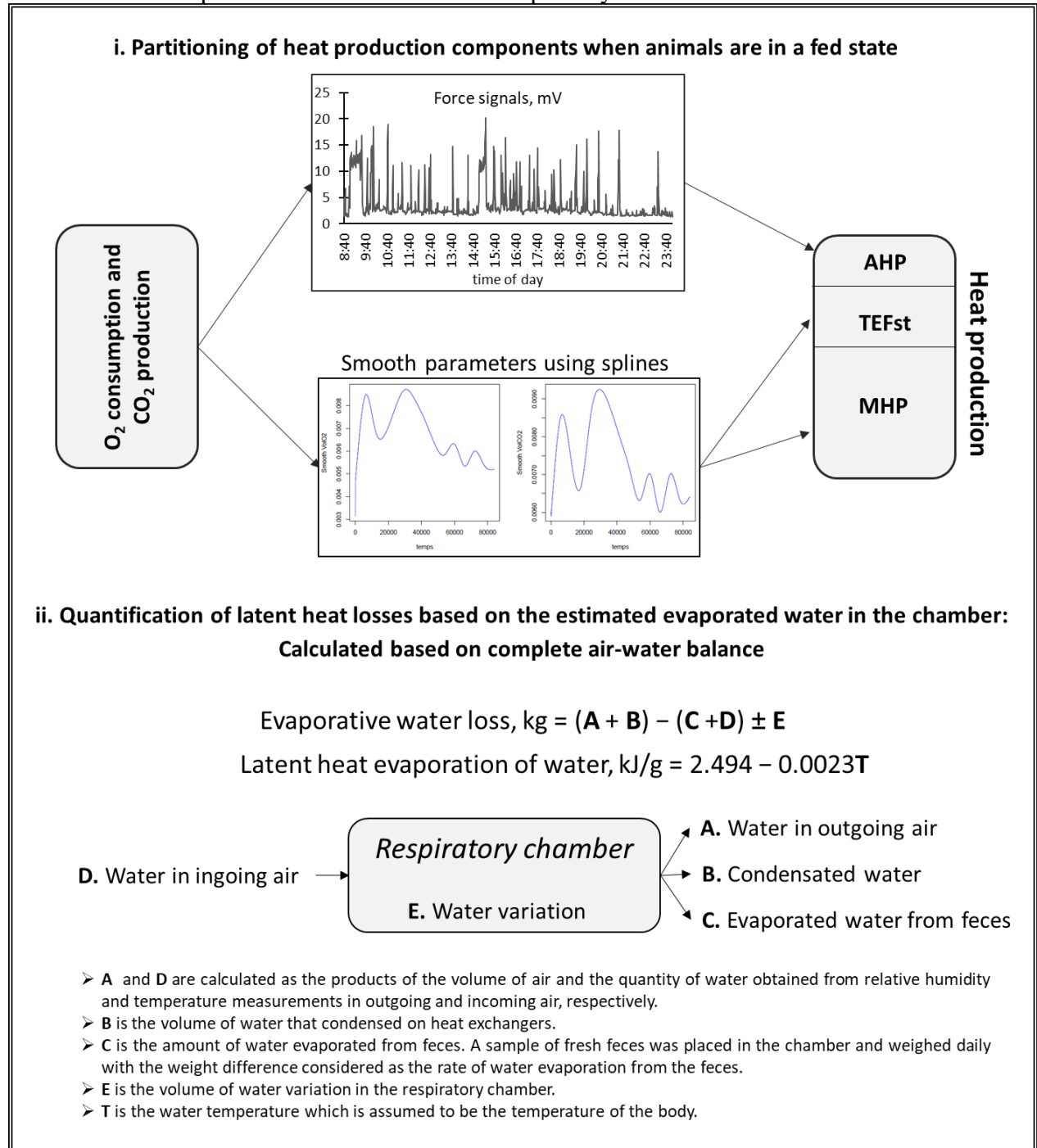
$$4) \text{HP} = 16.175 \text{ VO}_2 + 5.021 \text{ VCO}_2 - 0.958 \text{ P} - 2.167 \text{ VCH}_4$$

pentose phosphate pathway² for the addition of two-carbon units in the hydrocarbon chain for fatty acid synthesis (Cooper, 2000). Meanwhile, protein synthesis is a much more energetically costly process as it can take at least 5 moles of ATP only for the incorporation of one amino acid into protein (Stouthamer, 1973), not even taking account the cost of protein turnover which is the continuous renewal and replacement of protein in the body (Schutz, 2011). Pigs like most mammals have evolved to be homeothermic animals, characterized by their ability to regulate their core body temperature in a narrow range (which is generally higher than the environment, i.e. from 37-39°C in swine depending on the physiological stage) through complex neural controls and mechanisms (Bligh, 1998; Morrison and Nakamura, 2019). However, maintaining a constant and elevated internal temperature is energetically expensive and requires large amounts of heat (Bennet, 1988; Levesque and Lovegrove, 2014).

From the methodological point of view, one method for assessing energy expenditure is the direct calorimetry technique (Lavoisier and Laplace, 1783). This method is considered the gold standard for heat production (**HP**) measurement as it allows direct quantification of heat produced by the organism (whether coming from aerobic and anaerobic metabolism) via the temperature gradient between the body and the environment (Blaxter, 1967; Kaiyala and Ramsay, 2011; Kenny et al., 2017). However, direct calorimetry method requires expensive facilities and does not give an idea about the substrate being oxidized (Johnson and Coward-McKenzie, 2001). In livestock animals, rodents, and humans, a well-known alternative technique for HP measurement is the indirect calorimetry method which allows for continuous HP calculation via gas exchanges of animals placed in respiration chambers (O₂ consumption, and CO₂ and CH₄ production; **Text box 1**). Water evaporation at the level of the lungs and skin can also be considered in these methods which is important since the change of water from liquid to gaseous state consumes about 44.0 kJ/mole or 2.4 kJ/g of water (Lehninger et al., 2005). This is in contrast to the comparative slaughter technique that can only measure retained energy in the body over a rather long duration (Blaxter, 1967). The indirect calorimetry technique has some limitations such as the assumption that O₂ and CO₂ pools in the body does not change although some intermediates might be temporarily stored in the body (Burnett and Grobe, 2013; Gerrits et al., 2015). Nevertheless, this method is widely used and has been

² The pentose phosphate pathway branches out from glucose 6-phosphate (G6P) to produce sugars for nucleic acid synthesis and NADPH that provides reducing power for the synthesis of fatty acids, sterols, nucleotides, and non-essential amino acids. In contrast to glycolysis, it does not require ATP (Ge et al., 2020).

Text box 2. Examples of measurements in the respiratory chamber.



- Principle of the partitioning of total heat production from kinetics of O₂ consumption and CO₂ production as described by van Milgen et al. (1997) with modifications described by Quemeneur et al. (2020). Example shown for the force signals and smooth parameters using spline comes from the data of a pig with 2 feeding windows.
- Principle of the calculation of evaporative water loss described by Renaudeau et al. (2013) and of latent heat as the required amount of heat to evaporate 1 g of liquid water based on the equation of Curtis (1983).

reported to be a close match with other techniques (Burnett and Grobe, 2013; Gerrits et al., 2015).

Indirect calorimetry studies in growing pigs has demonstrated that HP is not constant during the day (Verstegen et al., 1986; van Milgen et al., 1997; van Milgen et al., 2001; Schrama et al., 2003; Bolhuis et al., 2008; Labussière et al., 2013; Brown-Brandl et al., 2014; Pedersen et al., 2014; van Erp et al., 2020; and Quemeneur et al., 2020 among others). The amount of heat produced differs according to the metabolic processes involved and mathematical modelling methods have thus been used to understand the dynamic changes in HP and its different components (van Milgen et al., 1997; Van Klinken et al., 2012; van Klinken et al., 2013; Labussière et al., 2013; Quemeneur et al., 2020). **Text box 2** shows the principle of total HP partitioning from the kinetics of O₂ consumption and CO₂ production. Regardless of the mathematical method used, total HP is generally partitioned in the aforementioned studies into different components (example shown in **Figure 2**): fasting HP (**FHP**), thermic effect of feeding (**TEF**), and activity related HP (**AHP**).

Fasting HP can be used to evaluate an animal's basal metabolic rate (**BMR**) and estimate the corresponding energy requirements for maintenance of vital body processes. Based on the concept of BMR (Du Bois, 1916; Lusk and Du Bois, 1924), it is assumed to be the minimum energy requirement for vital organs such as the heart, lungs, liver, kidneys, intestine, nervous system, sex organs, muscles, and skin and is measured in adult, inactive, and non-productive individual who is in a post-absorptive fasting state in a thermoneutral (**TN**) environment (McNab, 1997; Kumagai and Yahagi, 2013). The FHP is generally expressed according to metabolic body weight (**BW**) with an exponent that varies according to the age, i.e. $BW^{0.75}$ for sows and $BW^{0.60}$ for growing pigs (Kleiber, 1961; Brown and Mount, 1982; Noblet et al., 1999). As previously mentioned, BMR is generally measured in adults and can be difficult to estimate in growing pigs as they can still gain weight and deposit protein even with no positive energy retention as a result of fat mobilization (Le Dividich et al., 1980). Nevertheless, the concept of maintenance requirements has been accepted even in growing animals to estimate energy needs wherein there is zero net energy gained or lost and no change in body pools (van Milgen and Noblet, 2003).

The other two components (AHP and TEF) are collectively referred to as the heat increment in pig nutrition (Noblet et al., 2010; Labussière et al., 2015). Heat from physical activity (AHP) comes from the discussed ATP hydrolysis during muscle contraction (Loiselle

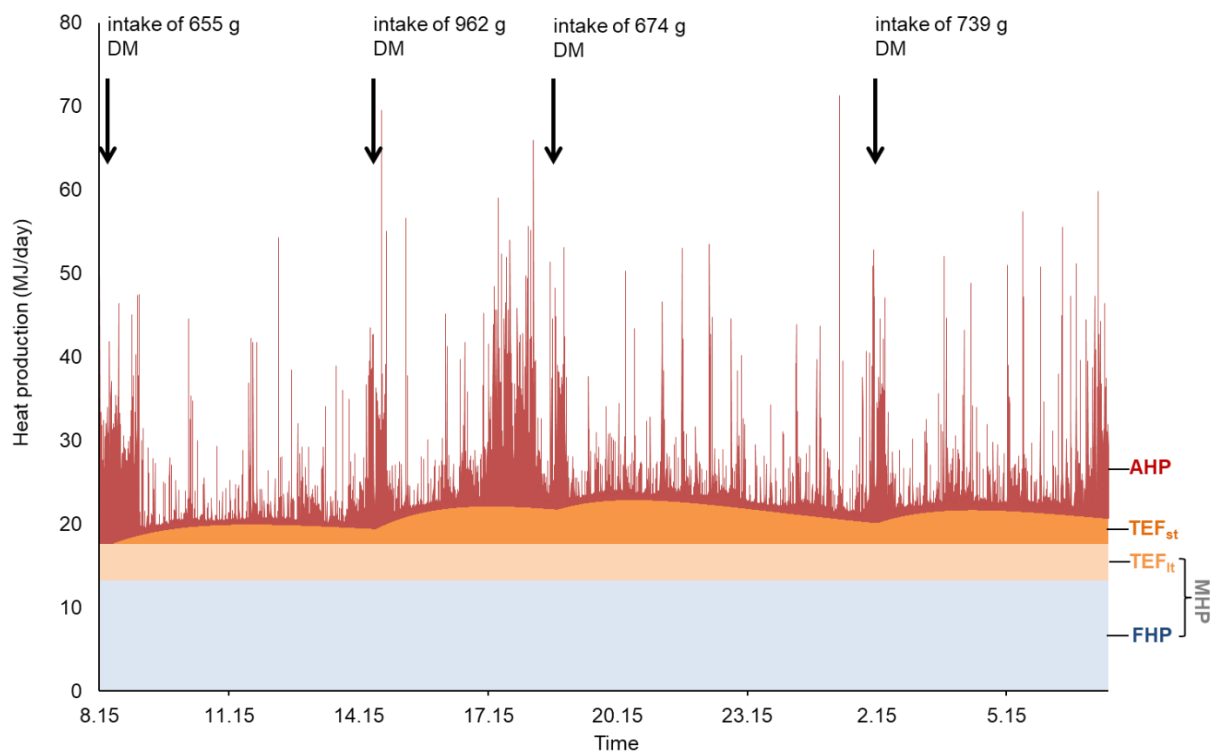


Figure 2. Example of heat production partitioning between components due to the basal metabolic rate (fasting heat production; FHP), the short- and long-term thermic effect of feeding (TEF_{st} and TEF_{lt}), and the physical activity (AHP). MHP (or RHP; minimal or resting HP) is the sum of the FHP and the TEF_{lt}. (Adapted from Labussière et al., 2013).

et al., 2015) e.g. when animals move, stand up, walk, or eat. As shown in **Text box 2**, the changes in O₂ consumption and CO₂ production used for calculating AHP are assumed to be proportional to a measurement of physical activity (e.g. electrical signals from force sensors) with different parameters for O₂ and CO₂ (Labussière et al., 2013). The last component of total HP is the TEF which is also referred to as dietary induced thermogenesis and is commonly split into components, i.e. the short-term and the long-term TEF (Rothwell and Stock, 1981; van Milgen and Noblet, 2003; Armbruszt and Garami, 2014; Calcagno et al., 2019). In modelling energy partitioning, short-term TEF is assumed to represent the dynamic variations in HP after meal ingestion that are related to the act of ingestion, the digestion and absorption of food, and a part of intermediary metabolism (Labussière et al., 2015). Meanwhile, long-term TEF is generally associated with nutrient deposition and hindgut fermentation (van Milgen and Noblet, 2003; Labussière et al., 2015). While BMR is generally estimated after one day of fasting (FHP), there is also the so-called resting metabolism rate (**RMR**) that is similar to the BMR except that it is measured in non-fasted individuals that is not in post-absorptive state. The RMR is thus the BMR plus the heat increment from a previous feeding activity (Brody and Lardy, 1946). The resting (**RHP**) or minimal HP (**MHP**) can be considered as the RMR of the pigs measured during a fed state and is therefore calculated as the sum of the FHP and the long-term TEF (Labussière et al., 2015). In estimating energy partitioning, the basal metabolism is generally assumed to be stable and the observed variations in HP during the day are assumed to be associated to the instantaneous peaks of AHP and the short-term TEF (van Milgen et al., 1997; Labussière et al., 2013; Quemeneur et al., 2020).

While FHP and MHP are usually reported as singular values, it must be noted that they only serve as estimates especially since BMR and RMR varies throughout the day and has been reported to follow a circadian rhythm in pigs and in other mammalian species (Benedict, 1915; Ingram and Dauncey, 1985; Nagashima et al., 2003; Mortola, 2007; Bailey et al., 2014; McHill and Wright Jr, 2017). Indeed, RMR is reported to be higher in the morning than at night which is associated to the endogenous circadian effect on energy expenditure (Romon et al., 1993; Bo et al., 2015; Morris et al., 2015; Richter et al., 2020; Ruddick-Collins et al., 2022). Thermal demand was also found to be lower in pigs at night than during the day (Verstegen et al., 1986) suggesting a diurnal difference in metabolic rate. Nevertheless, mathematical modeling of the dynamic variations in HP helps understand the factors that contribute and that affect the total HP, examples of which are shown in **Table 1**.

Table 1. Examples of factors that affect energy partitioning of growing pigs such as previous feeding level, sex, diet composition, and high ambient temperature.¹

Factor	Category	Ave. BW, kg	Heat production							Heat production					
			ME intake	FHP	TEF _{lt}	TEF _{st}	TEF _{total}	AHP	RE	FHP	TEF _{lt}	TEF _{st}	TEF _{total}	AHP	RE
			(kJ kg BW ^{-0.60} ·d ⁻¹)							%total heat production					
Previous FL ²	60% FL	68.3	1552	609	133	127	260	188	491	57	13	12	25	18	32
	80% FL	69.3	2053	644	202	195	398	185	832	53	17	16	33	15	41
	100% FL	74.6	2543	729	226	267	493	195	1137	52	16	19	35	14	45
	<i>P</i>		<0.01	<0.01	<0.01	<0.01	<0.01	0.75	<0.01	-	-	-	-	-	-
Sex ³	Entire male	114.0	2396	856	-	-	315	218	1020	62	-	-	23	16	43
	SC male	111.0	2632	735	-	-	464	212	1216	52	-	-	33	15	46
	IC male	120.1	2864	783	-	-	484	250	1346	52	-	-	32	16	47
	<i>P</i>		<0.01	<0.01	-	-	<0.01	0.22	<0.01	-	-	-	-	-	-
Diet ⁴	Diet 1	56.5	1701	734	85	107	192	226	549	64	7	9	17	20	32
	Diet 2	60.1	2600	789	135	210	345	193	1272	59	10	16	26	15	49
	Diet 3	60.9	2542	774	173	273	446	204	1117	54	12	19	31	14	44
	Diet 4	61.9	2512	778	198	243	441	179	1114	56	14	17	32	13	44
	Diet 5	59.2	2505	754	157	152	309	206	1235	59	12	12	24	16	49
	<i>P</i>		<0.01	0.64	<0.01	<0.01	<0.01	0.50	<0.01	-	-	-	-	-	-
High ambient temperature ⁵	23-AL	26.1	2524	808	-	-	431	214	1070	56	-	-	30	15	43
	23-PF	24.9	1949	755	-	-	259	258	677	59	-	-	20	20	46
	33-AL	25.2	1948	642	-	-	263	262	781	55	-	-	23	22	47
	<i>P</i>		<0.01	<0.01	-	-	<0.01	-	<0.01	-	-	-	-	-	-

¹BW^{0.60}: metabolic body weight; ME: metabolizable energy; FHP: fasting heat production; TEF: thermic effect of feeding; AHP: activity heat production; RE: retained energy; FL: feeding level; SC: surgically castrated; IC: immunocastrated males

²68-75 kg male growing pigs were fed at 60, 80, or 100% of their estimated *ad libitum* feed intake level (at 1.56, 2.08, and 2.60 MJ ME kg BW^{-0.60}·d⁻¹, respectively (de Lange et al., 2006).

³114-120 kg male growing pigs were kept as entire males, were surgically castrated, or were immunocastrated (Labussière et al., 2013).

⁴56-61 kg male growing pigs were fed a basal diet limiting in lysine supply at 1.7 MJ ME kg BW^{-0.60}·d⁻¹ (Diet 1) or one of the four other diets at 2.55 MJ ME kg BW^{-0.60}·d⁻¹: the basal diet and a dietary supplement that consisted of starch (Diet 2), of starch and corn gluten meal (Diet 3), of starch and casein (Diet 4), or of starch and lipid (Diet 5) (van Milgen et al., 2001).⁴25-26 kg castrated male pigs were reared at 23°C and fed *ad libitum* (23-AL), at 33°C and fed *ad libitum* (33-AL:), and at 23°C and pair fed to the level of 33-AL pigs (23-PF).

In pigs, FHP generally represents 50 to 65% of the total HP depending on different factors. For example, FHP can increase with a higher previous feeding level and plane of nutrition (Koong et al., 1983; de Lange et al., 2006) due to the weight of metabolically active organs (Koong et al., 1983; Koong et al., 1985; Pekas and Wray, 1991) as these are vital organs needed to sustain life. A higher FHP in entire males compared to surgically castrated males (Labussière et al., 2013) has been associated, among other factors, to a higher visceral weight (Quiniou and Noblet, 1995) as visceral organs largely contribute to FHP (van Milgen et al., 1998) and can constitute up to 40-50% of animal energy expenditure (Ferrell, 1988). Leaner pigs can have higher FHP due to the contribution of muscle energy expenditure (Tess et al., 1984; van Milgen et al., 1998) which might be related to the previously discussed energy cost of protein turnover. The genetic selection for higher feed intake and lean growth can thus be related to the higher FHP reported observed in modern and leaner breeds (Brown-Brandl et al., 2004; Renaudeau et al., 2006a; Barea et al., 2010). Environmental conditions can also influence the FHP and RHP with lower values when pigs are exposed to high ambient temperature (Collin et al., 2001b; Quiniou et al., 2001a; Campos et al., 2014a) that is highly related to the reduction of the feeding level (Labussière et al., 2016). Immune challenge can also increase the RHP of pigs and it can be linked to the energetic cost of inflammatory and immune responses (Campos et al., 2014a).

Most of the observed diurnal changes in HP are related to the physical activity (AHP) and TEF (Labussière et al., 2013; Pedersen et al., 2014) which comprises about 10 to 20% and 20 to 25% of HP, respectively. In pigs, AHP is closely associated to the feeding activity (Noblet et al., 1993; Labussière et al., 2013; Pedersen et al., 2014) as a result of the physical activity from standing up and walking to the feeder (van Milgen et al., 1998). AHP can constitute up to 18-19% of HP when pigs have >10 meals/day compared to only 13-14% when they have ~7 meals/day (Quemeneur et al., 2020). AHP also increases with low ambient temperature from 17 to 23% in 22°C and 12°C, respectively (Quiniou et al., 2001b), related to the increased feeding activity and possibly shivering in cold conditions to maintain homeothermic body temperature. As the measure of AHP relies on signals of force sensors, AHP in high ambient temperature can increase despite the lower physical activity because of increased panting (Quiniou et al., 2001b). However, AHP proportion can also vary due to housing conditions which is slightly higher in individually housed pigs (~17% of HP) possibly due to less comfortable conditions compared to group housed pigs (~13% of HP) (Quiniou et al., 2001b).

Text box 3. The heat exchange between the pig and its environment via the sensible (convection, radiation, and conduction) and the latent (evaporation) routes.

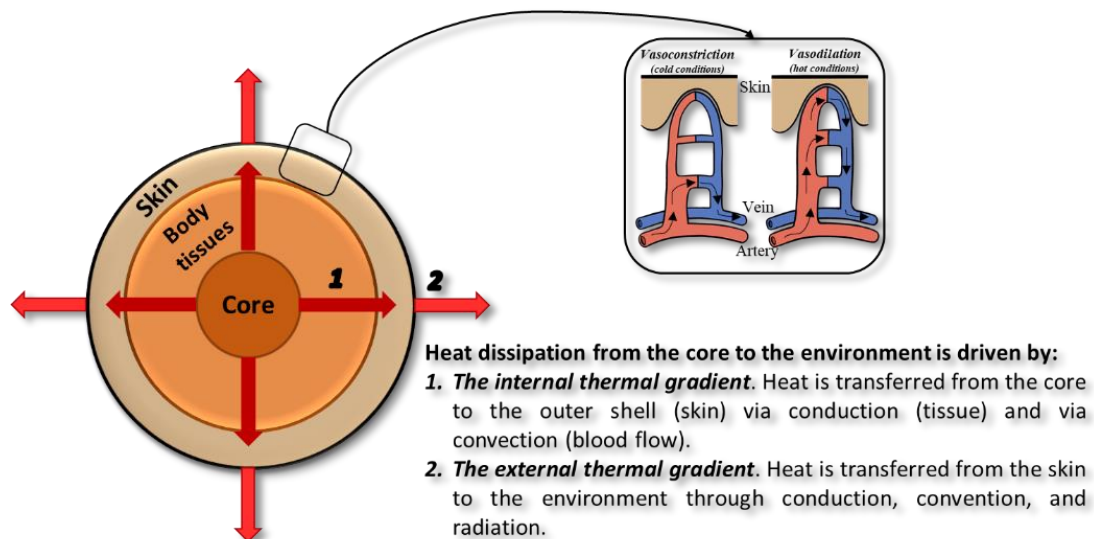
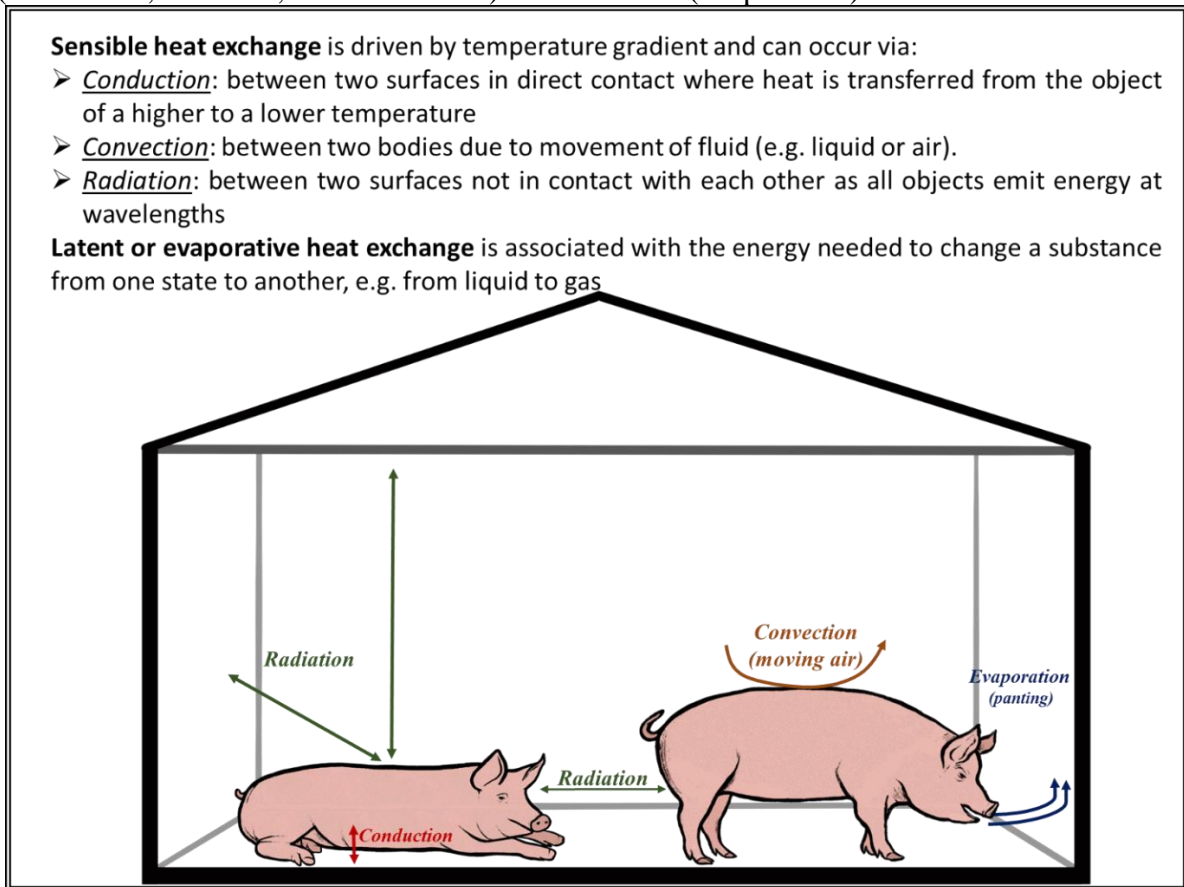


Figure 3. The core and shell model for heat transfer driven by (1) the internal and (2) the external thermal gradient.

Meanwhile, TEF, as suggested by its name, is highly influenced by the diet and the utilization of nutrients. As seen in **Table 1**, dietary nutrients have different metabolizable energy (**ME**) utilization efficiencies³. For example, starch and lipid utilization have relatively high ME efficiency at 0.842 and 0.883, respectively, but protein utilization has only 0.520 (van Milgen et al., 2001) because of the high heat increment associated to the previously mentioned energetic cost of deamination process and of urea synthesis and excretion. Heat increment related to TEF can also be related to nutrient deposition and maintenance wherein lipid deposition (**LD**) is more energy efficient than protein deposition (**PD**) at 0.84 vs 0.62, respectively (Noblet et al., 1999) which is linked to the discussed energetically costly process of protein turnover [breakdown and (re)synthesis].

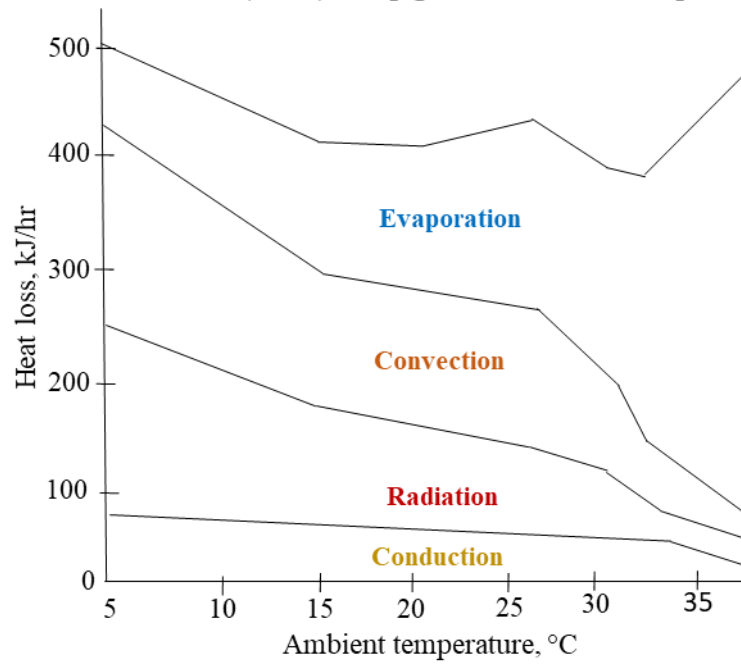
Heat loss

Following the principles of thermodynamics, there is a constant heat exchange within the animal, and between the animal and its surroundings that is driven by the temperature or thermal gradient (Wiegert, 1968; Curtis, 1983; DeShazer et al., 2013; Taylor et al., 2014; Boldrini et al., 2018). Heat exchanges can be achieved by sensible routes (i.e. conduction, convection, and radiation) which highly relies on temperature gradient, and the latent route which is mainly done by water evaporation (Curtis, 1983; DeShazer et al., 2013; Collier and Gebremedhin, 2015) (**Text box 3**). Although animals can still gain heat from the environment when environmental temperature exceeds core body temperature, this subsection focuses mainly on the heat losses (**HL**) of the animal to the environment (NRC, 1981; Curtis, 1983; McCafferty et al., 2017).

As most of the heat produced by the animal comes from its core where most of the vital and visceral organs are located, heat transfer is 1) from the core to the skin (internal thermal gradient) and 2) then from the skin to the environment (external thermal gradient) (Precht et al., 1973; Curtis, 1983; Speakman and Krol, 2010) (**Figure 3**). Within the body, heat is transferred via conduction between the tissues, the skin, and the external insulation (e.g. fur or feathers), and via convection through the blood flow in the arterial-venous system as blood carries heat to the skin surface and brings “cooled” blood to the core (Stolwijk and Hardy, 1966; Precht et al., 1973; Charkoudian, 2010; Witzman, 2012; McCafferty et al., 2017; Boldrini

³Metabolizable energy (ME) utilization efficiency is a function of the net energy (NE) which is the difference between the ME and the heat increment. ME utilization efficiency is calculated as ME/NE.

a. Heat loss (kJ/hr) of a pig at different air temperatures



b. Heat loss (%) of a pig at different air temperatures

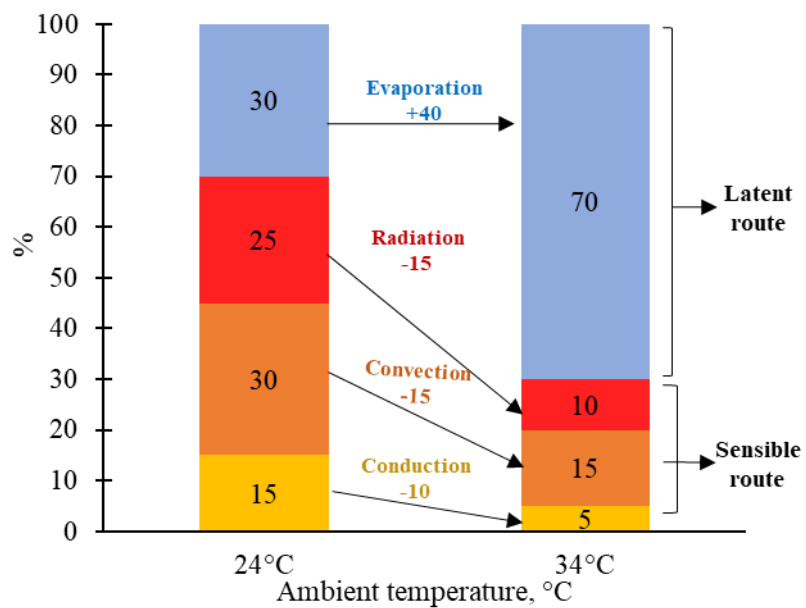


Figure 4. Proportion of heat loss via the sensible route decreases and via the latent route increases with increasing ambient temperature. Adapted from a) Bond et al. (1952) and b) Renaudeau (unpublished results).

et al., 2018). Thus, heat transfer from core to skin depends on the conductivity of the tissues and the skin blood flow rate (Stolwijk and Hardy, 1966; Huang et al., 2021).

From the animal's surface, heat can be dissipated to the environment by the sensible route (**Text box 3**). Heat exchanges can be achieved by conduction such as between the body surface to the floor when the pig is lying down, by convection which is highly influenced by the surrounding air temperature and velocity, or by radiation between the animal and the walls or ceilings, or even between animals (Precht et al., 1973; Curtis, 1983; DeShazer et al., 2013). Since conventional pigs are relatively hairless (or have very fine hair) (Ingram, 1965; Mota-Rojas et al., 2021), the skin acts as the main boundary between the pig's body and its surroundings and thus serves as the site for heat exchange. As already mentioned, sensible routes principally rely on the temperature gradient of the body surface and the environment thus the pig's capacity for sensible HL decreases when ambient temperature increase, driving pigs to rely more on the latent pathway to lose heat (Bond et al., 1952; Whittow, 1971; National Research Council (NRC), 1981; Curtis, 1983; Pedersen et al., 1998; Huynh et al., 2005a; Collier and Gebremedhin, 2015) (**Figure 4**).

Animals can generally lose heat by the latent evaporative pathway through the skin by sweating and wallowing, and through the respiratory tract by panting (Collier and Gebremedhin, 2015). However, pigs not only have fewer sweat glands than other livestock species (30/cm² compared to 800 to 2000/cm² in cattle and 250/cm² in sheep) (Renaudeau et al., 2012) but these sweat glands are also non-functional (Ingram, 1967). Thus, heat is lost through the evaporative route mainly by panting and wallowing in pigs (Ingram, 1965; Morrison and Mount, 1971; Huynh et al., 2007). Whereas sensible HL is limited by temperature gradient, the latent route can be influenced by both the ambient temperature and the relative humidity (**RH**) since high RH slows down the evaporation rate (Huynh et al., 2005b). However, it has been suggested that HL efficiency in growing pigs by evaporation may not be limited until an ambient temperature above 30°C and at 90% RH (Renaudeau, 2005). Indeed, the effect of humidity on HL is more apparent under higher air temperatures (as shown by Fialho et al., 2004) (**Figure 5**) possibly due to the higher reliance on the evaporative HL pathway when the thermal gradient is decreased.

There are fewer studies on HL compared to HP (Pedersen and Sällvik, 2002). Along with ambient temperature, skin and rectal temperatures are usually used to quantify internal and external thermal gradients (Renaudeau et al., 2012; Taylor et al., 2014) but there can be a

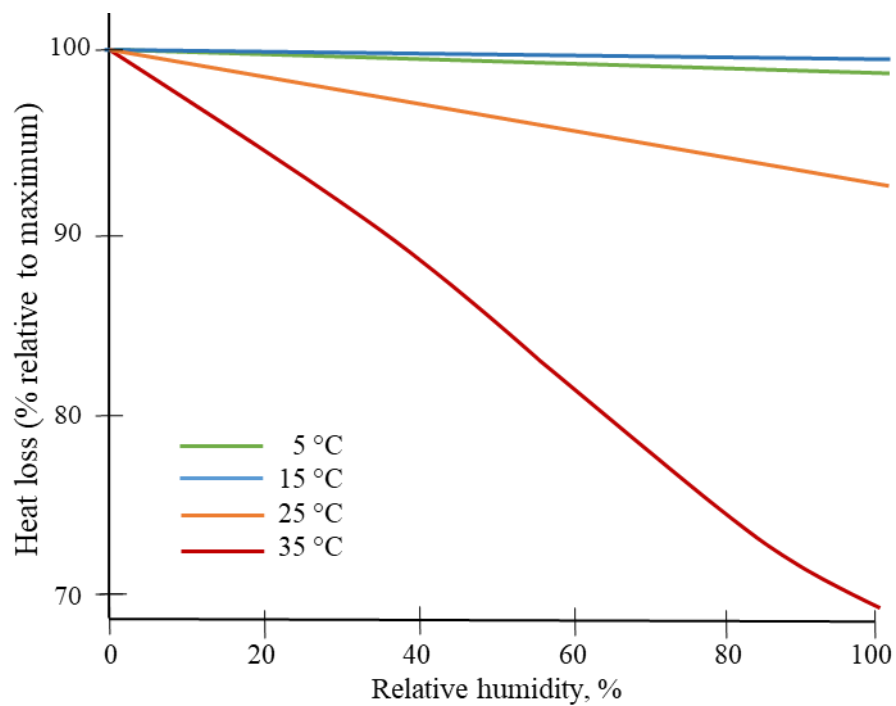


Figure 5. Relative heat loss modeled as a function of relative humidity at different ambient temperatures. (Adapted from Fialho et al., 2004).

delay in the responses between the two temperatures (Huynh et al., 2005b). Furthermore, skin temperature varies depending on body part where it is measured and depending on the position of the pig (Warriss et al., 2006; Mortola, 2007; Magnani et al., 2011; Soerensen and Pedersen, 2015; Teixeira et al., 2020; Stukelj et al., 2022). Meanwhile, respiration rate is usually associated to a higher evaporative water loss (Kamada and Notsuki, 1987) but direct quantification of evaporative HL can be difficult. Mathematical models that aim to estimate the partition between sensible and latent HL according to the ambient temperature have been published by the CGIR⁴ group in different livestock animals (Pedersen and Sällvik, 2002). Alternatively, latent HL can be measured directly in respiratory chambers (**Text box 2**). By using this method, sensible HL is calculated by difference between total HP and latent HL (Kamada and Notsuki, 1987; Huynh et al., 2005b; Huynh et al., 2007; Renaudeau et al., 2013).

⁴ International Commission of Agricultural and Biosystems Engineering (CIGR).

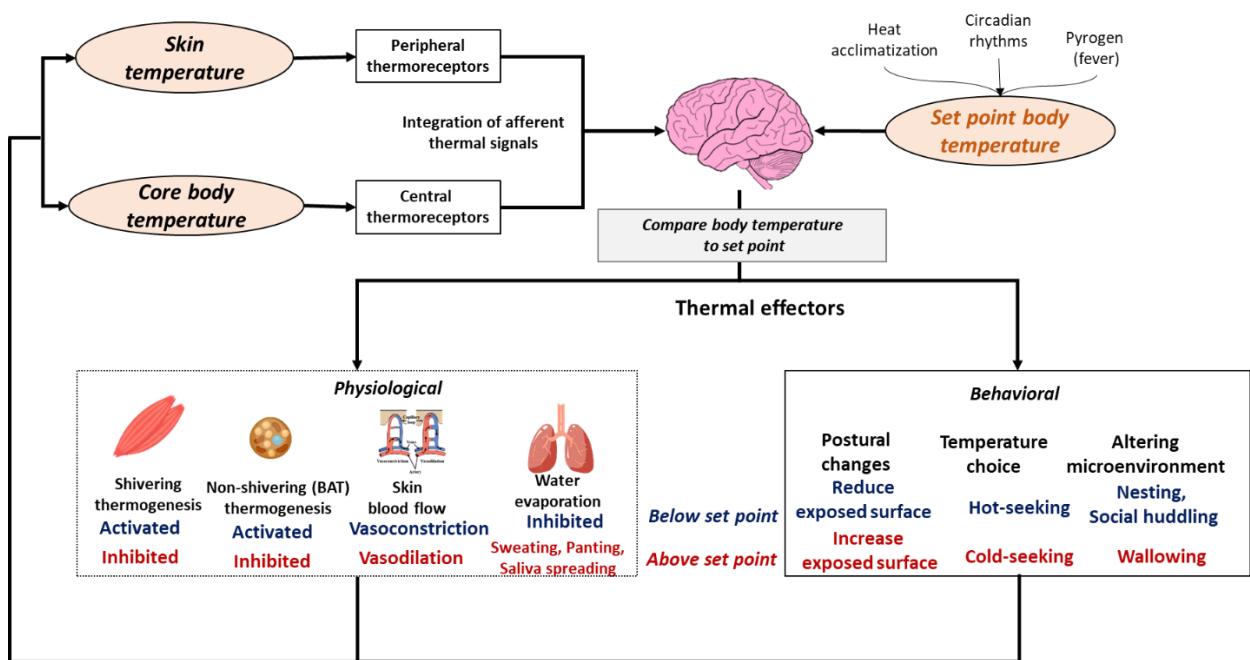


Figure 6. Model of thermoregulation as a negative feedback system for maintaining a homeothermic body temperature. If there is a deviation sensed in the body temperature, the system makes alterations to restore it toward its set point value. BAT: brown adipose tissue. (Adapted from Sawka and Wenger, 1988; Weller, 2005; Modell et al., 2015; and Fossion et al., 2018; Tan and Knight, 2018).

Finding the equilibrium of heat production and losses

$$\text{Heat storage} = \text{metabolic HP} \pm \text{sensible heat exchange}^5 - \text{evaporative heat loss}$$

Equation 1

The concept of homeostasis is based on the ability of an animal (or any living organism) to maintain a stable state of its internal environment through self-regulating processes (Cannon, 1932). As defined in **Equation 1**, core body temperature is indicative of the balance (or imbalance) of HP and HL and it is kept within narrow limits when the net body heat storage (or heat load) is near zero (Weller, 2005; Tansey and Johnson, 2015). In a homeostatic system, there is a *regulated variable*⁶ (i.e., core body temperature) that has to be maintained within a certain range (i.e., set point or threshold) through different *controlled variables* (i.e., thermoregulation responses) that can be altered when the feedback system senses deviation from the specified set point (Kotas and Medzhitov, 2015; Modell et al., 2015; Fossion et al., 2018). Thermoregulation mechanisms are controlled by the hypothalamus (Campbell, 2008) based on the information sent by the central and peripheral thermoreceptors.⁷ When a deviation from the set point is sensed, physiological and behavioral thermal effectors are employed to maintain the core body temperature (Hellon, 1972; Morrison and Nakamura, 2011; Tansey and Johnson, 2015; Tan and Knight, 2018; Morrison and Nakamura, 2019) (**Figure 6**). There are four most important physiological effectors in mammals which are involuntary responses that either generate or dissipate heat: *i.* the shivering and non-shivering thermogenesis which are activated in cold conditions, *ii.* Cardiovascular adjustments via the skin blood flow wherein there is increased vasoconstriction to conserve heat in cold conditions and vasodilation to increase blood flow to the peripheral tissues in order to maximize HL in hot conditions, and *iii.* An increased water evaporation to increase evaporative HL which can include sweating and salivary spreading in other mammals but, as discussed, is mainly via panting in pigs (Ingram, 1965; Charkoudian, 2010; González-Alonso, 2012; Tan and Knight, 2018).

⁵ Sum of all conductive, convective, and radiation heat exchanges.

⁶This can pertain to different variables such as body temperature, blood glucose, salt and water balance, blood pressure, pH concentration, among others.

⁷ Central thermoreceptors are found in the hypothalamus, spinal cord, viscera, and great veins while peripheral receptors are found in the skin.

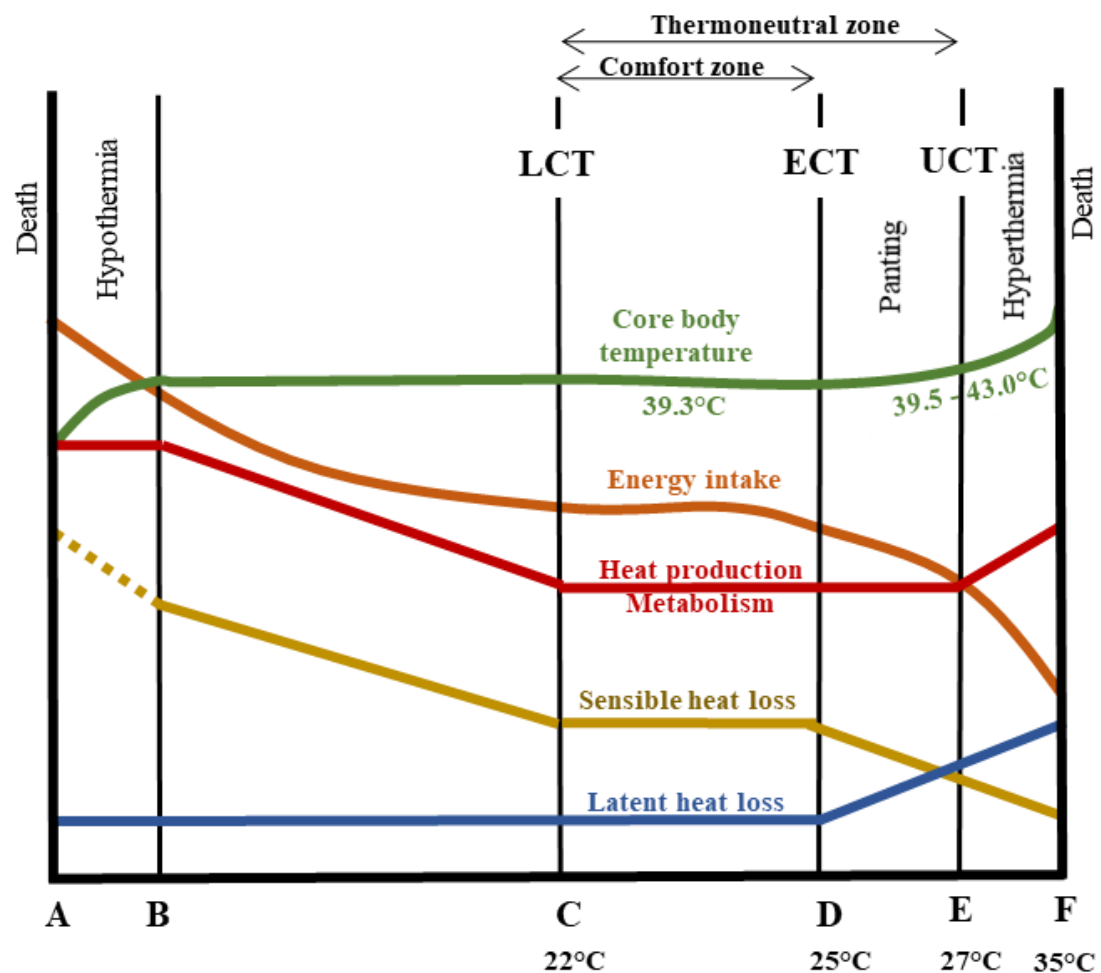


Figure 7. General relationship of the core body temperature, heat production, and heat losses according to the ambient temperature in growing pigs. LCT: Lower critical temperature, ECT: evaporative critical temperature, UCT: upper critical temperature. Adapted from Mount (1979), Renaudeau et al. (2004), Renaudeau et al. (2012), and Gourdine et al. (2021).

General static view of the balance between heat production and heat losses

Ambient temperature highly influences heat exchanges of the animal with its surroundings. The animal must have a higher body temperature than the environment to be able to lose heat, otherwise heat storage will constantly be positive and body temperature will continuously increase which can lead to hyperthermia ($>42^{\circ}\text{C}$ body temperature). On the other hand, when ambient temperature is too low, the thermal gradient between the body surface may be too high and the animal may lose more heat than it produces leading to hypothermia ($<34^{\circ}\text{C}$ body temperature) wherein metabolic processes are slowed down further impairing the animal's ability to produce heat and thermoregulate (Dickson et al., 1979; Weller, 2005; Taylor et al., 2014; Collier and Gebremedhin, 2015).

Classical relationships between body temperature, energy intake, HP, and HL according to ambient temperature variation is shown in **Figure 7**. This diagram presents the standard view of how body temperature is maintained in different ambient temperatures. To briefly describe the figure, there is the concept of the thermoneutral zone (**TNZ**), generally defined as the range of ambient temperatures delimited by the lower and upper critical temperatures (**LCT** and **UCT**; denoted by points C and E, respectively) wherein a stable body temperature can be maintained with HP remaining relatively constant. Within the TNZ is the evaporative critical temperature (**ECT**; point D) that theoretically divides the TNZ into two parts. In the thermal comfort zone (**points C-D**), thermoregulation effort is minimal and pigs mainly rely on the sensible HL to maintain thermal balance. Meanwhile, between ECT and the UCT (**points D-E**), pigs are still able to maintain HP but have started to rely more on the latent HL pathways as thermal gradient between skin surface and environment decreases. Outside the TNZ, HP is increased below LCT (by increasing feed intake or by inducing shivering or nonshivering thermogenesis) and is decreased above UCT (by decreasing feed intake and metabolic rate) to maintain body temperature. Thus, the pigs start to expend energy outside the TNZ to ensure homeothermia. Meanwhile, below **point A** (lower extreme) and above **point F** (upper extreme), the pig can no longer do further thermoregulation which can lead to extreme thermal injury or death (Ingram and Legge, 1971; Mount and Monteith, 1974; Ingram and Mount, 1975; Blaxter, 1977; Curtis, 1983).

Critical temperatures can be influenced by many factors related to the animal (age, physiological stage), the management (feeding level, stocking density), and the environment (floor type, ventilation rate) (example in **Table 2**). For example, LCT can be from $9\text{--}16^{\circ}\text{C}$ and

Table 2. Critical ambient temperature according to age, body weight, physiological stage, floor type, air speed, and skin wetness.

Animal	Age	Wt	Floor	Air	Feeding	Skin	Temperature, °C		
	(week)						(kg)	type	speed
Piglet	1	2	mesh	still	ad lib	15	26	35	41
	4	5					24	33	39
Weaners	5	7	mesh	still	ad lib	15	26	35	41
	6	10					24	33	39
	8	16					22	30	37
	5	7	concrete	still	ad lib	15	27	36	42
	6	10					25	34	40
	8	16					25	31	38
Growers	9	20	concrete	still	ad lib	15	16	30	38
	15	50					12	28	36
	21	90					9	27	36
	9	20	concrete	draughty	ad lib	15	18	32	39
	15	50					15	30	37
	21	90					14	29	36
	9	20	concrete	draughty + spray	ad lib	60	20	34	42
	15	50					17	32	40
	21	90					16	31	39
Dry sows									
Individual housing			concrete	still	restricted	15	15	27	36
				draughty	restricted	15	18	29	38
				draught+ spray	restricted	60	20	33	40
Group housing			concrete	still	restricted	15	12	26	35
				draughty	restricted	15	15	28	38
				draught+ spray	restricted	60	18	32	40
Lactating sow		150	mesh	still	ad lib	15	8	22	32
				still+drip		30	9	26	33
			concrete	still	ad lib	15	10	23	33
				still+drip		30	11	25	34

Adapted from Kruger et al. (1992) and Lorsch (2005)

UCT from 36-39°C in 90-kg growing pigs. However, in practice, critical temperatures are not fixed and they merely serve as guidelines since the pig's thermoregulatory response to ambient temperature is dynamic and can even be influenced by the duration of exposure. For example, even though 42°C is far above the UCT, pigs are able to recover from a repeated heat challenge of 42°C when exposed for 6 hours at a time but not when the challenge was constant for 24 hours (Dickson et al., 1979). Indeed, summarizing the relationship of heat exchanges and core body temperature as such in the graph is an oversimplification of the reality because it gives a static view of thermoregulation. Heat loss proportions reported and modelled by cited literature in the previous subsections are usually values calculated based on the total HP averaged on a long period in already acclimated animal but does not account for the HP variations that happens within a day or according to the duration of exposure to elevated temperature. Indeed, the continuous and varied metabolic heat produced by living organisms means that there is an ever-changing heat exchange with the environment and that thermal equilibrium (heat storage) is never constant (Weller, 2005; Mendoza and Griffin, 2010; Taylor et al., 2014).

Maintaining thermal equilibrium is a dynamic process

In the following parts, the dynamics of thermoregulatory responses of pigs to high ambient temperature are discussed regarding their daily variations according to the duration of heat exposure and their within-day variations in simulated constant or cyclic heat challenges.

Variations of thermoregulatory responses during acclimation to heat exposure

Similar to other stress responses, thermoregulatory responses can be divided into the acute and the chronic phases. Pigs have a biphasic acclimation response to heat stress (**HS**) depending on the length of exposure and the ambient temperature (Renaudeau et al., 2010): the short term-term heat acclimation (**STHA**) and the long-term heat acclimation (**LTHA**). The STHA responses to a high ambient temperature involve the activation of the autonomic sympathetic system that diverts energy to processes that increases heat dissipation (e.g., increase cardiovascular activity with blood redirection to the skin and other peripheral tissues) (Collier and Gebremedhin, 2015; Mota-Rojas et al., 2021). These are highly accelerated responses that aims to alleviate the initial strain, even at the expense of homeostasis (Horowitz, 2002). Thus, STHA includes inefficient, rapid changes in the first 1-2 days of heat exposure to maintain thermal balance, characterized by a sudden drop in feed intake and a rapid increase

in water intake, skin (cutaneous) temperature, respiratory frequency, and rectal temperature (Bernabucci et al., 2010; Renaudeau et al., 2010). Meanwhile, the LTHA is defined by the gradual reduction in body temperature over time as the pigs acclimate to HS and it is characterized by increased thermal effector efficiency (Horowitz, 2002; Renaudeau et al., 2010). Depending on the ambient temperature, skin temperature may remain high as long as pigs are in hot conditions as it is affected both by the internal (core) and ambient temperature (Renaudeau et al., 2010). Reduction in core body temperature may likely be attributed to the lower HP due to the reduced feeding and activity levels that are closely related to the lower TEF and AHP (lower meal frequency and shorter time spent in the feeder), and possibly a lower FHP from the reduced sizes of metabolically active organs in those subjected to chronic HS for longer periods (Koong et al., 1982; Brown-Brandl et al., 2000; Quiniou et al., 2000b; Collin et al., 2001c). These acclimation responses can also be related to different endocrine changes during HS since chronic responses are coordinated by the endocrine and homeorhetic system (Collier and Gebremedhin, 2015).

Long-term exposure of the animal to a stressor results to endocrine-mediated chronic responses that can change tissue sensitivity to homeostatic signals resulting in a new physiological state with a modified set point (Collier et al., 2005; Baumgard and Rhoads, 2013; Monaghan and Spencer, 2014). The hypothalamic–pituitary–adrenal axis (HPA) axis plays a central role in the acclimation response of animals to thermal stress (Bernabucci et al., 2010) and is involved in growth hormone secretion which is reported to be reduced in HS pigs (Cui and Gu, 2015). Activation of the HPA axis is also implicated in the release of common stress biomarkers in swine such epinephrine, glucagon, and cortisol (Rhoads et al., 2013; Martínez-Miró et al., 2016). Pigs exposed to high ambient temperature (Alves et al., 2008; Yu et al., 2010; Hao et al., 2014; Hung et al., 2014; Cui and Gu, 2015) showed increased levels of blood cortisol which is a primary stress hormone involved in catabolism and increased glucose production from body protein and fat, increased cardiovascular activity (heart rate), and increased mitochondrial oxygen uptake (Ulrich, 1959; Geverink et al., 1999; Dallman and Hellhammer, 2011; Pradhan et al., 2013; Kuo et al., 2015). Increased inflammatory signaling was found in oxidative muscle from 4 to 12 hours after heat exposure but not any more after 24 hours wherein there was instead found an increased tumor necrosis factor alpha (TNF- α) (Montilla et al., 2014; Ganesan et al., 2016; Ganesan et al., 2017), a known cytokine that promotes pro-inflammatory and pro-apoptotic (cell death) signaling cascades (Kale et al.,

2018). Indeed, increased inflammatory responses such as cytokine production (Kpodo et al., 2021) and circulating endotoxins (Pearce et al., 2013b; Gabler et al., 2018) have been reported in pigs subjected to high ambient temperature.

Although not yet fully understood, animals under HS have altered physiological state which has been previously reviewed (Bernabucci et al., 2010; Baumgard and Rhoads, 2013; Ross et al., 2017). For example, heat exposure has been widely reported to decrease circulating levels of thyroid hormones in pigs specifically the pro-hormone thyroxine (**T4**) and its active form triiodothyronine (**T3**) (Marple et al., 1974; Kouba et al., 2001; Collin et al., 2002; Cui and Gu, 2015; Sanz Fernandez et al., 2015a) and this is associated with a decreased feed intake, basal metabolic rate, and HP (Kim, 2008). In terms of energy metabolism, there have been reports of decreased non-esterified fatty acids (**NEFA**) levels (Kouba et al., 2001; Sanz Fernandez et al., 2015a; Sanz Fernandez et al., 2015b; Cottrell et al., 2020) in HS pigs that are associated with a reduced ability for lipid mobilization despite the decreased feed intake and the elevated stress hormones during HS (Zhao et al., 2018). Muscle or protein catabolism seems to be preferred during HS in line with the increased creatinine (Pearce et al., 2013a; Kim et al., 2021), increased muscle oxidation (Fausnacht et al., 2021) and plasma Nt-methyl histidine, a marker of muscle catabolism, (Pearce et al., 2013a) during acute HS. In humans, exogenous glucose (i.e. from the diet) has been reported to be a less preferred energy source in hot conditions than muscle glycogen (Jentjens et al., 2002). The effect of HS on insulin has also been largely investigated especially in the past decade. Heat exposure has been reported to decrease insulin sensitivity (Yuen et al., 2013; Liu et al., 2017; Cottrell et al., 2020) or reduced glucose clearance rate (Sanz Fernandez et al., 2015a) with pigs exhibiting hyperinsulinemia demonstrated by increased basal and circulating insulin concentrations (Pearce et al., 2013a; Sanz Fernandez et al., 2015a; Mayorga et al., 2018; Seibert et al., 2018). Meanwhile, some studies also demonstrated an increased insulin sensitivity in HS pigs (Sanz Fernandez et al., 2015b; Campos et al., 2019) indicating that the effect of heat exposure on pig insulin levels is not yet fully elucidated. One theory on increased insulin secretion is that it may be a response to the increased endotoxins during HS (Baumgard and Rhoads, 2013). The contradiction between the roles of increased cortisol levels (promoting gluconeogenesis and body depot mobilization) and increased insulin (promoting glycolysis and nutrient deposition) can also be hypothesized to be a factor in the reported insulin resistance. Nevertheless, insulin is a key

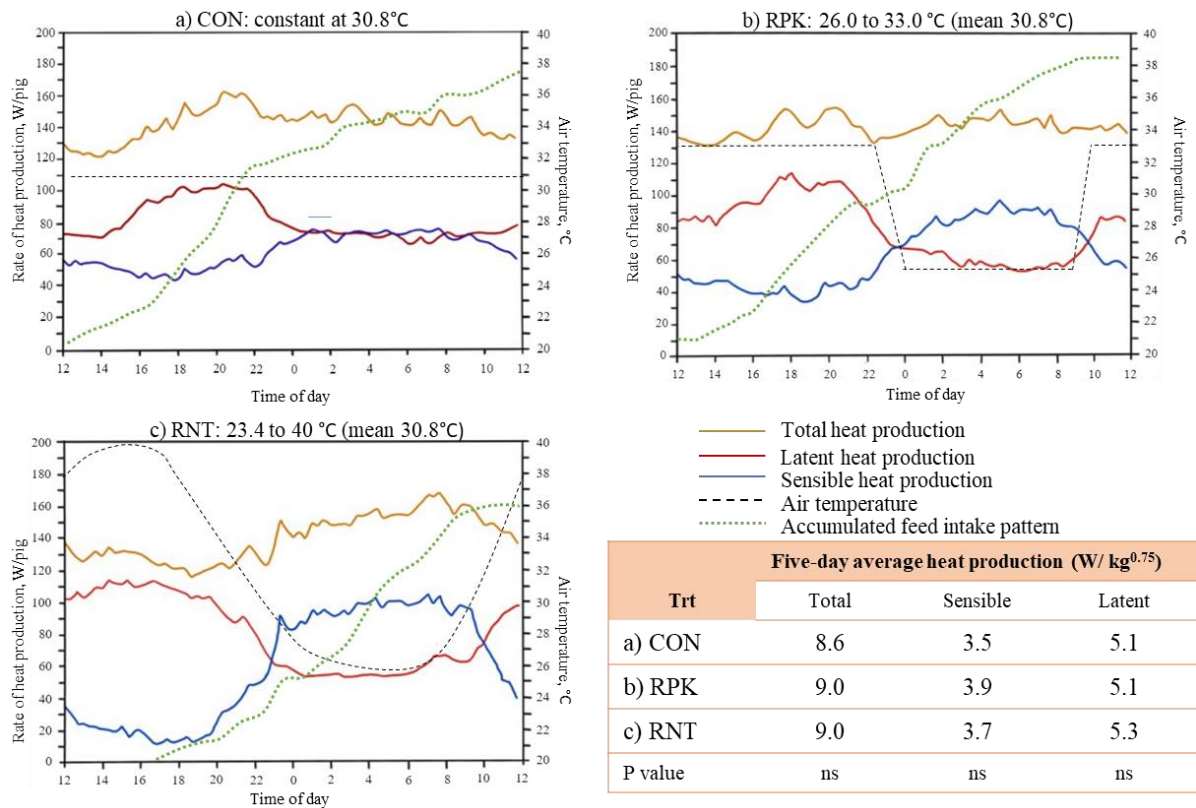


Figure 8. Heat production, and sensible and latent heat losses of pigs subjected to a mean air temperature of 30.8°C but with different temperature fluctuations: a) CON or constant at 30.8°C, b) RPK or reduced peak temperature of 26 to 33°C, and c) RNT or reduced night temperature of 23.4 to 40°C. (Adapted from Xin and DeShazer, 1991)

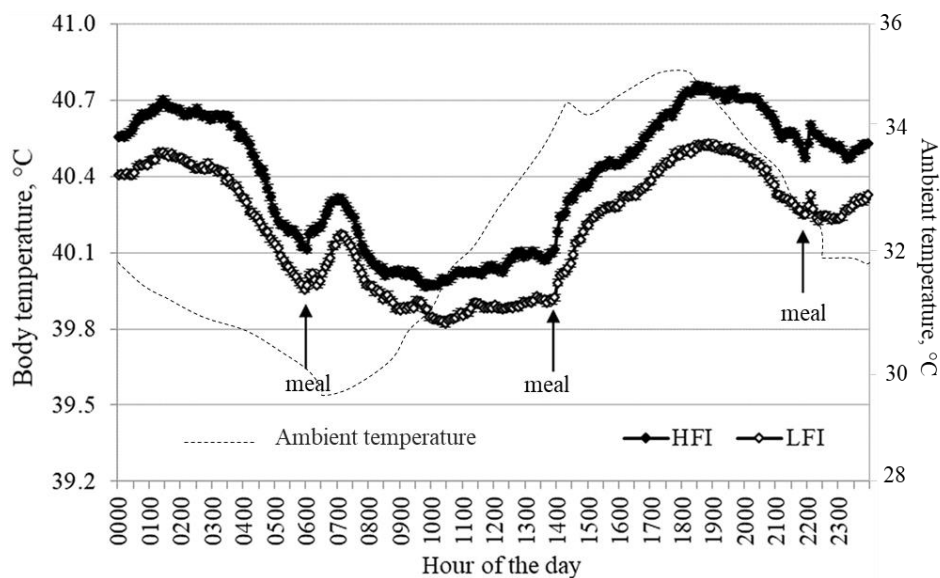


Figure 9. Body (intestinal) temperature of pigs with a high (400 g/meal) or low (320 g/meal) feed intake recorded at 5-min intervals. Pigs received their meals (0600, 1400, and 2200 h) and were exposed to a natural ambient temperature from around 29 to 36°C. (Adapted from Cervantes et al., 2018).

hormone in many metabolic processes and is theorized to play a key role in the adaptation of pigs and other mammals in coping with a heat challenge (Baumgard and Rhoads, 2013).

Nychthemeral variations of thermoregulatory responses

Pigs exposed to a diurnal or cyclic heat challenge shift their feeding and other heat-producing activities in the cooler parts of the day (Xin and DeShazer, 1991; Nienaber et al., 1996; Quiniou et al., 1996; Patience et al., 2005; Cross et al., 2020). Wide temperature variation measured throughout the day (23.4 to 40.0°C) also results to largely fluctuating patterns of sensible and latent HP responses in pigs (**Figure 8**). In the mentioned study, rate of latent HP increases when ambient temperature increases or with increasing cumulated feed intake throughout the day. This further evidences what has been previously discussed on how pigs increase their reliance in latent HL when temperature gradient decreases whether from the increased ambient temperature of the environment or the higher TEF-related HP of the animal. Fluctuating ambient temperature outside the TNZ can cause cyclic acute responses to the cyclic thermal challenge which can increase immune and inflammatory responses as indicated by increased leukocytes, cytokine responses, insulin resistance in pigs exposed to rapid changes in ambient temperature (Nienaber et al., 1989; Johnson et al., 2016a; Johnson et al., 2016b). These studies demonstrate that the thermoregulation ability of pigs can be impaired with an exposure to a fluctuating ambient temperature due to the repeated activation of acute stress responses possibly preventing their ability to reach the LTHA state.

Dynamic variations of pig responses throughout the day

As described before, thermoregulatory responses and heat exchanges of the animal varies throughout the day according to the diurnal variation in ambient temperature. While most studies on body temperature and HP are set in thermoneutral conditions as cited above, only a few studies have looked at the dynamics of body temperature throughout the day when the pigs are exposed to a simulated or naturally cyclical high ambient temperature (Ou et al., 2016; Cervantes et al., 2018). As shown in **Figure 9** intestinal temperature variations closely follow the pattern of ambient temperature and are influenced by the timing and the amount of feed intake. Indeed, there was a postprandial increase of body temperature each time the pigs ate which was higher in the afternoon and the evening meal during the warmer periods of the day (Cervantes et al., 2018). This higher extent in body temperature increase during the hotter

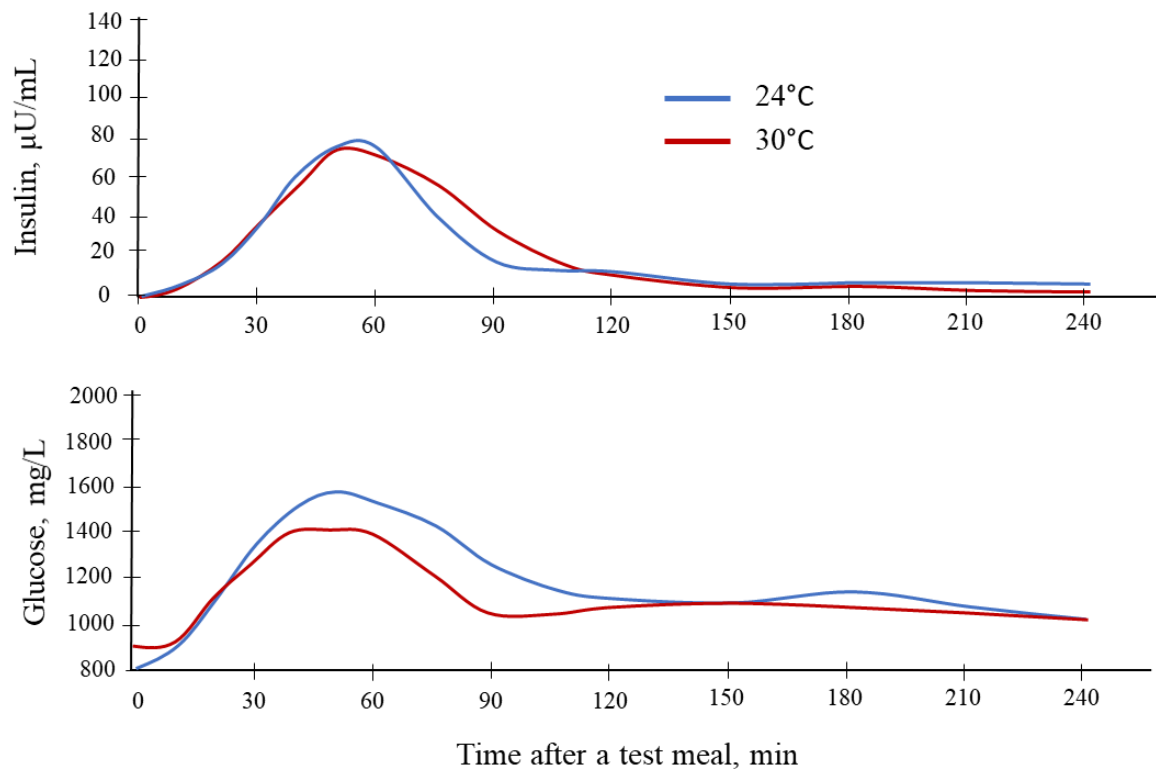


Figure 10. Postprandial plasma kinetics of insulin and glucose exposed to different ambient temperatures after 17 d at 24°C or 30°C (Adapted from Campos et al., 2019).

parts of the day must mean HL pathways are more quickly saturated due to the lower sensible HL ability especially after a larger meal due to the TEF-related heat (recall Equation 1 on heat storage in the body).

Logically, postprandial kinetics are not only observed in body temperature but also in the plasma hormones and metabolites (Liu et al., 2017; Pluschke et al., 2018; Campos et al., 2019; Li et al., 2019; Cottrell et al., 2020; Rodríguez-López et al., 2021 among others) (**Figure 10**). Among the plasma parameters in the cited studies, glucose and insulin are the most widely reported proxies of the general state of energy metabolism. In general, blood glucose (along with other nutrients) rises after a meal and insulin is released by the pancreas in order to promote glucose uptake (Röder et al., 2016). There is also a decrease in NEFA levels after a meal which is related to the insulin-mediated suppression of adipocyte lipolysis (Burdge et al., 2003). Nevertheless, these suggest that changes in the dynamic pattern of HP, of body temperature, and of different hormones and metabolites are also highly related to the feeding activity and, possibly, the size and the timing of the meals.

Section summary

Maintaining a homoeothermic (set point) body temperature is a dynamic process that involves finding a balance between heat production and loss especially since heat is constantly produced in the pig's body and is highly variable depending on the metabolic processes involved. Exposure to a high ambient temperature decreases the thermal gradient between the body surface and the environment which decreases the efficiency of the animal to dissipate heat. When HL pathways are saturated and the pigs can no longer dissipate further heat to maintain body temperature, they enter into a state of HS which results to a cascade of thermoregulatory and physiological responses. The heat associated to physical activity and the TEF largely contributes to the instantaneous peaks of HP and body temperature during the day and is thus decreased during HS. However, pig responses to HS depends also on the characteristics of the heat challenge employed in different experimental conditions (e.g., duration, constant or cyclic, timing and size of meals), which probably have a huge impact on the thermoregulatory responses measured on the animal and on its performance.

Text box 4. Brief description of the pair-feeding technique

What is the pair-feeding method?

Pair-feeding is an experimental technique that aims to remove confounding effects among experimental groups that is due to the feeding level (same plane of nutrition). Since exposure to high ambient temperature decreases feed intake, pair-feeding in heat stress studies means *restricting the feed intake of the thermoneutral control group* to a level similar to those under heat stress.

In pig studies, feed allowance of pair-fed thermoneutral pigs are usually calculated based on the average daily feed intake of the heat stressed animals with the ration divided into two or three distributions per day. Caution must therefore be taken in *comparing pair-fed /restricted-fed thermoneutral models to ad libitum-fed heat stressed animals* because they are not subjected to the same types of stress. Imposed feed restriction can increase stress responses due to prolonged fasting or can disrupt the natural feeding behavior.

Nevertheless, pair-feeding has been a widely accepted technique especially in determining direct effects of heat stress in pigs, especially since there is a significant difference in feed intake between *ad libitum*-fed pigs raised in thermoneutral and in heat stressed conditions.

(Sources: Mitchell and Beadles, 1930; Russell et al., 2008; Ellacott et al., 2010)

Failure to maintain thermal equilibrium during heat stress: consequences on pig performance

Maintaining core body temperature in HS conditions generally reduce economically important traits such as reproduction and production performance in pigs and other livestock species (DeShazer et al., 2009; Ross et al., 2017; Liu et al., 2021). In growing pigs, the most described negative effect of HS is on feed intake reduction (Renaudeau et al., 2011; da Fonseca de Oliveira et al., 2019). Other metabolic and physiological changes induced by heat exposure also contribute to the impairment of animal performance, body composition, and health (Baumgard and Rhoads, 2013; Ross et al., 2015; Ross et al., 2017; Gonzalez-Rivas et al., 2020; Liu et al., 2021). This subsection tackles how pig responses to a heat challenge compromise pig performance directly (physiological changes due to heat exposure) and indirectly (induced by feed intake reduction). Some factors affecting severity of HS and possible interventions to mitigate HS effects are also discussed.

Direct effects of heat stress

Due to negative effect of HS on feed intake, most of the pig studies that evaluated direct effects of a heat challenge and that will be mentioned in this subsection attempt to remove the confounding HS effect of feeding level by using pair-feeding techniques (**Text box 4**).

Consequence of increased peripheral blood flow

As mentioned before, in high ambient temperatures, blood is redistributed towards organs implicated in HL dissipation such as the skin, ears, diaphragm, and lungs but away from the core and intestinal tissues (Collin et al., 2001a). This can cause a cascade of reactions (**Figure 11**) such as hypoxia due to the insufficient blood flow to these organs leading to increased production of reactive oxygen species and decreased cellular antioxidant activity, and thus to oxidative stress and to tissue damage (Turrens, 2003; Oliver et al., 2012; Belhadj Slimen et al., 2014; Hao et al., 2021). There have already been many reports of impaired intestinal barrier integrity and weakening of tight junctions that results to “leaky gut” syndrome in pigs subjected to short-term HS (Pearce et al., 2013c; Liu et al., 2016; Abuajamieh et al., 2018; Gabler et al., 2018; Kpodo et al., 2020b; Xia et al., 2022). Gastrointestinal tract mucosa represents the barrier between the body and the external environment allowing nutrient uptake and restricting translocation of pathogenic molecules and bacteria through the tight junction

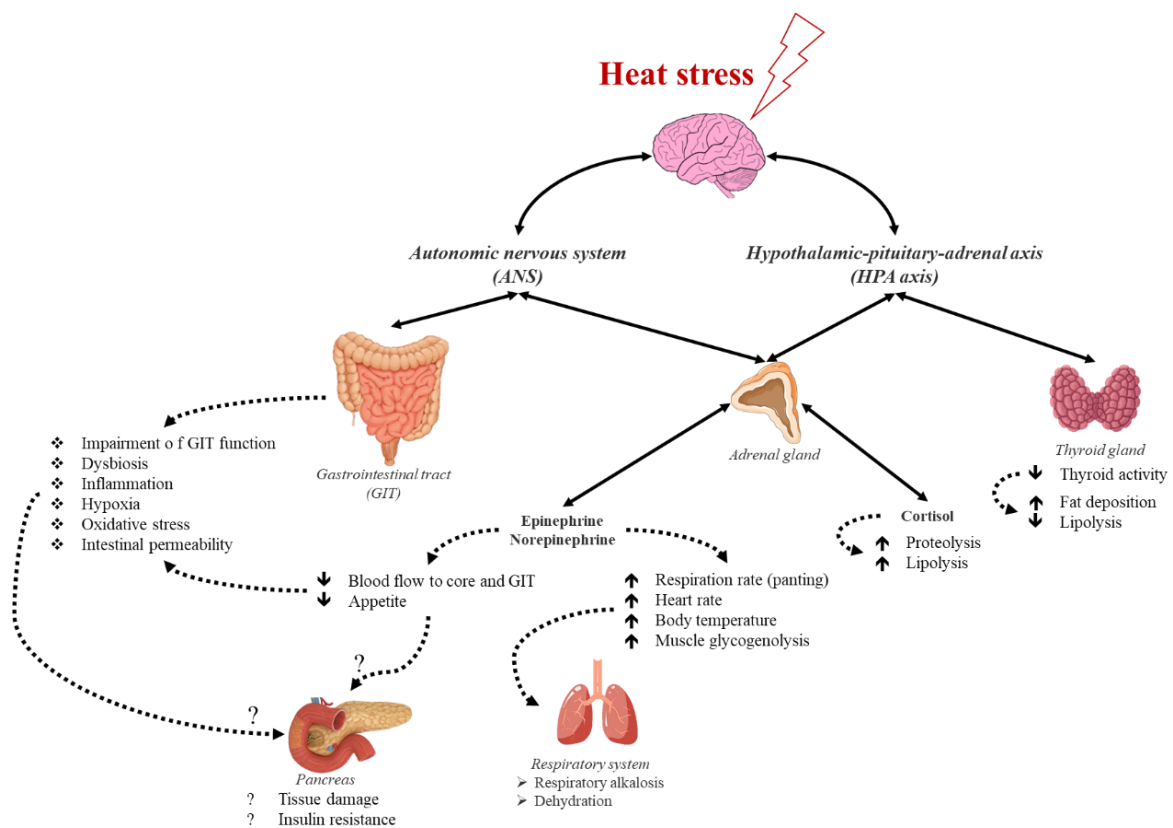


Figure 11. Relationship between heat stress and the autonomic nervous system (ANS), and the hypothalamic-pituitary-adrenal (HPA) axis which are implicated in thermoregulation and thermal acclimation responses. (Adapted from Gonzalez-Rivas et al., 2020)

barrier (Wiest and Rath, 2003; Turner, 2009; Vancamelbeke and Vermeire, 2017; Assimakopoulos et al., 2018; Pluske et al., 2018). Thus, impairment of gut integrity can compromise overall health and can affect other organs as it allows the passage of the bacteria and bacterial components such as endotoxins into intestinal tissue and then into the bloodstream (Terciolo et al., 2019; Massier et al., 2021). Not only does this impair gut health but oxidative stress also compromises immune system of animals exposed to HS (Morrow-Tesch et al., 1994; Chauhan et al., 2021) which has metabolic and energetic consequences (Gabler et al., 2017; Huntley et al., 2017) thereby decreasing efficiency of energy utilization.

Whether due to an increased endotoxin in the systemic circulation that impairs ovarian functions (Mayorga et al., 2020) or to a decreased uterine and umbilical blood flow (Dreiling et al., 1991; Limesand et al., 2018), heat exposure has been reported to reduce reproduction performance in pigs and in other mammalian species such as pregnancy loss (seasonal infertility) during early gestation, placental insufficiency during mid-gestation, and fetal growth retardation during late gestation (Hansen, 2009; Ross et al., 2017; Mayorga et al., 2020; Liu et al., 2021). As reviewed by Hansen (2009), maternal hyperthermia has also been reported to impair oocyte development and maturations, early embryonic development, cardiovascular and brain functions, and other physical and physiological defects. This is in line with reported endometrial integrity deterioration and uterine tissue damage (Lian et al., 2022), placental nutrient transport capacity impairment (Zhao et al., 2021), and placental insufficiency (Zhao et al., 2020) when sows are exposed to HS. Heat exposure can thus negatively affect not just the pregnant sow and but also the progeny.

Consequence of elevated evaporative heat loss

As the ambient temperature increases, sensible HL pathways becomes limited leading to panting. The related rise in respiratory frequency is accompanied by a decreased tidal volume that increases ventilation of the upper respiratory tract and preserves alveolar ventilation (Robertshaw, 2006; Tsuji et al., 2016). However, in cases of extreme heat and body temperature continues to rise, there is a shift to a slower deeper panting phase characterized by an increase of alveolar ventilation rate (Hales and Webster, 1967; Renaudeau et al., 2012). As reported in HS pigs, a significant increase in respiratory rate can result to hypocapnia (low blood CO₂) and respiratory alkalosis in the blood (Liu et al., 2017; Liu et al., 2018a; Le et al., 2019; Cottrell et al., 2020). The increased evaporative HL can also lead to dehydration and electrolyte imbalance

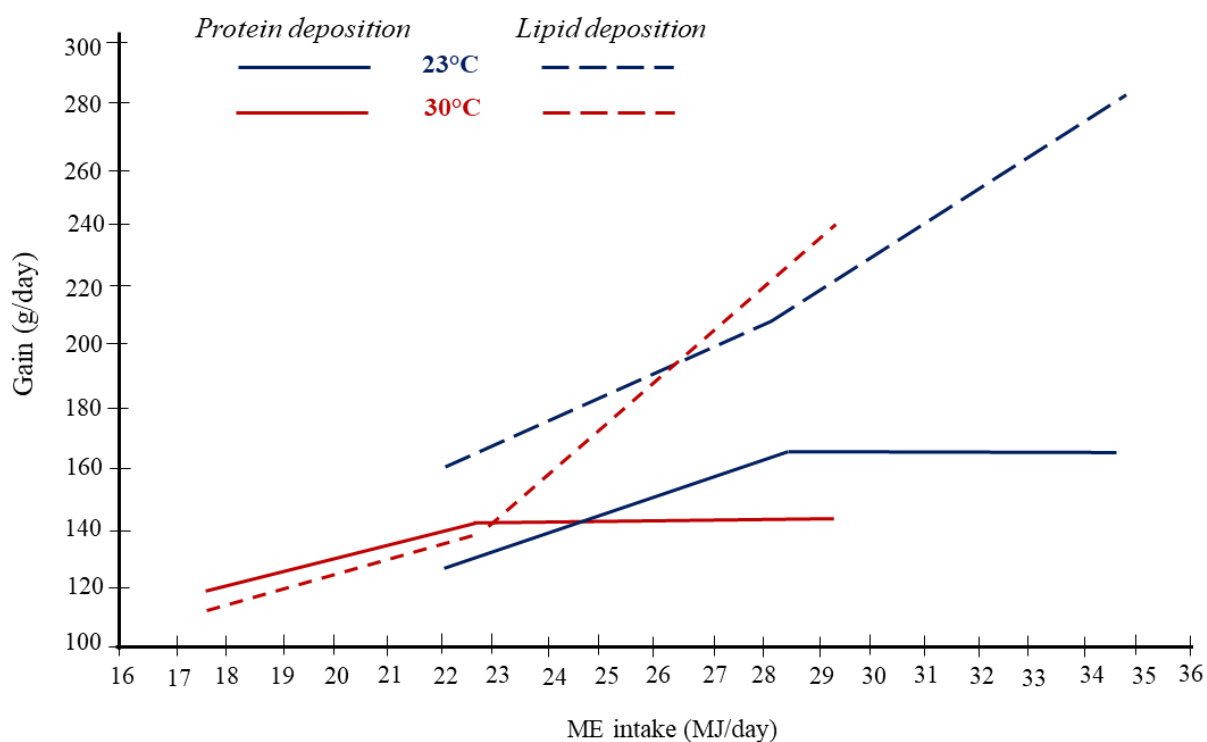


Figure 12. Effect of temperature and ME intake on protein and lipid deposition in 24 to 65 kg live weight barrows. Predicted data for protein deposition (PD, g/day) at 23°C (—), at 30°C (—) and lipid deposition (LD, g/day) at 23°C (- - -), at 30°C (- - -). (Adapted from Le Bellego et al., 2002b).

with negative subsequent effects on growth performance (Patience et al., 1987; Haydon et al., 1990; Ahmad and Sarwar, 2006; Brêtas et al., 2019; Ortega et al., 2022).

Consequences of the metabolic and physiological changes

In thermoneutral conditions, pigs are inclined to prioritize and use higher proportion of ME intake for protein instead of lipid deposition even in restricted-fed pigs until maximum PD (PD_{max})⁸ has been reached (Campbell et al., 1985; Bikker et al., 1996; Quiniou et al., 1996; Moughan et al., 2006). However, heat exposure would limit PD_{max} in growing pigs (Le Bellego et al., 2002b). As shown in **Figure 12**, this effect seems to be unrelated to the feeding level and could be related to higher energetic cost of PD or may also be due to the increased insulin responses as discussed in a previous section of the literature review. This modified energy partitioning for PD and LD upon heat exposure is also observed in HS pigs when compared to pair-fed TN group (Pearce et al., 2013a) with fat deposition generally occurring in internal compartments (Christon, 1988; Kouba et al., 2001; Wu et al., 2016). This may be related to effect of high ambient temperature on the decreased activity of stearoyl-CoA-desaturase⁹ in the fat and muscle depots but not in the liver (Kouba et al., 1999) alongside the increased triglycerides transported by VLDL¹⁰ (Kouba et al., 2001). The increased internal rather than external fat deposition has been hypothesized to be an acclimation response to reduce body insulation (thus increasing heat dissipation capacity) during HS (Le Dividich et al., 1998). As insulin inhibits hydrolysis of triglycerides in adipose cells into glycerol and free fatty acids (Laws et al., 1997), the direct effect of HS on fat deposition might be connected to the reported altered insulin response during HS. On the other hand, presence of NEFA also inhibits insulin-stimulated glucose uptake and glycolysis (Boden et al., 1994; Roden, 2004) causing insulin resistance which is also correlated with visceral adiposity in HS animals (Frayn, 2000; Bergman et al., 2006; Usui et al., 2010). However, increased carcass fatness could also be an indirect consequence of a reduced net lean deposition rather than an increased fat deposition as indicated by previously discussed increased markers of muscle catabolism such as creatinine, leucine oxidation, and plasma Nt-methyl histidine (Pearce et al., 2013a; Zhao et al., 2018; Kim et al., 2021).

⁸Maximum protein deposition (PD_{max}) is the genetic upper limit to whole body protein deposition wherein excess energy intake after PD_{max} has been reached will be deposited as lipid (Moughan et al., 2006).

⁹Stearoyl-CoA-desaturase catalyzes biosynthesis of monosaturated fatty acids from saturated fatty acids.

¹⁰Very low lipoproteins are produced in the liver and carry triglycerides in the bloodstream to supply body tissues.

Table 3. Summary of gut microbiota alterations induced by heat stress in monogastric animals.

Animal	Age	Sampling location	Treatment description	Phylum changes	Genus changes	References
Pig	11 weeks	Feces	28-30°C vs. 25°C 3 weeks	<i>Firmicutes</i> (+) <i>Proteobacteria</i> (+) <i>Spirochaetes</i> (+) <i>Actinobacteria</i> (-) <i>Bacteroidetes</i> (-)	Not reported	(Le Sciellour et al., 2019)
	Not mentioned (29–31 kg BW)	Feces	34-36°C vs. 24-26°C	<i>Proteobacteria</i> (+) <i>Bacteroidetes</i> (-)	<i>Acinetobacter</i> (+)	(Xiong et al., 2020)
	Sow in 85d of pregnancy	Feces	28–32 °C vs. 22°C	No differences	<i>Coprococcus</i> 3 (+), <i>Coprostanoligenes</i> group (+), <i>Halomonas</i> (+), <i>Ruminococcaceae</i> UCG-005 (+), <i>Ruminococcaceae</i> UCG-013 (+), <i>Bacteroidales</i> RF16 group (-), <i>Streptococcus</i> (-), <i>Treponema</i> 2 (-)	(He et al., 2019a)
Broiler	2 weeks	Cecum	34-38°C vs. 24-26°C 4 weeks	<i>Firmicutes</i> (+) <i>Proteobacteria</i> (+) <i>Bacteroidetes</i> (-) <i>Cyanobacteria</i> (-)	<i>Lactobacillus</i> (+), <i>Bacteroides</i> (-), <i>Dorea</i> (-), <i>Faecalibacterium</i> (-), <i>Oscillospira</i> (-)	(Shi et al., 2019)
	3 weeks	Cecum	31-33°C vs. 21-23°C 2 weeks	<i>Firmicutes</i> (+) <i>Bacteroidetes</i> (-)	<i>Anaerofustis</i> (+), <i>Pseudonocardia</i> (+), <i>Rikenella</i> (+), <i>Tyzzerella</i> (+), <i>Parabacteroides</i> (-), <i>Romboutsia</i> (-), <i>Saccharimonas</i> (-), <i>Weissella</i> (-)	(Liu et al., 2020)
	4 weeks	Ileum	30-33°C vs. 20-22°C 2 weeks	<i>Firmicutes</i> (+) <i>Bacteroidetes</i> (-) <i>Proteobacteria</i> (-)	<i>Alistipes</i> (+), <i>Azospirillum</i> (+), <i>Clostridium</i> XIVb (+), <i>Faecalibacterium</i> (+), <i>Oscillibacter</i> (+), <i>Rothia</i> (+), <i>Streptophyta</i> (+), <i>Coprococcus</i> (-), <i>Streptococcus</i> (-)	(Wang et al., 2018)
	11 weeks	Cecum	24-30°C vs. 22°C 10 weeks	<i>Bacteroidetes</i> (+) <i>Euryarchaeota</i> (-)	<i>Akkermansia</i> (+), <i>Desulfovibrio</i> (+), <i>Faecalibacterium</i> (-)	(Wang et al., 2020)
Laying hen	28 weeks	cecum	29-35°C vs. 20-22°C 4 weeks	No differences	<i>Anaerosporeobacter</i> (+), <i>Barnesiella</i> (+), <i>Clostridium_sensu_stricto_1</i> (+), <i>Escherichia_Shigella</i> (+), <i>Gallibacterium</i> (+), <i>Odoribacter</i> (+), <i>Rikenellaceae_RC9_gut_group</i> (+), <i>Sphaerochaeta</i> (+), <i>Bacteroides</i> (-), <i>Lactobacillus</i> (-), <i>Prevotellaceae_Ga6AI_group</i> (-), <i>Ruminococcaceae_UCG_005</i> (-)	(Xing et al., 2019)
	45 weeks	feces	25-34°C vs. 21-28°C 6.6 weeks	<i>Bacteroidetes</i> (+) <i>Firmicutes</i> (-) <i>Fusobacteria</i> (-) <i>Proteobacteria</i> (-)	<i>norank_p_Candidatus_Saccharimonas</i> (+), <i>Cloacibacillus</i> (+), <i>Enterorhabdus</i> (+), <i>Flaviumibacter</i> (+), <i>Prolixibacter</i> (+), <i>Sunxiuqinia</i> (+), <i>norank_f_Synergistaceae</i> (+), <i>Synergistes</i> (+), <i>Dorea</i> (-), <i>Exiguobacterium</i> (-), <i>norank_f_Lachnospiraceae</i> (-), <i>Marvinbryantia</i> (-)	(Zhu et al., 2019)

Adapted from He et al. (2021)

Indirect effects of heat stress (mediated by a reduction in feed intake)

Average daily feed intake (**ADFI**) is the main determining factor of growth performance in pigs since tissue (lean and fat) deposition and subsequent maintenance needs dietary energy and nutrients (Nyachoti et al., 2004; Patience et al., 2015). The negative effect of HS on feed intake is well-documented and usually associated with the reduction in TEF-related HP (Collin et al., 2001b; Quiniou et al., 2001a; Renaudeau et al., 2013; Campos et al., 2014a; Labussière et al., 2021). Although there is little to no effect on feed efficiency except at very high ambient temperature (above 32°C), the main consequence is a reduction in growth [as reported in both meta-analyses by Renaudeau et al. (2011) and da Fonseca de Oliveira et al. (2019)]. For example in 50-kg BW pigs, feed conversion ratio (**FCR**) remains at an average of 2.7 kg/kg from 20 to 30°C (Renaudeau et al., 2011), but ADFI and average daily BW gain (**ADG**) are reduced by -32 and -18 g·d⁻¹·°C⁻¹, respectively (Renaudeau et al., 2011). The reduction in ADFI and the consequent reduction in ADG results in longer time to reach the market weight (Cruzen et al., 2015). The decreased slaughter BW of pigs exposed to high ambient temperature might also be related to a reduction in the absolute weight of the visceral organs (Collin et al., 2002) which can mean a positive effect on the dressing BW. Indeed, when expressed as a percentage of empty BW, carcass proportion increased by 0.9 points upon heat exposure while head, total viscera, and liver proportion (%) of empty BW decreased by 3.9, 5.6, and 7.3 points, respectively (Johnson et al., 2015b).

Changes in the gut microbiota

Gut microbiota is particularly important in monogastric species when considering feed utilization especially its influence on dietary fiber digestion (Le Sciellour et al., 2018; Niu et al., 2019). However, the past decade has provided a vast amount of pig gut microbiota research on health and production (as reviewed by Gresse et al., 2017; Nowland et al., 2019; and Duarte and Kim, 2022 among others). While it is still unclear, the effect of HS on pig microbiota may be related to the reduced feeding level since feed intake restriction in itself can modify microbiota composition (Le Floc'h et al., 2014; Xia et al., 2022) but comparing HS to pair-fed (**PF**) TN controls also suggest a direct effect of heat exposure (Xia et al., 2022; Xiong et al., 2022). Nevertheless, some studies looking at HS effects in monogastric animals are provided in **Table 3** (reviews by He et al., 2021; and Wen et al., 2021).

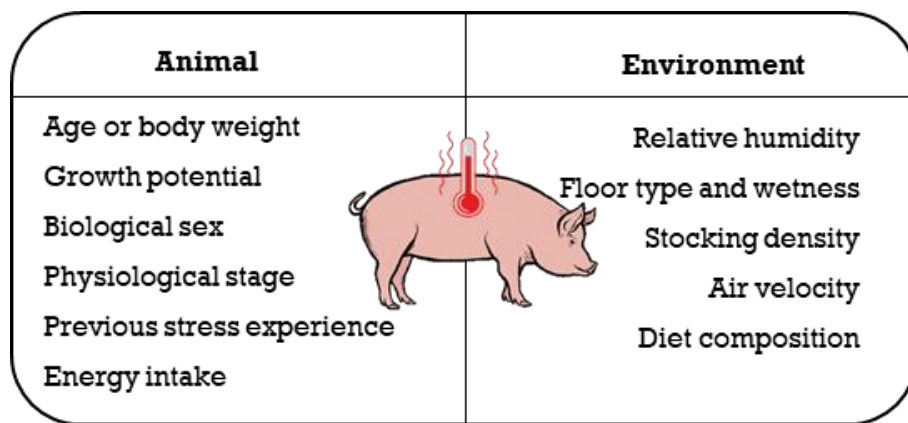


Figure 13. Some animal and environment related factors affecting severity of heat stress in pigs.

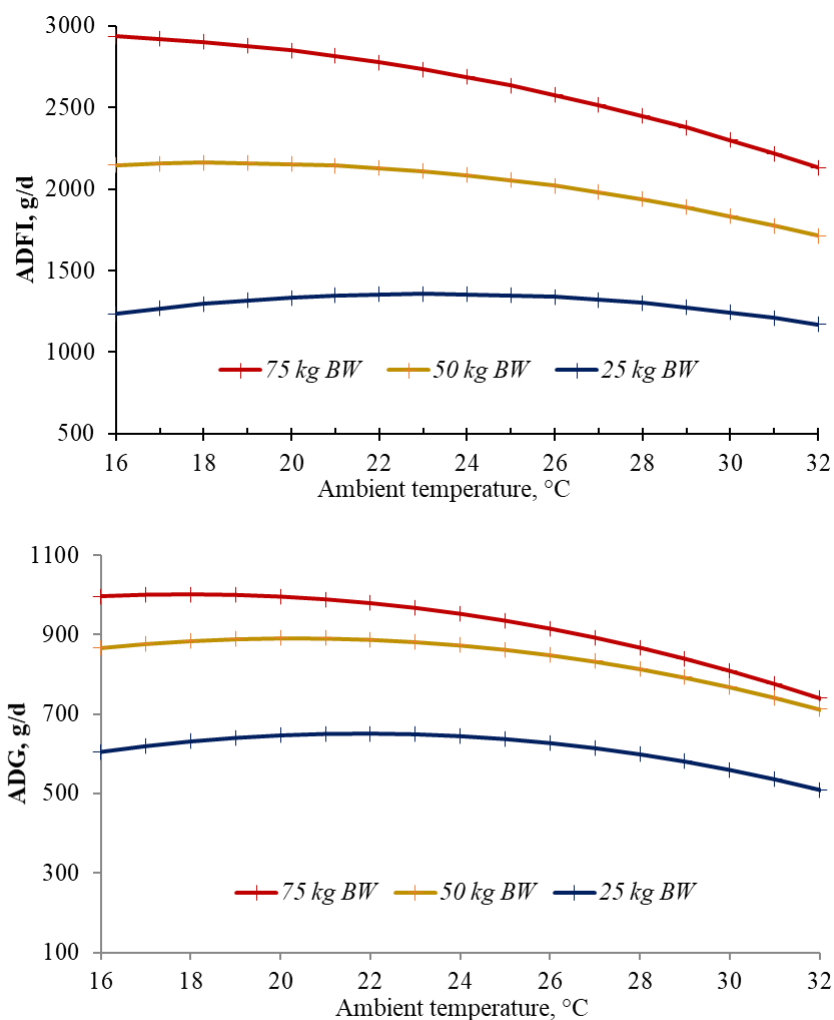


Figure 14. Effect of ambient temperature and body weight on ADFI and ADG of growing pigs. (Adapted from Renaudeau et al., 2011)

In growing pigs, heat exposure can result to increase in *Proteobacteria* relative to *Firmicutes* and *Bacteroidetes* (Xiong et al., 2020). Decrease in abundance of genera *Lactobacillus* and *Clostridium* has also been reported under HS conditions and was theorized to an increase in pathogenic bacteria colonization as it coincided with an increase in potentially pathogenic *Prevotellaceae* (Le Sciellour et al., 2019). Meanwhile, heat exposure increased bacterial genera *Chlamydia*, *Lactobacillus*, *Succinivibrio*, *Bifidobacterium*, and *Lachnoclostridium* which has been correlated with oxidative stress, intestinal barrier dysfunction, and inflammation and decreased *Lactobacillus* and *Bifidobacterium* which are suggested to be part of the initial responses in gut dysbiosis and inflammation (Xia et al., 2022). Meanwhile, some species such as *Lactobacillus reuteri*, *Clostridium chartatabidum*, and *Anaerovibrio lipolyticus* are suggested to be important during heat exposure as they are positively related to an improved feed intake and energy efficiency (Labussière et al., 2021).

Factors affecting severity of heat stress in growing pigs

Severity of HS effect on pigs depends on many factors relating both to the animal and the surrounding environment (**Figure 13**). While the goal of the literature review is not to enumerate all these factors, this subsection discusses some animal and environment related factors.

Animal related factors

Body weight

The severity of feed intake reduction increases not only with ambient temperature but also with BW (Quiniou et al., 2000a; Zhang et al., 2020). As shown in **Figure 14**, the curvilinear effect of elevated temperature on ADFI is dependent on the BW of the pigs wherein ADFI was reported to decline between 20 and 30°C with an average of -32 and -78 g·d⁻¹·°C⁻¹ for 50 kg and 100-kg pigs, respectively (Renaudeau et al., 2011). The non-linear model proposed by the latter paper clearly shows that the critical temperature (inflection point) in which ADFI and thus ADG is lower in heavier pigs. The higher susceptibility of heavy animals is usually explained by lower HL capacity due lower surface area to mass ratio and the thicker subcutaneous fat (thicker insulation) and to a higher metabolic rate (Bruce and Clark, 1979).

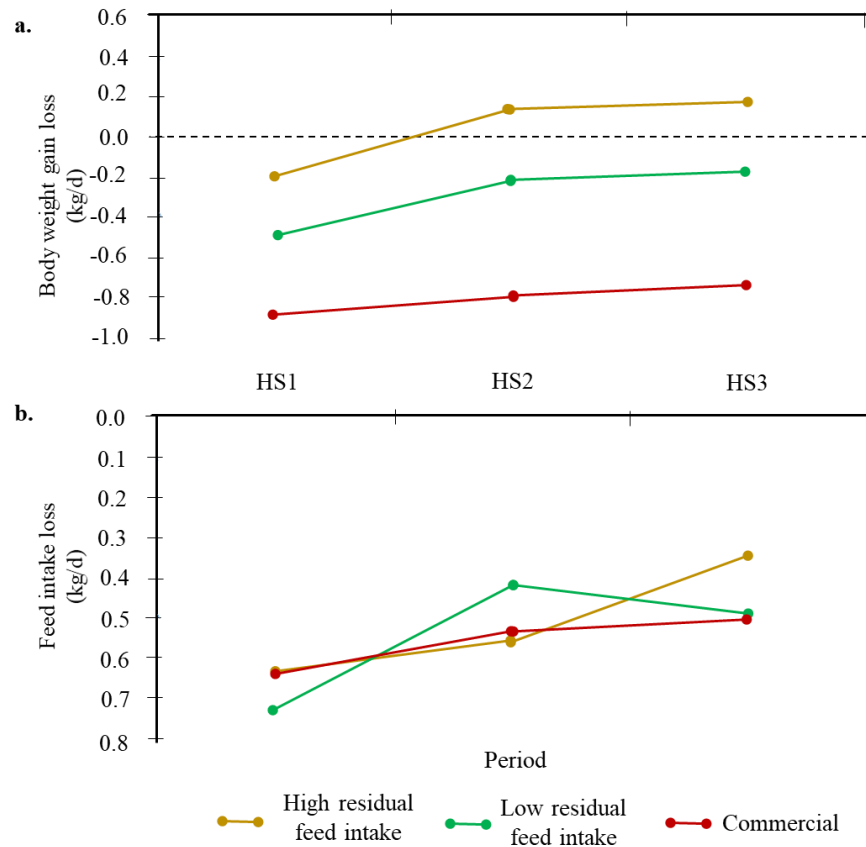


Figure 15. Absolute drop in body weight gain (a), and feed intake (b) between a constant TN (21.9°C) and cyclical HS (31.8°C average) HS environments for three pig lines subjected thrice to a 4-day heat exposure (HS1, HS2, and HS3). HS1 was preceded by a 9-day TN period and HS2 and HS3 by a 7-day TN period. (Adapted from Rauw et al., 2017).

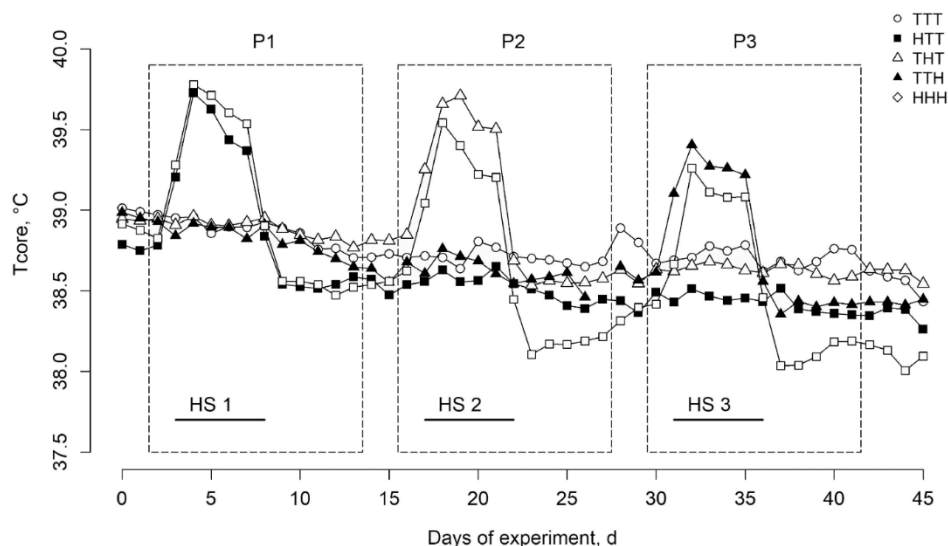


Figure 16. Effects of heat challenge (T at 22°C vs. H at 32°C) in the first (P1), second (P2), and third (P3) experimental periods on internal body temperature (Tcore, °C) profiles throughout the experiment. The challenge, which was repeated in P1, P2, and P3, consisted of a 3-d prechallenge period (22°C), a 5-d HS challenge (32°C), and a 7-d recovery period (22°C) (Adapted from Renaudeau, 2020).

Growth potential and higher productivity

It is generally accepted that selection for higher productivity increases environmental sensitivity (Kolmodin et al., 2003; Knap and Su, 2008) with modern pig breeds or pigs with higher growth potential being more susceptible to the negative effects of HS (Renaudeau et al., 2006b; Rauw et al., 2017; Gourdine et al., 2021). In comparing three pig lines, i.e., commercial, high and low residual feed intake (**RFI**)¹¹, pigs with lower growth potential (low and high RFI) grew slower at 0.72 and 0.66 kg·d⁻¹, respectively, than commercial pigs at 1.22 kg·d⁻¹ but maintained their growth performance better upon exposure to high ambient temperature (**Figure 15**). Similar results were reported in an earlier study comparing conventional Large White breed with a local Carribean breed (Creole) wherein the local breed with a lower lean deposition had lower reduction in ADFI and ADG (-5 and -3%, respectively) than the conventional breed (-12 and -9%, respectively) when exposed to a naturally high ambient temperature (Renaudeau et al., 2006b). Indeed, increased lean deposition in modern pig breeds translates to higher energy intake and HP rendering them more susceptible to HS (Brown-Brandl et al., 2001).

Sex

With the previously discussed higher protein deposition, growth rate, and HP of entire male pigs (Moughan et al., 1987; Campbell and Taverner, 1988; Labussière et al., 2013), they are assumed to be more likely negatively affected by HS than castrated males or female pigs. Sexual dimorphism on the response of pigs to HS has not been fully explored but barrows were reported to have lower feed efficiency in HS conditions than gilts (Renaudeau et al., 2006b). Meanwhile, studies from one laboratory have suggested that there might be an underlying effect of sex in the heat-induced oxidative stress status of pigs. Skeletal muscles of barrows were unaffected (Rudolph et al., 2021) while those of gilts showed increased oxidative stress upon HS (Montilla et al., 2014) although there might be the confounding effect of age with former study having used ~60 kg barrows and the latter ~35 kg gilts. However, these have yet to be demonstrated in the animal level and in pigs of similar ages.

¹¹ Residual feed intake (RFI) is a genetic measure of net feed efficiency and represents the difference between the total feed intake and part of the feed intake unexplained by maintenance and production energy cost. Thus, selection for a low RFI is a selection for improved feed efficiency without compromising production (Gilbert et al., 2007).

Table 4. Potential effects of in utero heat stress on the swine industry.

Production stage	Biological effect	Potential swine production effect	References
Gestation and farrowing	Reduced piglet birth weight	Reduced piglet survivability and weaning weight	(Lucy et al., 2012)
	Decreased boar fertility	Greater number of services per sow and reduced reproductive efficiency	(Lugar et al., 2018)
	Increased piglet cortisol	Greater risk of mortality at farrowing due to increased stress response	(Machado-Neto et al., 1987)
	Altered lactation performance	Impact on piglet growth from farrowing to weaning	(Safranski et al., 2015; Wiegert et al., 2015)
Post-weaning (nursery)	Greater stress response	Greater likelihood of morbidity and mortality following weaning	(Chapel et al., 2017)
	Altered immune function	Increased disease susceptibility	(Machado-Neto et al., 1987)
Growing-finishing	Increased maintenance costs	Reduced energy available for growth and lean meat production	(Chapel et al., 2017)
	Altered body composition	Increase adiposity and reduced lean accretion reduces meat yield and quality	(Boddicker et al., 2014; Johnson et al., 2015a)
	Reduced thermotolerance	Greater heat stress susceptibility resulting in increased morbidity and mortality	(Johnson et al., 2013; Johnson et al., 2015b)
	Decreased feed efficiency	Decreased profitability due to an increase in feed costs	(Johnson et al., 2015a)
	Altered postabsorptive metabolism	Impact on nutrient partitioning and carcass quality	(Boddicker et al., 2014)
	Altered body composition	Increase adiposity and reduced lean accretion reduces meat yield and quality	(Boddicker et al., 2014; Johnson et al., 2015a)
Market	Increased muscle fiber size	Tougher meat products	(Wilmoth et al., 2015)
	Reduced live weight	Less meat produced per pig harvested	(Wiegert et al., 2015)

Adapted from Johnson and Baumgard (2019)

Previous heat stress experiences

Another aspect that affects severity of HS effects does not come from the immediate internal or external factors but comes from a pig's previous exposure to a HS challenge. In pigs, a previous heat exposure resulted to an attenuated increase in body temperature upon a new heat challenge (Rauw et al., 2017; Renaudeau, 2020) (**Figure 16**). Pigs that were already subjected to a heat challenge twice (HHH) decreased ADFI and ADG when subjected to another HS challenge in Period 3 but to a lesser extent than pigs subjected to HS for the first time (TTH) (Renaudeau, 2020). Similarly, in pigs subjected three times to an ambient temperature of 32°C, the drop in feed intake and BW gain was larger in the first (−662 and −529 g·d^{−1}, respectively) than in the second (−506 and −294 g·d^{−1}) and third (−439 and −234 g·d^{−1}) heat challenge (Rauw et al., 2017). Meanwhile, a previous exposure to a heat challenge seems to limit the pig's ability for compensatory feed intake and growth (Mayorga et al., 2018; Renaudeau, 2020) that may be associated to gut integrity impairment (Mayorga et al., 2018).

Recent studies have also shown that the prenatal thermal environment can influence pig responses to a postnatal heat challenge. Studies in other mammalian species have reported that the HS during pregnancy can affect their progeny (Shiota and Kayamura, 1989; Edwards et al., 2003; Hansen, 2009). In farm animals, prenatal epigenetic adaptation and thermal manipulation has been well-studied in poultry species to improve the chick's ability to cope with a post-hatch thermal challenge (Yahav and McMurtry, 2001; Tzschentke and Basta, 2002; Moraes et al., 2003; Yahav et al., 2004; Tzschentke, 2007), or to influence carcass characteristics (Collin et al., 2007; Loyau et al., 2013; Loyau et al., 2016). In ruminants, maternal HS has been found to lower stress response of newborn calves and negatively affect the fetal development (Guo et al., 2016), and to reduce feed intake and thus growth of calves coming from cows subjected to HS during late gestation (Monteiro et al., 2016).

As previously discussed, direct effects of heat exposure in pregnant sows can include impaired endometrial integrity and reduced uterine blood flow which results to a cascade of negative effects to the offspring and thus to the overall pig production efficiency (as reviewed by Ross et al., 2017; Johnson and Baumgard, 2019; and Liu et al., 2021) (**Table 4**). Some studies have reported that pigs born from HS pregnant sows are reported to be less heat tolerant due to increased basal body temperature (Johnson et al., 2013; Johnson et al., 2015c) and increased cortisol response of pigs when subjected to a postnatal stressor (Chapel et al., 2017; Johnson et al., 2018). Carcass fatness is also associated with pigs that were exposed to a

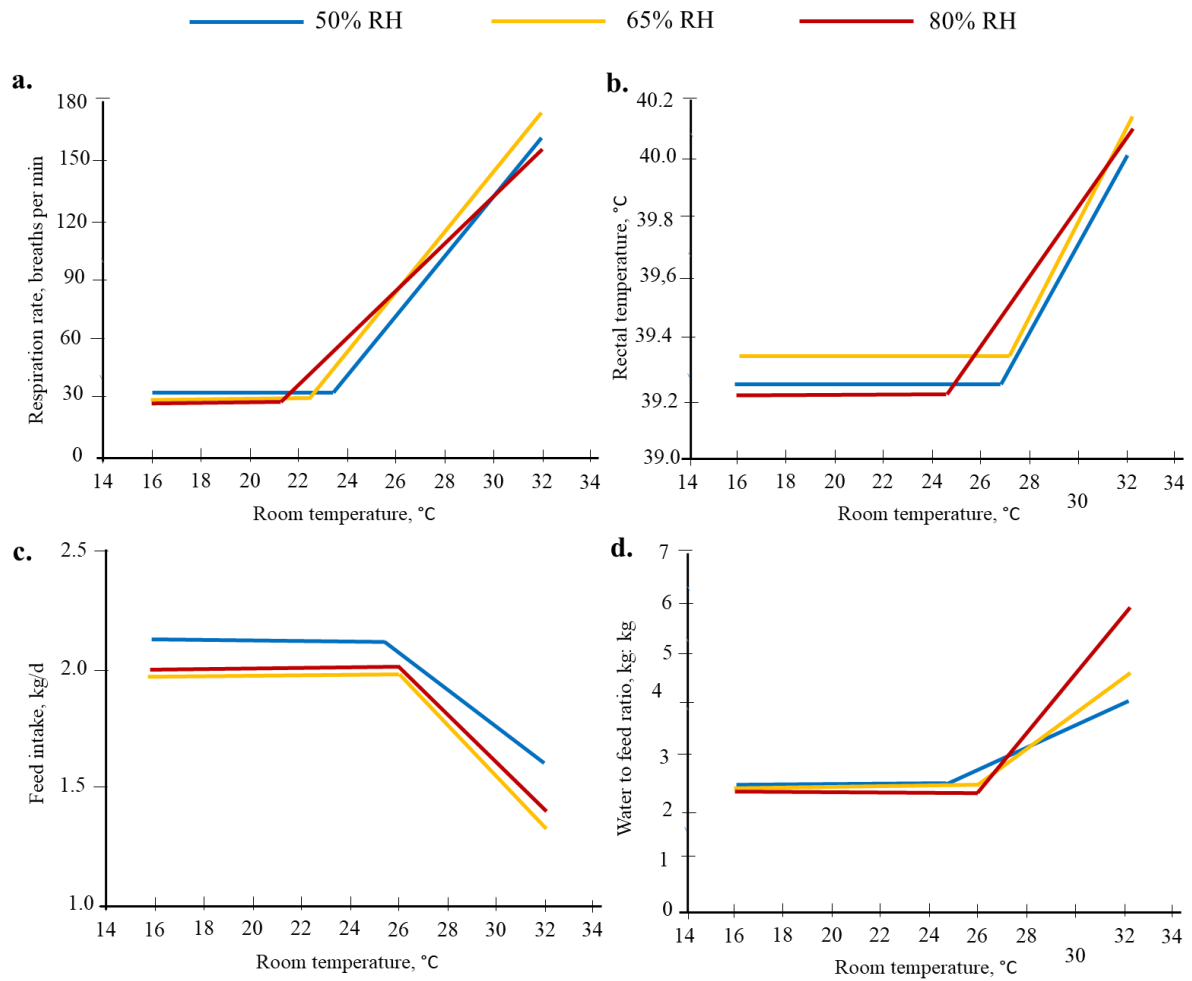


Figure 17. Relationship between ambient temperature and respiration rate (a), rectal temperature (b), feed intake (c), and water to feed ratio (d) according different relative humidities (50% RH, 65% RH, and 80% RH) (Adapted from Huynh et al., 2005).

prenatal HS environment and has been associated to seasonal fatness of pigs born during the summer season (Trezona et al., 2004; Rodríguez-Sánchez et al., 2009; Choi et al., 2019a; Liu et al., 2020a). However, results differ across studies with increased fatness *in utero* HS pigs observed in some studies (Boddicker et al., 2014; Johnson et al., 2015b) but not in others (Cruzen et al., 2015; Johnson et al., 2015a) which might be related to many factors such as the stage and length of the heat challenge during gestation and the growth stage of the progeny. The reported decrease in placental sufficiency and decrease in the number of skeletal muscle fibers of the offspring of sows subjected to mid-gestation HS (Zhao et al., 2020) also lend support to the altered body composition of prenatal HS pigs. Nevertheless, most of the pig studies related to prenatal HS that have been published considered only primiparous sows (Boddicker et al., 2014; Cruzen et al., 2015; Johnson et al., 2015c; Johnson et al., 2015a; Johnson et al., 2015b; Chapel et al., 2017; Zhao et al., 2020) thus results cannot be easily extrapolated to multiparous sows that might be more susceptible to HS due to the higher BW.

Environmental related factors

Relative humidity

As previously discussed, high air humidity slows down evaporation and can exacerbate HS effects especially in high ambient conditions. For example, increase in respiratory rate and subsequent increase in rectal temperature happens at a lower temperature (inflection point) in 60-kg group-housed pigs at 80% RH (21.3 and 24.6°C, respectively) than at 65% RH (22.6 and 27.1°C, respectively) or at 50% RH (23.4 and 26.6°C, respectively) (Huynh et al., 2005b) (**Figure 17**). Indeed, it has been suggested that an 18% increase in RH at 30°C is similar to a 1°C increase in air temperature (Curtis, 1983). This had evidently consequences on feed intake (decrease of -81, -99, and -106 g·d⁻¹ per 1°C above inflection point at 50, 65, and 80% RH, respectively) and thus on growth (ADG of 721, 644, and 630 g·d⁻¹ at 50, 65, and 80% RH, respectively) (Huynh et al., 2005b). Meanwhile, in individually housed pigs, decreased HL efficiency by panting only started at 90% RH and at ambient temperature above 30°C (Renaudeau, 2005).

Housing conditions (stocking density, floor type)

A higher stocking density can increase HS susceptibility of pigs since heat is conserved better with surrounding warm bodies. This can be one of the many factors explaining the discrepancy in the previously mentioned RH effects wherein respiratory rate increased at 80%

Table 5. Examples of nutritional and management strategies to mitigate heat stress in pigs.

Strategy	Principle and benefits	References
Reduce heat production		
Increase diet density (Decreased crude protein and fiber, use of synthetic amino acids, addition of fat)	Reduce heat increment Compensate for feed intake reduction	(Renaudeau et al., 2001; Le Bellego et al., 2002a; Wang et al., 2022)
Feed withdrawal after acute HS	More rapid decrease in body temperature due to no further TEF-related heat production	(Kpodo et al., 2020)
Increase heat losses		
Floor cooling	Increase temperature gradient thereby increasing conductive heat loss	(Silva et al., 2009; Cabezon et al., 2017a)
Wetting of the skin or water provision to increase wallowing (fogging, misting, etc.)	Increase evaporative heat loss through the skin	(Black et al., 1993; Huynh et al., 2007; Justino et al., 2014; Godyn et al., 2018)
Provision of chilled drinking water	Increase heat loss (by warming water to body temperature level)	(Jeon et al., 2006)
Increasing air speed	Increase convective heat loss	(Bjerg et al., 2017)
Night feeding	Increase feeding during the cooler parts of the day (higher temperature gradient)	(Choi et al., 2019)
Attenuate direct effects of heat stress		
Antioxidant supplementation (Vit. E, Selenium, Mitoquinol)	Reduce oxidative stress Improve intestinal integrity	(Liu et al., 2016; Liu et al., 2018a; Liu et al., 2018b; Le et al., 2019; Rudolph et al., 2021)
Pre- and probiotic supplementation	Gut microbiota modulation Improve intestinal integrity Improve immune functions Reduce pro-inflammatory responses	(Review by He et al., 2021)
Betaine supplementation	Energy-sparing (as osmolyte) to compensate for feed intake reduction Decrease intestinal permeability	(Schrama et al., 2003; Cabezon et al., 2017b; Le et al., 2020)
Electrolyte provision	Maintain electrolyte balance	(Ortega et al., 2022)
Plant extracts	Decrease intestinal permeability	(Le et al., 2020)
Chromium supplementation	Improve insulin sensitivity	(Liu et al., 2017) (Hung et al., 2014)
Cinnamon supplementation	Improve insulin sensitivity	(Cottrell et al., 2020)
Thiazolidinedione or lipoic acid supplementation	Improve insulin sensitivity (theorized; not yet studied)	(Rhoads et al., 2013)

RH at 21°C with group-housed pigs (Huynh et al., 2005b) compared to 90%RH at >30°C in individually housed pigs (Renaudeau, 2005). An environment that allows pigs to modify temperature gradient, e.g. decreased huddling in case of group housing or increased contact surface that can increase sensible HL (such as lying on the lateral instead of sternal side) (Huynh et al., 2005a) can attenuate severity of HS effects. Studies also suggest that floor type influences pig response to HS with pigs increasing their preference to lie in slatted floors than in solid floors in high ambient temperatures (Huynh et al., 2005c).

Possible interventions to mitigate heat stress effects

Many nutritional and management solutions have already been proposed and studied to mitigate HS effects in pigs and in other livestock species; some of which are presented in **Table 5**. Some of these interventions target the improvement of body heat balance (increase temperature gradient) either by reducing HP (e.g., decreasing heat increment) or increasing capacity for HL of the animal. Meanwhile, some aim to attenuate direct effects of HS in terms of reducing oxidative stress, improving intestinal integrity, increasing insulin sensitivity, and improving immune functions among others. While the ideal is to improve facilities and improve climate control of the building, depending on the energy cost it can be much more expensive than to incorporate nutritional and feeding management techniques to current intensive production systems (Mayorga et al., 2019). Nutritional interventions to mitigate HS effects in livestock animals have indeed been reviewed extensively (Cottrell et al., 2015) with other reviews focusing on the importance of improving insulin sensitivity (Rhoads et al., 2013) or improving gut health and integrity (He et al., 2021; Wen et al., 2021). Based on current literature, there are no studies to our knowledge on modifying feeding frequency to improve performance during a heat challenge. In TN conditions, modifying feeding behavior via feeding frequency has been shown to improve growth performance and alter metabolic responses (Schneider et al., 2011; Le Naou et al., 2014). With the large contribution of TEF and AHP to the instantaneous peaks of HP, increasing feeding frequency might be worth studying with the idea that splitting HP into smaller increments via provision of feed into smaller portions might help avoid overwhelming HL pathways.

Meanwhile, one nutritional intervention (supplementation of live yeast *Saccharomyces cerevisiae* var. *boulardii* or *S. boulardii*) has been demonstrated to modify feeding behavior in dairy cows (DeVries and Chevaux, 2014; Perdomo et al., 2020) and in pigs (Labussière et al.,

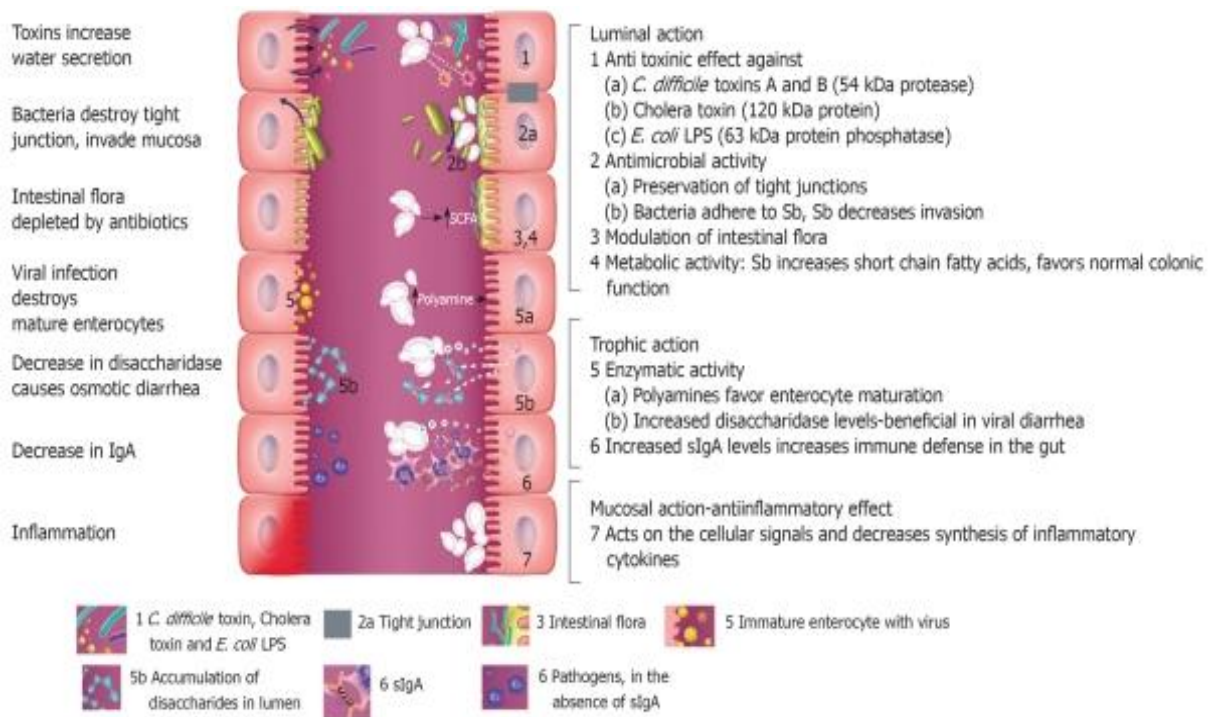


Figure 18. Potential modes of action of *Saccharomyces cerevisiae* var. *boulardii*
(Source: McFarland, 2010)

2021) in both TN and HS conditions. With reported mechanisms such as gut microbiota modulation and homeostasis (Chaucheyras-Durand and Durand, 2010), live yeasts (LY) supplementation is indeed a promising nutritional intervention during HS. Live yeast supplementation has already been reported to mitigate HS effects on feed intake in growing and finishing pigs, lactating sows, young calves, and dairy cows (Brewster et al., 2017; Lee et al., 2019; Perdomo et al., 2020; Domingos et al., 2021; Labussière et al., 2021). This translated to improved feed intake and energy efficiency in finishing pigs (Labussière et al., 2021), and milk production and piglet weight in lactating sows (Lee et al., 2019; Domingos et al., 2021; Rocha et al., 2022) subjected to high ambient temperature.

Potential mechanisms of live yeast in livestock animals during heat stress

Live yeasts belong to the category of probiotics which are live microorganisms beneficial to the hosts when ingested in appropriate and regular quantities (Chaucheyras-Durand and Durand, 2010). Probiotic activity is strain-dependent; thus this subsection mostly focuses on *Saccharomyces cerevisiae* var. *boulardii* or *S. boulardii* and the term live yeast (LY) will refer to this strain unless stated otherwise. This particular yeast is considered to be the probiotic strain because of its known thermotolerance with optimum temperature at 37°C, its ability to survive gastrotintestinal (GIT) transit (more resistant than *S. cerevisiae* in a gastric environment), and its inhibition of microbial pathogenic growth (Czerucka et al., 2007). The evidenced-based mode of action of *S. boulardii* is mainly in the GIT and has been reviewed by McFarland (2010) (**Figure 18**). In brief, *S. boulardii* has anti-toxin (blocking pathogen receptor site or acting as decoy receptor for pathogens), and antimicrobial effect (inhibition of pathogenic invasion by improvement of tight junctions and direct inhibition of pathogenic growth). Aside from gut microbiota modulation, LY has been shown to support normal microbiota establishment and to improve gut fermentation by increasing short chain fatty acids in times of disruption and disease. Other mechanisms also include improvement in nutrient utilization by secreting brush border enzymes and in immune responses by reducing pro-inflammatory responses and increasing antibody secretion such as secretory IgA.

Mechanism of *S. boulardii* during HS is still unclear but supplemented HS animals showed altered thermoregulatory responses (e.g. decreased rectal or skin temperature), altered meal behavior (increased meal frequency or shorter inter-meal intervals), decreased inflammatory responses, and modulated gut microbiota with an increase in *Lactococcus lactis*

that was correlated to an improved feed intake during HS (Lee et al., 2019; Perdomo et al., 2020; Labussière et al., 2021; Mayorga et al., 2021). Other potential mechanisms of *S. boulardii* may be related to decreased bacterial adhesion in intestinal cells, reduced pro-inflammatory responses, and increased immune responses of pigs (Lessard et al., 2009; Collier et al., 2011; Daudelin et al., 2011; Oswald et al., 2015; Gresse et al., 2021) which may attenuate discussed compromised immune system of HS animals. Intestinal and mucosal integrity were also improved by LY supplementation in healthy and immunocompromised pigs (Bontempo et al., 2006; Collier et al., 2011; Oswald et al., 2015; Trevisi et al., 2017) which might help with the discussed leaky gut symptoms observed in hot conditions. The beneficial effect in the GIT of *S. boulardii* that makes it an established therapeutic treatment for gut symbiosis, diarrhea, and constipation in humans (Kelesidis and Pothoulakis, 2012) might also apply to pigs especially since HS pigs seem to show symptoms like in humans with inflammatory bowel disease and diabetes (Jurjus et al., 2016; Hu et al., 2021). Reported attenuation of diabetes-related complications by *S. boulardii* such as hyperglycemia (insulin resistance) and leaky gut by gut modulation, decreased inflammatory and oxidative responses in the gut and liver, and an overall improved cardiovascular health (Brandão et al., 2018; Albuquerque et al., 2019; Cunha et al., 2020) thus might also be potential mechanisms during HS.

Section summary

Physiological and metabolic responses of growing pigs to maintain their body temperature in high ambient temperature results to reduced growth performance which is mainly due to the reduction in feed intake. However, heat exposure has also many less known direct effects on the metabolic and physiological responses of pigs that are usually evaluated using pair-feeding models in which *ad libitum*-fed HS pigs are usually compared with restricted-fed TN controls with strict feeding schedule. While severity of HS effects depends on many factors related to the animal and to the environment, recent studies show that a pig's prenatal thermal environment can also influence the pig's postnatal performance although this remains to be elucidated. Possible nutritional and management interventions have also been discussed but none seem to consider the importance of feeding behavior (timing, size, or frequency of meals) in the saturation of HL pathways during the course of the day. Thus, LY supplementation seems to be a potential nutritional intervention during HS especially with its reported effects on feeding behavior modification and energy efficiency.

Conclusion of the literature review

This literature review demonstrates that regardless of environmental conditions, pigs will strive to maintain a constant homeothermic body temperature conducive for biological process because failure to do so can cause heat injury and, in extreme cases, death. During a thermal challenge, animals maintain a thermal balance via biochemical, physiological, and/or behavioral adjustments collectively known as thermoregulation. Exposure to high ambient temperature limits their HL ability thereby disrupting the thermal equilibrium. When their heat load surpasses their capacity for heat dissipation and they can no longer maintain body temperature, they enter a state of HS. Decreased growth during a heat challenge is mainly related to the reduced feed intake since it is the pig's main adaptation response to limit heat production. However, in the recent years, more aspects of HS in pigs have been coming into light that have been previously unexplored.

Recent studies have shown that it is not just the immediate surroundings but also the prenatal thermal environment that affects pig performance in terms of their core body temperature, body composition, and their responses to a postnatal heat challenge. Results, however, are inconsistent perhaps due to differences in duration and stages of prenatal heat challenge across studies. Increasing number of studies have also been looking on the direct effects of heat exposure unrelated to feed intake such as increased adiposity and decreased metabolic flexibility that are demonstrated using pair-feeding models. However, it is difficult to assess which effects are due to heat exposure and which are confounded with the feeding pattern since TN pair-fed models with strict feeding schedule are usually compared with *ad libitum*-fed HS pigs.

Another aspect of HS in pigs often overlooked is its impact on feeding behavior even though both feeding level and behavior largely contribute to the instantaneous peaks of HP due to feeding-related AHP and TEF. These variations in HP and body temperature must also mean that maintaining thermal balance is a dynamic process throughout the day. Yet, most nutritional strategies to mitigate HS effects focus mainly on reducing the global heat load (e.g. improving energy utilization or reducing heat increment) and there are only a few studies on pigs investigating feeding management strategies (e.g. time or frequency of feeding). One nutritional intervention, however, has been recently demonstrated to modify feeding behavior in pigs and in dairy cows. Still, it is unclear whether the positive effects of LY (*Saccharomyces cerevisiae* var *boulardii* CNCM I-1079) supplementation on feed intake and energy efficiency

of HS pigs were due to an altered feeding behavior (higher meal frequency) and/or energy metabolism.

There are certainly many more elements about HS which need to be understood. The main goal of this thesis project is to contribute in bridging knowledge gaps in some emerging aspects of HS in pigs in four parts:

1. To investigate effects of a chronic prenatal heat challenge on the growth, body composition, and physiological responses of pigs in postnatal TN or HS conditions,
2. To evaluate and to confirm indirect (caused by a reduced feed intake) and direct HS effects (regardless of feed intake and pattern) on pig performance,
3. To determine whether the positive effects of LY supplementation in HS pigs is due to changes in feeding behavior or energy metabolism, and if these effects can be replicated by imposing an increased meal frequency, and
4. To further understand the dynamic process of how body heat balance is maintained by looking at variations in thermoregulatory and physiological responses during the day of pigs subjected to high ambient temperature.

General experimental strategy

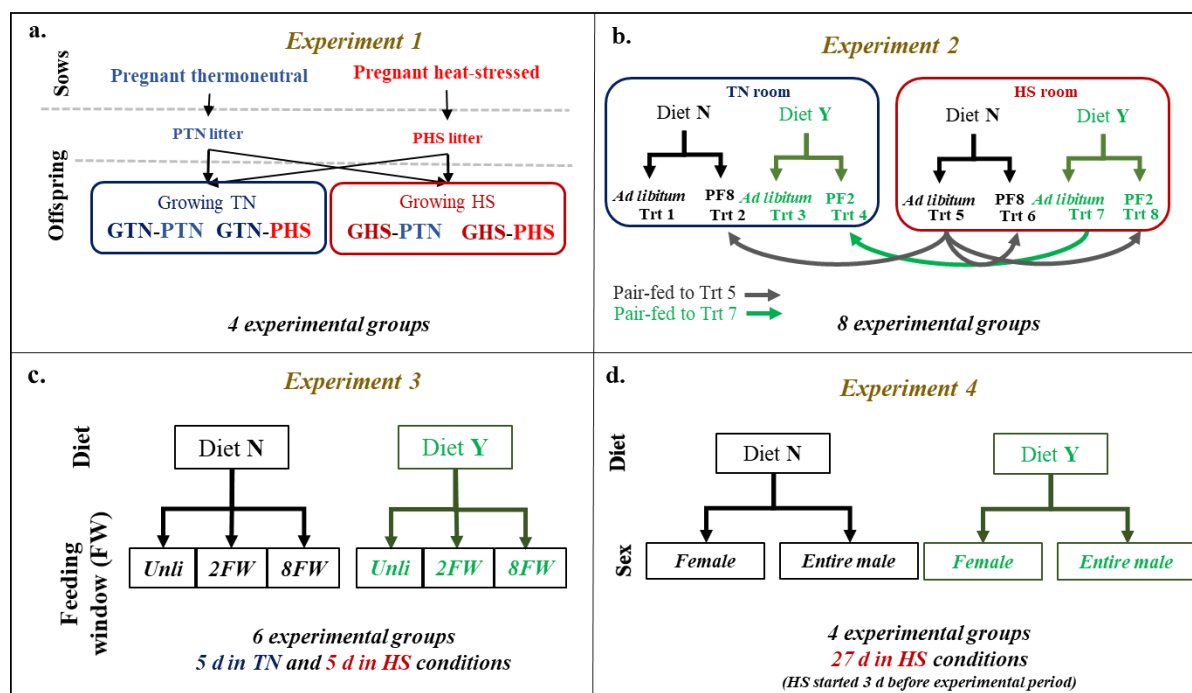


Figure 19. Summary of the experimental designs of the trials performed in the thesis project. TN: thermoneutral; HS: heat stressed; Diet N: control (0 g live yeast/ton); Diet Y: supplemented with live yeast (100 g live yeast/ton); PF2: pair-fed with 2 feed distributions; PF8: pair-fed with 8 feeding distributions; FW: feeding window.

General experimental strategy

Overview of the experiments

All experiments were conducted in the INRAE experimental facilities at the Unité Expérimentale Physiologie et Phénotypage des Porcs (UE3P) located in Saint-Gilles, France in accordance with the French legislation on animal experimentation. The French National Committee for Consideration of Ethics in Animal Experimentation approved all studies with authorization numbers APAFiS 2017080718212019 for Exp. 1, 2019020622003043 for Exp. 2 and 3, and 202106041718963 for Exp. 4. In this section, the experimental strategies used in the different experiments are explained regarding the type of animals, heat challenges, feeding managements, and phenotyping strategies employed in this study.

The overview of the experiments is presented in **Figure 19** (experimental treatments) and in **Table 6** (animals, housing, and heat challenge employed). Four experiments were planned that aimed to answer to the main questions of the thesis. The effects of a chronic prenatal HS were evaluated in Exp. 1 and the possible mechanisms and factors affecting positive effects of LY supplementation during HS were evaluated in Exp. 2, 3 and 4. Exp. 2 was designed to evaluate if positive effects of LY were related to a higher number of meals. This experiment was also designed to distinguish direct and indirect HS effects using a pair-feeding approach based on both feed restriction and change in meal frequency distribution. Exp. 3 was conducted in the respiratory chambers in order to determine whether or not LY effect was due to the altered feeding behavior or to modified energy metabolism that can explain improved energy efficiency (Labussière et al., 2021). In contrast to Exp. 2, feeding restriction in Exp. 3 was only limited to the time and duration of feeder access but not the amount of intake. Lastly, Exp. 4 was designed to have a link between Exp. 2 (all females) and Exp. 3 (all entire males) and with the idea that heat tolerance and response to LY supplementation adaptation may differ between sexes (Tower et al., 2020; Rudolph et al., 2021).

Animals

The sows used in Exp. 1 were all Large White × Landrace from the UE3P experimental herd. Most pig studies related to prenatal HS considered only primiparous sows (Boddicker et

Table 6. Summary of the experiments included in this thesis project¹

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
General idea	Prenatal HS	Live yeast and feeding management	Live yeast and feeding frequency	Live yeast and sex
Animal				
Number	24 mixed parity sows and 48 growing pigs	128 growing pigs	36 growing pigs	64 growing pigs
Sex	Females	Females	Entire males	Females & Entire males
Initial BW, kg	35.9 ± 0.8	66.1 ± 0.6	62.7 ± 1.1	60.9 ± 0.6
Final BW, kg	101.1 ± 1.1	92.7 ± 0.7	85.7 ± 1.2	106.1 ± 1.2
Housing	Group-housed 3 pigs in a 4 m ² pen	Individual 1 pig in a 2 m ² pen	Individual 3.3 m ²	Individual 1 pig in a 2 m ² pen
Environmental temperature				
Type	Cyclic 18 to 24°C vs. 28 to 34°C	Constant 22°C vs. 32°C	Constant 22°C then 28°C	Constant 32°C
Heat challenge duration	109 d for the sows; 58 d for the growing pigs	20 d	5 d	27 d
Treatments				
Factors	Pregnancy environment (TN vs. HS)	Ambient temperature (TN vs. HS)	<i>all groups experienced both TN and HS conditions</i>	<i>all groups were subjected to HS conditions</i>
	Growing environment (TN vs. HS)	Feeding management (Ad libitum vs. PF2 vs. PF8)	Feeding windows: Unlimited vs. 2 vs. 8	Female vs. Entire male
	-	LY supplementation (0 vs. 100 g/ton of feed provided on top)	LY supplementation (0 vs. 100 g/ton of feed)	LY supplementation (0 vs. 100 g/ton of feed provided on top)

¹TN= thermoneutral; HS=heat stress; LY=live yeast; PF2: pair-fed with 2 feed provisions; PF8: pair-fed with 8 feeding provisions.

al., 2014; Johnson et al., 2015c; Chapel et al., 2017; Zhao et al., 2020), but parity can affect sow response to high ambient temperature (Spencer et al., 2003; Iida et al., 2021; Renaudeau et al., 2021). Thus, mixed parity sisters (or at least half-sister sows) were used in the experiment to consider effects that might be related to age or parity and to remove the confounding effect of the litter origin of the sows. In addition, within each parity and experimental groups, females were inseminated using the same Pietrain sire origin (4 in total). In all experiments, pigs were blocked according to litter origin (full- or half-siblings) and according to the BW in order to have a uniform origin and average initial BW among the experimental groups.

For Exp. 1, the plan was to be able to compare also the offspring microbiota composition to that of their sow origin thus it was decided to use only female offspring (a total of 48 pigs) in the experiment since sex can have an effect on microbiota profile (Zhang et al., 2021). In Exp. 2, according to the numerous variation factors to be evaluated (ambient temperature, feeding management, and LY supplementation) it was not possible to test a sex effect. Thus, only female growing pigs were used (a total of 128 pigs). Due to building constraints, only 32 individual pens (2 rooms) were available at a given time and thus the experiment was conducted in 4 successive replicates (32 pigs per replicate). The plan for the experiment in the respiratory chambers (Exp. 3) was already to use entire male pigs to provide a link with the previous study which was the basis of the experimental design on LY and feeding frequency (Labussière et al., 2021). Since only two pigs can be measured at the same time and because pigs from the experimental herd were only available every 3 weeks, Exp.3 was conducted in 9 replicates with 4 pigs coming from the same litter per replicate for a total of 36 entire male growing pigs. Finally, both females and entire males (a total of 64 pigs; 32 each sex) blocked according to their litter origin were used in Exp. 4 to connect the experiments with LY (Exp. 2 and Exp. 3) in terms of similarities and differences of HS responses based on the sex of the animal. Exp. 4 was conducted in two successive replicates [16 female (**F**) pigs and 16 entire male (**EM**) pigs per replicate] because to similar building constraints as described for Exp. 2).

Housing

All experimental rooms used in the study were climate controlled and the ambient temperature of the rooms are presented in **Figure 20**. Experiments 1, 2, and 4 were conducted in the same experimental facilities in which two rooms were used. For Exp. 1 and 2, one room

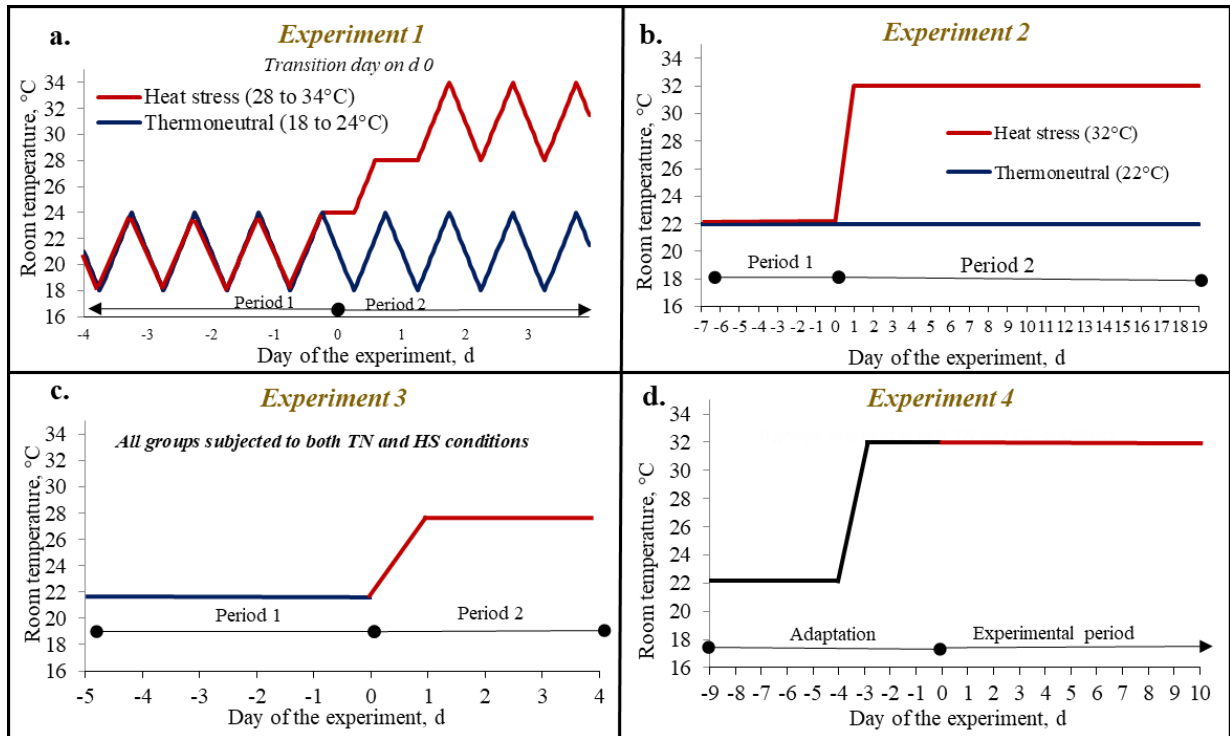


Figure 20. Ambient temperature of the rooms in the experiments. In Exp. 1, 2, and 3, period 1 was considered to be where all groups were in TN environment and period 2 when the HS challenge already started with d 0 considered as the transition between the periods.

(a) Exp. 1: period 1 from d -7 to -1; period 2 was from d 0 to 57. (b) Exp. 2: period 1 from d -7 to d -1; period 2 was from d 0 to 19. (c) Exp. 3: period 1 from d -5 to -1; period 2 was from d 0 to 4. (d) Exp. 4: Experimental period started after a 3-day adaptation to the HS challenge from d 1 to d 27.

was maintained in TN conditions (18 to 24°C for Exp. 1 or 22°C for Exp. 2) for the whole experimental period, while the other room was in TN conditions during the adaptation and pre-challenge period and was afterwards maintained in HS conditions (28 to 34°C for Exp. 1 or 32°C for Exp. 2). These same two rooms were used in Exp. 4 but with both rooms maintained at 22°C for 7 d and then at 32°C for 3 d during the adaptation period before the 27 d experimental period at 32°C. For the transition to the heat challenge period, ambient temperature in Exp. 1 gradually changed at a rate of 1°C/h from 0600 to 1800 hours and was thereafter maintained in cyclic HS conditions of 28 to 34 °C while ambient temperature in Exp. 2 and 4 was gradually changed at a rate of 2°C/h from 1200 to 1800 hours and was thereafter maintained in constant HS conditions of 32°C. Relative humidity was not controlled but was measured for the aforementioned experiments. For Exp. 3, ambient temperature was maintained during the experimental period at TN conditions of 22°C during the first 5 d of measurement and was increased for the last 5 d to 28°C with the transition on d 0 (fixed at 25°C at 0800h and an increase of 1°C/h from 0800h to 1100h) regardless of the experimental group. Relative humidity was kept constant at 65%.

In Exp. 1, each room had 8 slatted pens (4 m²) designed for housing three pigs each and equipped with a feeder and nipple drinker. In Exp. 2 and 4, the same experimental rooms were used but were partitioned into 16 slatted pens (2 m²) per room in which pigs were individually housed. These two rooms had different climate control systems in which one room has a full air-conditioning system while the other only has a heating system (thus less homogeneity in the room). In Exp. 2, a makeshift automatic feed distributor system was placed in the pens that were assigned for pair-fed groups with 8 feeding distributions (**Figure 21**). In experiment 3, pigs were individually housed in metabolic crates equipped with an automatic feeder (with a feeding trap) and a bowl drinker during the adaptation period. They were then moved to another metabolic crate (3.3 m²) in a 12-m³ open circuit respiration chamber (as described by Vermorel et al., 1973) for the duration of the experimental period (**Figure 22**). Automatic feeder and bowl drinker was also provided in inside the respiratory chamber similar to those used during the adaptation period.

Heat challenge

As discussed in the literature review, responses elicited can differ depending on the type (constant or cyclic ambient temperature) and duration (acute or chronic) of the heat

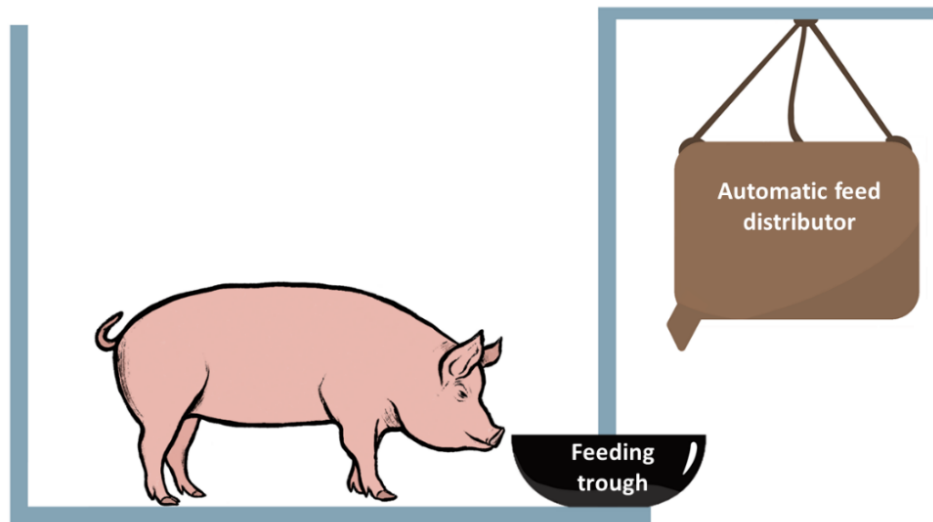


Figure 21. Diagram of the set up in the experimental pen for Experiments 1, 2, and 4 where pigs were individually housed. A makeshift automatic feed distributor was provided only for Experiment 2 for pigs with 8 feeding distributions.

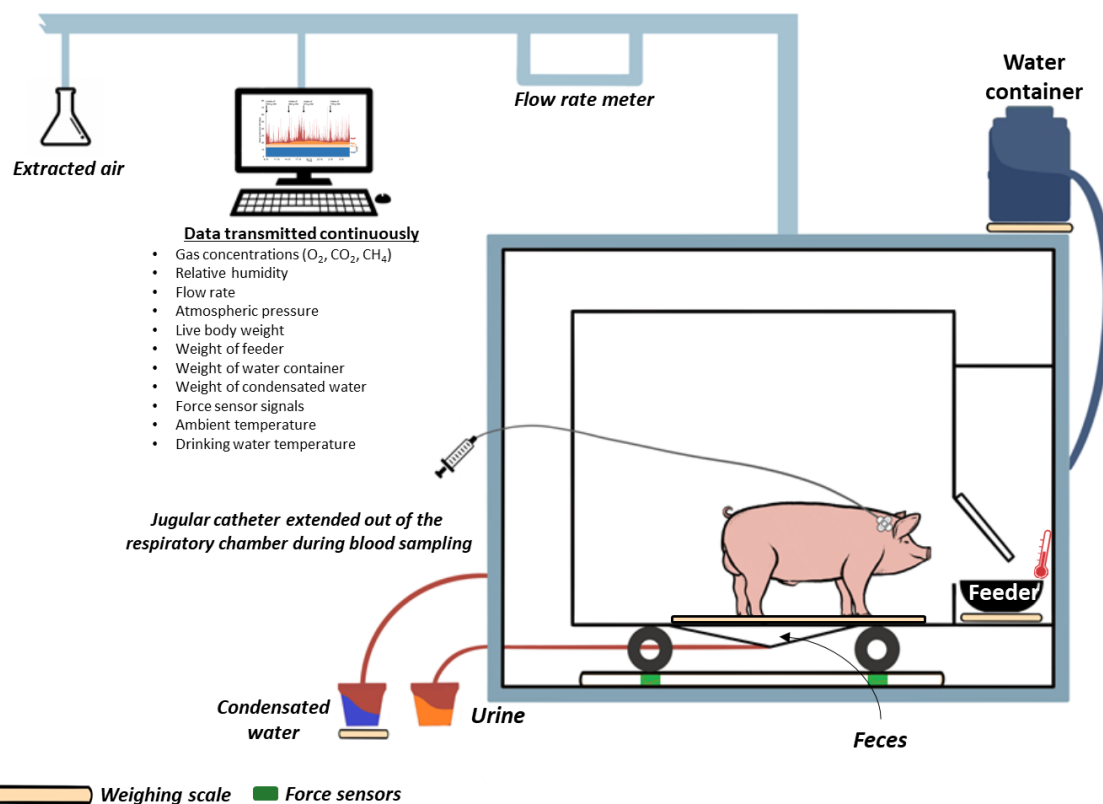


Figure 22. Diagram of the set up in the respiratory chamber for Experiment 3.
 Not pictured: drinking bowl (wherein drinking water temperature was continuously measured).

challenge. In Exp. 1, the sows and offspring growing pigs were subjected to the same cyclic and chronic thermal challenge to simulate the natural diurnal variations in ambient temperature during summer. Meanwhile, a constant ambient temperature was chosen for the experiments with LY supplementation (Exp. 2, 3, and 4) due to its hypothesized effect on feeding behavior and thus to avoid possible confounding effect of a cyclic heat challenge on feeding behavior, e.g. increased feeding activity in cooler parts of the day (Xin and DeShazer, 1991; Quiniou et al., 2000c; Patience et al., 2005). The chosen ambient temperature considered to be the heat challenge for Exp.3 was also different. Due to the climate-controlled, relatively small, and fully insulated compartment of the respiratory chamber (Exp. 3) compared to the climate-controlled, mechanically ventilated rooms in Exp. 2 and 4, the air was at the same temperature as all the objects and equipment inside the chamber which could be why pigs already have trouble coping with an ambient temperature of 28°C in this housing condition as described in a previous experiment (Labussière et al., 2021). Thus, an ambient temperature of 28°C was deemed sufficient to elicit HS responses in pigs housed in respiratory chambers compared to 32°C in the climate-controlled rooms used in the other experiments.

Diet and feeding management

Experimental diets

The experimental diets are presented in **Table 7** and were all provided in pelleted form. Commercial diet formulations were used for Exp. 1 (growing diets) and for Exp. 2 and 4 (finishing diets), while Exp. 3 diets were especially formulated for the experiment and was produced in the UE3P experimental feed mill. For the supplemented diets, LY was incorporated at 100 g/ton of feed (1×10^6 CFU *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079/g of feed) in the feed before pelleting in the experimental feed mill for Exp. 2 and 4, and within the formula for Exp. 3. In order to ensure survival of the live yeast in feed, pelleting temperature did not exceed 65°C. Live yeast count in the feed was also conducted before each experiment by an external laboratory before feeding to the animals to ensure that the feed actually contained the minimum CFU count. In a previous experiment (Labussière et al., 2021), improved feed intake and retained energy with live yeast supplementation was mainly associated to increased deposition of fat and only tended to increase that of protein. Thus, the experimental finishing diets for Exp. 3 were formulated to be limiting in protein (0.65 vs. 0.84

Table 7. Basal composition of the experimental growing and finishing diets.^{1,2}

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Ingredients, % as-fed				
Wheat	26.20	34.00	26.73	33.82
Barley	25.55	25.00	26.73	25.00
Corn	16.00	15.00	26.73	15.00
Soybean meal	19.00	5.06	4.66	5.00
Rapeseed meal	-	9.00	4.62	9.00
Soybean hulls	-	-	1.71	-
Wheat bran	5.00	8.00	1.71	8.00
Vegetable oil	2.00	1.00	0.63	1.00
Micro ingredients	6.25	2.94	6.13	3.18
Stearin	-	-	0.05	-
TiO ₂	-	-	0.30	-
Chemical composition, % as fed⁴				
Dry matter	88.4	88.7	88.9	88.8
Crude Protein	16.3	14.8	12.4	15.6
Crude Ash	4.6	4.5	4.4	4.1
Crude Fat	3.3	2.8	2.7	3.2
NDF	12.3	14.6	11.7	14.7
ADF	3.9	5.0	4.1	4.6
ADL	1.10	1.5	0.8	1.2
Starch	45.3	40.6	47.8	-
Formulated values, % as fed				
Net energy, MJ/kg	9.66	9.62	9.60	9.62
SID lysine : NE (g/MJ NE)	0.83	0.84	0.65	0.84

¹All diets fed in pellet form.

²Diet formulation shown for experiments 2, 3, and 4 are the control (non-supplemented) diets. For the supplemented diet, live yeast was added on top (0.10%) for the diets of Exp. 2 and 4, and was included in the formulation for Exp. 3 (0.10% live yeast instead of 0.05% stearin).

³Including synthetic amino acids, vitamins and minerals.

SID Lys:NE of the diets in the other experiments) in order to more clearly evaluate differences in protein deposition among the experimental groups.

Feeding management

All pigs were adapted to their assigned experimental diets and feeding distribution schedule before the beginning of measurements during the experimental period (5, 7, 17, and 10 d of adaptation for Exp. 1, 2, 3, and 4 respectively). Feeding management was kept simple for Exp. 1 and 4 with all pigs having free access to feed during the whole day (*ad libitum* feeding), but with 2 manual feed distributions per day (one at 0900h and one at 15h00) in order to avoid spillage and soiling of the feed.

Three feeding managements were employed in Exp. 2 depending on the experimental groups: *ad libitum* feeding, and pair-feeding with 2 (**PF2**) or 8 (**PF8**) feeding provisions. Feed was distributed twice at 0900h and at 1500h for the *ad libitum*-fed and PF2 groups, while it was distributed at every 90-min interval from 0900h to 1930h in the PF8 groups. Despite its limitations, pair-feeding is a widely accepted technique to serve as a control for feed intake (Russell et al., 2008; Curfs et al., 2011) and the different number of feed provisions were added to allow evaluation of the effects of feeding frequency. In this experiment, pair-feeding was based on the pig's predicted BW and on the 3-d average feed intake per kg BW of either the non-supplemented *ad libitum* or live yeast supplemented *ad libitum* group in the HS room to smooth the inter-day variations. The calculated daily ration of the PF groups was then divided into two or eight feed provisions depending on their experimental group. On d 1 and 2 of the heat challenge, the HS *ad libitum* groups were exceptionally given feed allowance of –20% and –10% of their pre-challenge ADFI to remove the risk of inaccurate pair-feeding on the first days of the thermal challenge. The values used (–20% and –10% ADFI reduction on the first 2 days) were based on the results obtained in Exp. 1.

Many comparisons were considered in designing Exp. 2 as follows:

1. *Ad libitum* groups were used to compare the effect of HS in non-supplemented (**Trt 1 vs. 5**) and in supplemented (**Trt. 3 and 7**) pigs,
2. *Ad libitum* groups were also used to compare the effects of LY supplementation in TN (**Trt 1 vs. 3**) and HS (**Trt 5 vs. 7**) conditions,
3. Pair-fed groups were used to determine the direct effects of HS regardless of the feeding level and behavior [(**Trt 2 vs. 6**) and (**Trt. 4 vs. 7**)],

Table 8. Summary of the measurements taken during the experiments in this thesis project¹

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
General idea	Prenatal HS	Live yeast and feeding management	Live yeast and feeding frequency	Live yeast and sex
Live BW	Manual	Manual	Automatic and manual	Manual
Growth parameters	Yes	Yes	Yes	Yes
Slaughter	Yes, comprehensive	Yes, comprehensive	n/a	Yes, only carcass weight & lean meat %
Nitrogen balance	n/a	n/a	Yes	n/a
Feeding and drinking behavior	n/a	Infrared barrier (feeder presence)	Weight of feeding trough and water container (weight differences)	n/a
Physical activity	n/a	n/a	Yes	n/a
Thermoregulation	Rectal and skin temperature	Rectal and skin temperature	Heat loss partitioning (evaporative, sensible, etc.)	n/a
	-	Continuous measure of body temperature	Continuous measure of body temperature	-
	-	Respiratory frequency	-	-
Energy utilization	n/a	n/a	Yes	n/a
Blood sampling	Spot sampling at 1330h on d -4, 3, 49	Spot sampling at 1330h on d -1, 1 8, 20	Periprandial kinetics on d -1 and 1	n/a
Microbiota	Spot fecal sampling	Spot fecal sampling	Spot fecal sampling	Spot fecal sampling

¹TN= thermoneutral; HS=heat stress; LY=live yeast

4. The non-supplemented groups in the HS room (*Trt 5 vs. 6*) were used to assess if increasing meal frequency can improve HS response of pigs,
5. The PF2 supplemented group in the HS room was used to evaluate the effect of LY supplementation regardless of feed intake and without allowing them to increase feeding frequency (*Trt 5 vs. 8*), and
6. PF8 non-supplemented pigs and PF2 supplemented pigs (*Trt 6 vs. 8*) were used to evaluate if increasing feeding frequency is comparable to supplementing LY in HS conditions.

Although time and amount of feed provision were regulated in Exp. 2, it must be noted that due to the limitations of the facilities, pigs still had access to the feeding trough any time of the day regardless of if there was feed in trough or not.

Exp. 3 was also designed to evaluate LY and feeding frequency in hot conditions. A total of: 3 feeding management practices called feeding windows (**FW**) was applied: unlimited access (Unli; 0900h to 1545h and 1600h to 0700h), 2FW of 1 h each (from 0900h to 1000h and from 1500h to 1600h), and 8FW of 15 min each (at every 90-min interval from 0900h to 1930h), in which both the 2FW and 8FW groups were still allowed a total of 2 h of feed access per day. In contrast to Exp. 2, only time and duration of feeding were restricted in Exp. 3 but not the amount of feed available in the feeder. This was made possible due to the automated feeding traps designed to provide or restrict access to the feeder. Time of feed distribution were at 0855h and 1455h for the Unli and 2FW groups, and 5 min before each feeding window for the 8FW groups.

Measurements

Summary of the measurements taken in the four experiments are shown in **Table 8**. Description of the methodology for the measurements and calculations done in Exp. 1, 2, and 3 are described in more details in Publications 1, 2, and 3 respectively. For Exp. 4, growth and feed intake measurements are similar to the method applied as described in Publication 2.

Growth and body composition

Individual pig BW was measured manually at the beginning and at the end of adaptation period and then every 2 weeks for Exp. 1 and every week for Exp. 2 during the experimental

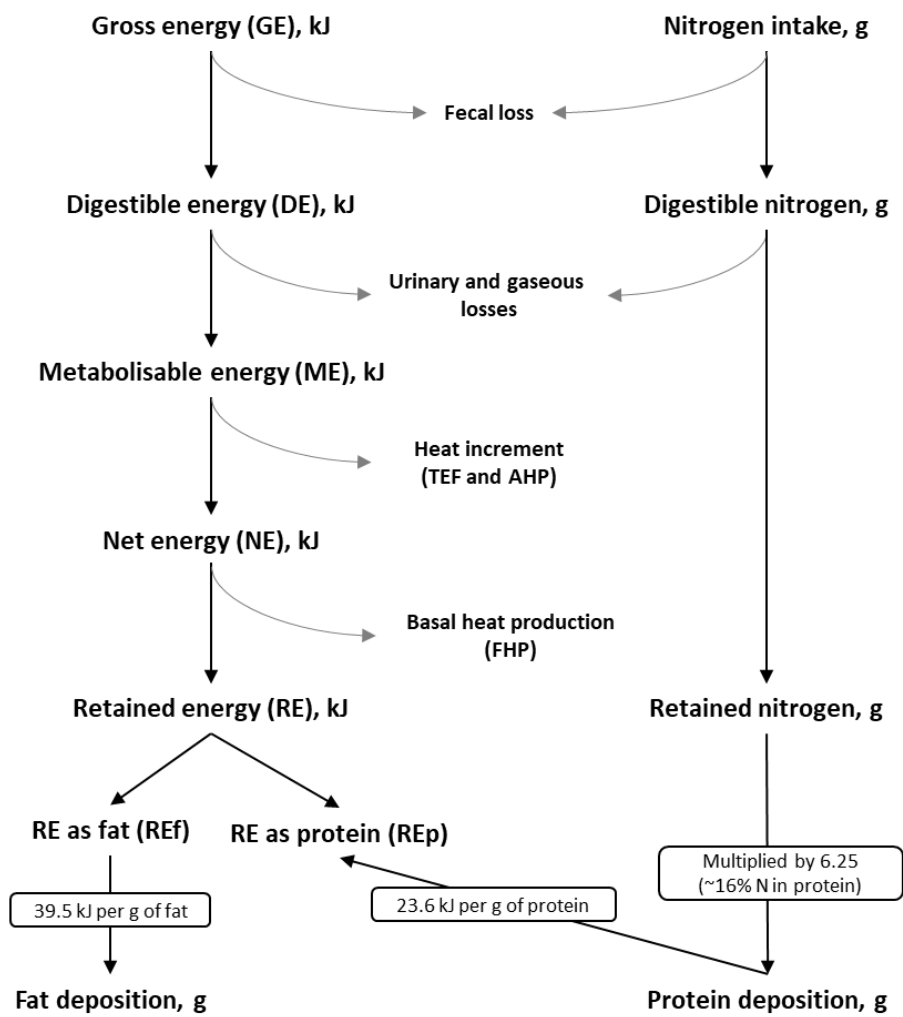


Figure 23. Energy and nitrogen balance

period. In Exp. 4, pigs were manually weighed at the beginning of adaptation, 3 d after the heat challenge has started, and then every week during the experimental period. For Exp. 1, 2, and 4, pigs were weighed on the day of slaughter after a 24 hour fasting period. For Exp. 3, pigs were manually weighed before entering and after exiting the respiration chambers but during the experimental period, BW was automatically transmitted and registered each time the pig stepped in front of the feeding trough through a platform equipped with a set of calibrated load sensors. The general practice in an experiment in the respiratory chamber is to take the pig out of the respiratory chamber to weigh them manually in between periods. However, it was necessary to have enough days wherein the pigs were adapted in the chambers with the least disturbance as possible to the animal since time was limited (only 5 d each for periods 1 and 2). Furthermore, moving the pigs out to be weighed can be a stressful event which can likely affect adaptation to HS, thus the automatic body weighing system was put in place to still be able to measure the BW without the pigs exiting the chambers.

In Exp. 1 and 2, a comprehensive slaughter performance measurement was conducted in the experimental abattoir of INRAE- UE3P which included measurement of different body components, while in Exp. 4, only slaughter body weight and lean meat percentage were measured in a commercial slaughterhouse. In Exp. 3, protein and fat deposition were calculated based on the nitrogen and energy balance measurements (**Figure 23**). For digestibility measurements, the indigestible marker method was chosen over the total collection method to have the least disturbance to the animal during the transition in between periods.

Behavior (feeding behavior and physical activity)

Feed intake was measured in all the four experiments, feeding behavior in Exp. 2 and 3, and drinking behavior in Exp. 3. For feed intake measurement, in brief, feed allowance and refusals were weighed per day. The daily feed intake was measured as the difference between offered and refused feed and, depending on the experiment, was then averaged per day and per period. For feeding behavior measurement in Exp. 2, an infrared barrier system was installed in each of the individual feeding trough. Feeder presence was thus measured each time the infrared barrier was interrupted by the pig when eating. For Exp. 3, the feeding trough and the water container (outside the chamber) were equipped with weight sensors whose data was continuously transmitted and registered. Thus, feeding and drinking behavior were detected based on weight differences. For the measurement of physical activity in Exp. 3, the metabolic

Table 9. Plasma hormones and metabolites analyzed in Experiments 1, 2, and 3¹.

Ingredients, % as-fed	Exp. 1	Exp. 2	Exp. 3²
Hormones			
Insulin	-	Yes	Yes
Thyroid hormones (T3, T4)	Yes	Yes	Yes
Metabolites			
Glucose	Yes	Yes	Yes
Lactate	-	-	Yes
Creatinine	Yes	Yes	Yes
NEFA	Yes	Yes	Yes
Urea	-	-	Yes
BAP	Yes	-	-
SOD	Yes	-	-
Enzyme activity			
Creatinine kinase	Yes	-	-
Lactate dehydrogenase	Yes	-	-

¹NEFA: Non-esterified fatty acids; BAP: biological antioxidant potential; SOD: Superoxide dismutase.

²Postprandial plasma kinetics measurement except for the thyroid hormones which was measured once at the beginning of the meal.

crate was mounted on force sensors producing an electrical signal proportional to the physical activity of the pig which was also continuously transmitted and registered.

Thermoregulation responses

In order to evaluate the thermal status of the pigs, rectal and skin temperature in both Exp. 1 and 2, and respiratory frequency (in Exp. 2 only) were measured on certain days of periods 1 and 2. Additional rectal and skin temperature, and respiratory frequency measurements were conducted in Exp. 2 at 0, 2, 4, and 6 h after the initial increase of ambient temperature during the transition period in order to evaluate acute responses in Exp. 2. In addition, a temperature probe that was implanted within the brachiocephalic muscle also allowed continuous measurements of internal body temperature in Exp. 2 and 3. A dynamic view of the partitioning of evaporative and sensible HL were also made possible by the continuous measurement of RH and air in the chambers based on the principle described by Renaudeau et al. (2013) and the measurement of water temperature.

Animal metabolism

The use of respiratory chambers in Exp. 3 allowed continuous measurement of the pig's heat production calculated based on respiratory gas exchanges, CH₄, and urinary nitrogen (including evaporated nitrogen) (**Text boxes 1 and 2**). Dynamic measurement of the HP partitioning into AHP, TEF, and MHP were also possible due to the simultaneous measurements of O₂ and CO₂ concentrations in the respiration chamber, of force sensor signals, and of physical characteristics of the gas in the chamber based on the modeling approach by van Milgen et al. (1997) but with the modifications described by Quemeneur et al. (2020).

Blood sampling and subsequent analysis of plasma hormones and metabolites were also conducted in order to evaluate physiological, metabolic, and oxidative status of the animals in different ambient temperatures in Exp. 1, 2, and 3 (**Table 9**). Blood sampling was done at 1330h in Exp. 1 and 2 once in TN conditions (d -4 and d-1, respectively) and on certain days of HS (d 3 and 49 of HS in Exp.1 and d 1, 8 and 20 of Hs in Exp. 2) in order to evaluate the effect of heat exposure duration on the plasma parameters. In Exp. 3, a catheter was inserted in the external jugular vein through a collateral vein under general anesthesia as described by Melchior et al. (2004) to be able to do successive periprandial blood samplings (on d -1 and d 1 of HS) with the least disturbance to the animal. This allowed evaluation of the metabolic

status of the animal during its fasted and fed state, and the kinetics of the plasma hormones and metabolites during and after a meal.

Microbiota

Spot fecal samples were collected in all experiments but only those in Exp. 1 were thus far analyzed and presented in this thesis project. In order to evaluate if prenatal and postnatal HS affects the microbiota of the pigs, spot fecal samples were collected on d 4 and d 105 of gestation in the sows (pre-challenge and after 96 d of chronic HS exposure, respectively), and on 78 and 136 d of age (pre-challenge and after 58 d of chronic HS exposure, respectively) in the growing pigs. The samples were then analyzed for 16S rRNA analyses of microbiota composition.

Results

Results

This section consists of four chapters corresponding to each of the four experiments:

Chapter 1. Chronic prenatal heat stress alters growth, carcass composition, and physiological response of growing pigs subjected to postnatal heat stress

Chapter 2. Effect of heat stress and feeding management on growth performance and physiological responses of finishing pigs

Chapter 3. Effect of live yeast supplementation and feeding frequency in heat stressed pigs

Chapter 4. Effect of sex and live yeast supplementation in heat stressed pigs

After a short introduction for each chapter, principal findings are presented. In case of supporting or supplementary information, these can be found in the Annex.

Chapter 1. Chronic prenatal heat stress alters growth, carcass composition, and physiological response of growing pigs subjected to postnatal heat stress

This chapter presents the results of Experiment 1 with focus only on the female growing offspring. The results dealing with growth, carcass composition, and physiological responses were initially presented in the 6th EAAP International Symposium on Energy and Protein Metabolism (ISEP 2019) and were afterwards published in the Journal of Animal Science in 2020 as presented in the succeeding pages. Meanwhile, the preliminary results of the microbiota analyses were presented in the 72nd Annual Meeting of the European Federation of Animal Science (EAAP) in 2021 as shown in *Annex 3*.



FETAL PROGRAMMING

Chronic prenatal heat stress alters growth, carcass composition, and physiological response of growing pigs subjected to postnatal heat stress

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Abstract

Postnatal heat stress (HS) effects on pig physiology and performance are widely studied but prenatal HS studies, albeit increasing, are still limited. The objective of this study was to evaluate the chronic prenatal HS effects in growing pigs raised in postnatal thermoneutral (TN) or in HS environment. For prenatal environment (PE), mixed-parity pregnant sows were exposed to either TN (PTN; cyclic 18 to 24 °C; $n = 12$) or HS (PHS; cyclic 28 to 34 °C; $n = 12$) conditions from day 9 to 109 of gestation. Two female offspring per sow were selected at 10 wk of age and allotted to one of two postnatal growing environments (GE): GTN (cyclic 18 to 24 °C; $n = 24$) and GHS (cyclic 28 to 34 °C; $n = 24$). From 75 to 140 d of age, GTN pigs remained in GTN conditions, while GHS pigs were in GTN conditions from 75 to 81 d of age and in GHS conditions from 82 to 140 d of age. Regardless of PE, postnatal HS increased rectal and skin temperatures (+0.30 and +1.61 °C on average, respectively; $P < 0.01$) and decreased ADFI (−332 g/d; $P < 0.01$), resulting in lower ADG and final BW (−127 g/d and −7.9 kg, respectively; $P < 0.01$). The GHS pigs exhibited thicker backfat ($P < 0.01$), lower carcass loin percentage ($P < 0.01$), increased plasma creatinine levels ($P < 0.01$), and decreased plasma glucose, nonesterified fatty acids, T3, and T4 levels ($P < 0.05$). Prenatal HS increased feed intake in an age-dependent manner (+10 g/kg BW^{0.60}·d^{−1} for PHS pigs in the last 2 wk of the trial; $P = 0.02$) but did not influence BW gain ($P > 0.10$). Prenatal HS decreased the plasma levels of superoxide dismutase on day 3 of GHS (trend at $P = 0.08$) and of T4 on day 49 ($P < 0.01$) but did not affect T3 on day 3 nor 49 ($P > 0.10$). Prenatal HS increased rectal and skin temperatures and decreased temperature gradient between rectal and skin temperatures in GTN pigs (+0.10, +0.33 and −0.22 °C, respectively; $P < 0.05$) but not in GHS pigs ($P > 0.10$). There were also PE × GE interactions found with lower BW ($P = 0.06$) and higher backfat ($P < 0.01$) and perirenal adiposity ($P < 0.05$) for GHS–PHS pigs than the other groups. Overall, increased body temperature and altered thyroid functions and physiological stress responses suggest decreased heat tolerance and dissipation ability of pigs submitted to a whole-gestation chronic prenatal HS. Postnatal HS decreased growth performance, increased carcass adiposity, and affected metabolic traits and thyroid functions especially in pigs previously submitted to prenatal HS.

Key words: carcass adiposity, growth, pig, postnatal heat stress, prenatal heat stress, thermoregulation

Introduction

As extreme heat events become longer, more frequent, and more intense, the impact on swine production also increases (Luber and McGeehin, 2008; IPCC, 2014). During postnatal heat

stress (HS), pigs decrease their feed intake (FI) as an adaptive response to reduce heat production (HP) (Renaudeau et al., 2008, 2013). These negative effects are heightened with the selection for higher lean percentage as HP increases with higher lean

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BAP	biological antioxidant potential
BFT	backfat thickness
BW	body weight
CGM	Capteur Gras Maigre (Lean Fat Sensor)
CK	creatine kinase
CP	crude protein
DM	dry matter
FCR	feed conversion ratio
FI	feed intake
GE	growing environment
GHS	growing heat stress
GTN	growing thermoneutral
HP	heat production
HPA	hypothalamic-pituitary-adrenal
HS	heat stress
LDH	lactate dehydrogenase
NE	net energy
NEFA	nonesterified fatty acids
PE	prenatal environment
PHS	prenatal heat stress
PTN	prenatal thermoneutral
sBW	slaughter BW
SOD	superoxide dismutase
TN	thermoneutral

tissue accretion rate (Brown-Brandl et al., 2014) resulting in less heat-tolerant pigs. In female mammals such as the sow, hyperthermia can induce physiological changes that can impair oocyte development, early embryonic development, fetal and placental growth, and nursing performance (as reviewed by Hansen, 2009) which can all affect the subsequent growth of the offspring. It is thus important to understand how prenatal HS can affect the pig's postnatal performance.

Prenatal HS can be defined as the in utero exposure of the offspring to maternal hyperthermia (Edwards, 1969; Lary, 1986). There are many studies in mice suggesting that prenatal HS can depress brain and body growth (Shiota and Kayamura, 1989; Hinoue et al., 2001), although the effects of prenatal stress, in general, can also depend on stress duration: acute prenatal stress enhances protection of fetus from maternal corticosteroids but chronic prenatal stress weakens it (Welberg et al., 2005). In farm animals, thermal conditioning during incubation have been reported to influence adaptive HS response of poultry species (Loyau et al., 2015), while in calves, prenatal HS can alter deoxyribonucleic acid (DNA) methylation profile and reduce growth performance (Monteiro et al., 2016; Skibieli et al., 2018). Recent studies in pigs suggest that sows exposed to gestational HS produced pigs with increased core body temperature and altered metabolic processes, body composition, and thermal responses (Boddicker et al., 2014; Cruzen et al., 2015; Johnson et al., 2015a, 2015b, 2015c; Chapel et al., 2017). The objective of this study was to evaluate the effects of chronic prenatal HS on the growth performance, body composition, and physiological responses of growing pigs in postnatal thermoneutral (TN) or HS environment.

Materials and Methods

The experiment was conducted in accordance with the French legislation on animal experimentation and was approved by the French National Committee for Consideration of Ethics in Animal Experimentation (Authorization: APAFiS #11016-2017080718212019 delivered on September 26, 2017).

Experimental design and animal management

The study was conducted in the INRAE experimental facilities at the Unité Expérimentale Porcs de Rennes (UEPR) located in Saint-Gilles, France from October 2017 to June 2018. The general framework of the experimental study is presented in Figure 1.

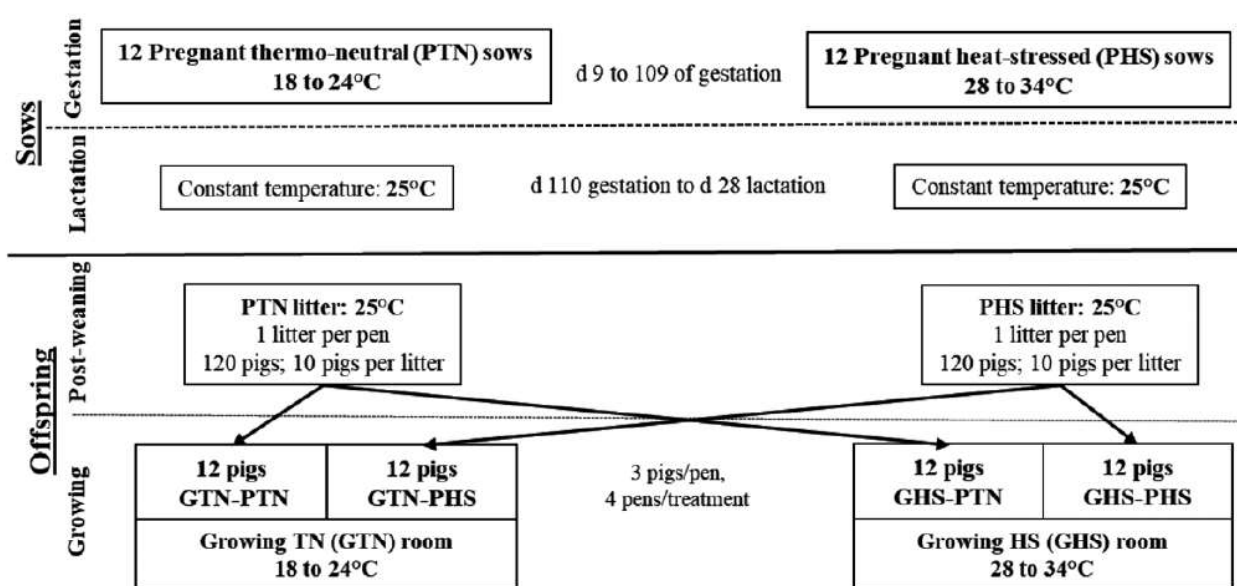


Figure 1. Framework of the experimental study from gestation to growing period. Pregnant sows (12 primiparous and 12 multiparous) were housed under either PTN or PHS conditions from 9 to 109 d of gestation. Their female offspring were subjected to GTN or GHS conditions from 82 to 140 d of age. Sows during lactation and piglets during post-weaning were housed under TN conditions.

Gestating and lactating sows

A total of 16 gilts and 16 multiparous sows (16 blocks of 2 sisters) were initially blocked according to parity and litter origin with additional blocking factors for the multiparous sows, that is, body weight (BW) and backfat thickness (BFT) at weaning of their respective litters. The animals were kept in one of two identical rooms during their pregnancy: one thermoneutral (PTN) room and one heat-stressed (PHS) room. Each room was equipped with two pens. In both rooms, gilts and sows were placed in separate pens (eight animals per pen of 4.5×4.8 m). The gilts were moved to the gestation rooms 3 wk prior to the expected date of breeding while the sows were blocked and transferred on the day of weaning immediately after weighing and BFT measurement. Sows were artificially inseminated with four different sire origins, with one sire used to inseminate four females (two multiparous and two primiparous sows) per treatment. One PHS primiparous sow was removed prior to insemination because of urogenital infection. From day 0 to 6 of gestation, the ambient temperature was kept under cyclic TN conditions (18 to 24 °C) in both experimental gestation rooms. The PTN sows were maintained at this environmental temperature regimen until day 109 of gestation. In the PHS room, the ambient temperature was gradually increased from day 6 to 9 and thereafter maintained under cyclic HS conditions (28 to 34 °C) from day 9 to 109 of gestation. Whatever the temperature treatment, the minimum and maximum temperatures were reached at 0600 and 1800 hours, respectively (Figure 2a). All animals were given a commercial gestation feed (13.6% crude protein (CP); 2,300 kcal/kg net energy [NE]) following a daily individual feed allowance calculated according to Dourmad et al. (1997). The daily ration was distributed in two meals at 0830 and at 1600 hours and water was provided ad libitum.

On day 110 of gestation, 12 sows (6 primiparous and 6 multiparous) from each temperature treatment were selected based on litter origin, BW, and BFT and were distributed equally to one of two identical farrowing rooms equipped with 12 pens and maintained in constant TN conditions of 25 °C. Cross-fostering was done within each pregnancy treatment (PTN or PHS) and within the parity, for example, piglet of a primiparous PTN sow can only be fostered to another primiparous PTN sow. Sows were placed in individual pens (1.79×2.38 m) until weaning (day 28 of lactation). From day 1 to 5 of lactation, sows

were given individual rations of 0.5, 1.5, 3.0, 5.0, and 6.0 kg/d, respectively. From day 6 to 28 of lactation, feed and water were provided ad libitum. The commercial lactation feed (16.5% CP; 2,343 kcal/kg NE) was distributed three times per day (0800, 1300, and 1600 hours).

Weanlings and growing pigs

At weaning, 10 littermate piglets (3 entire males, 4 castrated males, and 3 females) were selected from each of the 24 sows (total of 240 piglets). The pigs selected were those closest to average weaning BW of pigs from PTN and PHS groups (i.e., 9.7 and 9.5 kg, respectively). During post-weaning, the 10 selected littermates were housed in one pen (1.45×2.72 m), hence one litter per pen. The 24 litters were allocated to two rooms, with 6 PTN and 6 PHS litters in each room. The piglets were given a standard pre-starter diet (18.9% CP; 2,498 kcal/kg NE) for 1 wk and a standard starter diet (18.0% CP; 2,262 kcal/kg NE) until the end of post-weaning. Water and feed were provided ad libitum, and the rooms were maintained in constant TN conditions (25 °C) throughout the 6 wk of post-weaning.

At 10 wk of age, 48 females were randomly selected from these 240 piglets (choice of 2 out of the 3 females per litter). Considering the prenatal environment (PE: PTN vs. PHS), the two selected females per litter were allotted to one of two similar growing (G) rooms with two different thermal environments (GE: GTN or GHS). Each room was equipped with eight pens (2×2 m) designed for housing three pigs each. To balance out the experimental design, the pigs were blocked according to litter origin so that in one pen, all pigs were half-sisters (same sire). Moreover, each pig of one pen in the GTN room has a full sister in the corresponding pen of the GHS room.

The experiment, which started after 5 d of adaptation, was divided into two main periods. The first main period was from 75 to 81 d of age where all pigs were kept under cyclic TN conditions (18 to 24 °C). The second main period started at 82 d of age (day 0; transition day for the GHS pigs from TN to HS conditions) when the temperature in the GHS room was gradually changed at a rate of 1 °C/h from 0600 to 1800 hours and was thereafter maintained in cyclic HS conditions of 28 to 34 °C until 140 d of age (Figure 2b). The GTN room was maintained in cyclic TN conditions from 82 to 140 d of age. Ad libitum access

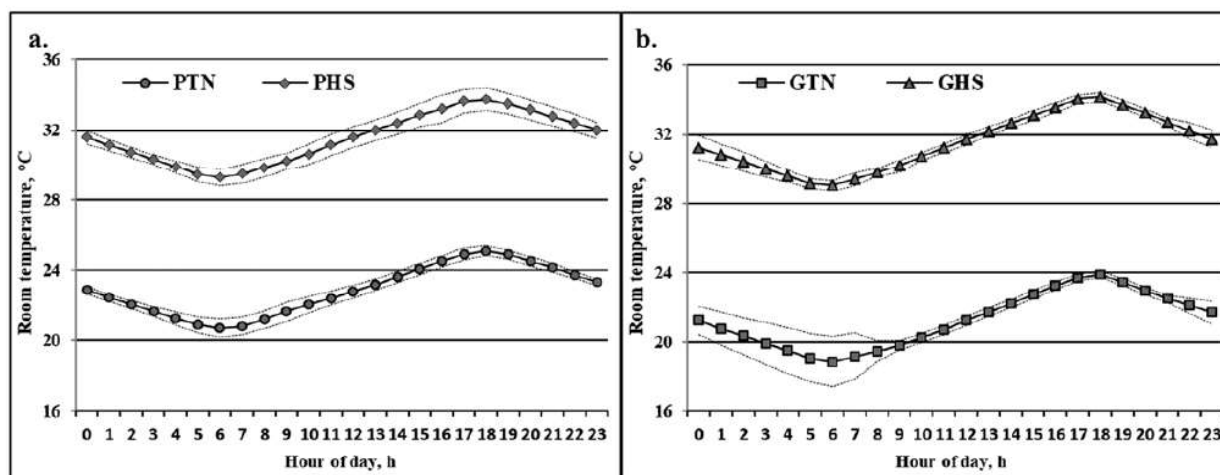


Figure 2. Actual average hourly ambient temperature (mean \pm SE) of the gestation rooms (a) from day 9 to 109 of gestation and of the growing rooms (b) from 83 to 140 d of age during the growth period.

to a standard growing-finishing feed (16.3% CP; 2,495 kcal/kg NE) and to water was provided throughout the growing period. Meals were distributed three times daily (0900, 1300, and 1600 hours). The pigs were slaughtered at 140 d of age.

Measurements

Growth and slaughter performance

For the overall growth performance, the two main periods previously described (i.e., 75 to 81 and 82 to 140 d of age) were considered. The pigs were individually weighed at the beginning of each main period, every 1 or 2 wk during the experimental period, and on the day before slaughter. The daily FI was measured for each pen as the difference between offered and refused feed. Refusals and spillages were collected daily at 0800 hours before the first feed distribution of the day; their dry matter (DM) (103 °C for 24 h) were also determined daily.

Pigs were slaughtered in the experimental slaughterhouse of INRAE-UEPR after 24 h fasting. Pigs were slaughtered by electrical stunning and exsanguination in compliance with the current national regulations applied in slaughterhouses. Hot carcass, perirenal fat, and head were weighed just after slaughter. Weights of the hypothalamus and pituitary gland were also recorded. Backfat (G2) and muscle (M2) depths were measured on one dorsal spot between the third and fourth last ribs at 6 cm of the spinal canal axis, using a Capteur Gras Maigre (Lean Fat Sensor) (DGM) device (Fives Syleps, Lorient, France). BFT was measured on carcass split at three different locations: on the first and last ribs and on the Gluteus muscle (minimum fat). The length of the left side of the carcass was also measured. On the day after slaughter, cold carcass and wholesale cuts from the right carcass side (ham, loin, shoulder, belly, and backfat) were weighed.

Physiological parameters

Rectal temperature was measured using a digital thermometer (Microlife Corporation, Paris, France; accuracy ± 0.1 °C) and skin temperature by a Type K thermocouple probe (HH-21 model, Omega, Stamford, CT, USA; accuracy ± 0.1 °C). These measurements were done on the sows at days 4, 9, 12, 29, 60, 106, and 110 of gestation and days 2, 6, 13, 20, and 26 of lactation. In the growing pigs, rectal and skin temperatures were measured at 1300 hours on days -5, 0, 2, 3, 7, 29, 43, and 53 of the second main experimental period. Skin temperature was not measured on day 3. On all pigs, at 1330 hours on days -4, 3, and 49, blood was collected at the jugular vein in heparin tubes, centrifuged (3,000 \times g; 10 min; 4 °C), and plasma was stored at -20 °C until analysis. Commercially available kits were used to measure the plasma levels of creatinine (Creatinine [Jaffe], Thermo Fisher Scientific Oy, Vantaa, Finland), glucose (Glucose [HK], Thermo Fisher Scientific Oy, Vantaa, Finland), nonesterified fatty acids or NEFA (FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany), and biological antioxidant potential or BAP (Diacron Labs srl, Grosseto, Italy). Intra-assay coefficient of variation (CV) was 4.9%, 1.7%, 0.4%, and 3.5%, respectively. Inter-assay CV was 7.0%, 8.4%, 2.4%, and 5.1%, respectively. For the enzymatic activities, creatine kinase or CK (CK [IFCC], Thermo Fisher Scientific Oy, Vantaa, Finland), lactate dehydrogenase or LDH (LDH [IFCC], Thermo Fisher Scientific Oy, Vantaa, Finland), and superoxide dismutase or SOD (Sigma-Aldrich, St Louis, MO) were measured. Intra-assay CV was 11.1%, 1.1%, and 4.1%, respectively. Inter-assay CV were 16.8% and 17.2% for CK and LDH, respectively. The plasma levels of thyroid hormones of T3 and T4 (ST AIA-PACK TT3 and ST AIA-PACK T4, Tosoh Corporation, Tokyo, Japan) were also determined. Intra-assay CV was 3.8% and 3.9%, respectively.

Calculations

Live BW measured on 75, 82, and 140 d of age were considered. Growth performance was calculated in two ways. First, for the overall growth performance, the average performance of the two main periods was considered (75 to 82 and 82 to 140 d of age). Since pen was the experimental unit, the average daily gain (ADG) for a given period corresponded to the mean of the three individual ADG. The average daily feed intake (ADFI) (measured per pen and divided by 3) was expressed in two ways: as the classical ADFI (g/d) and as the ADFI per metabolic BW ($\text{g} \cdot \text{kg}^{-0.60} \cdot \text{d}^{-1}$). The feed conversion ratio (FCR) was calculated as the FI divided by the BW gain for a given pen and for a given period. For data collected only from 82 to 140 d of age, this second main period was split into five subperiods: subperiod 1 (day 0 to 6; with day 0 as the transition of the GHS room to cyclic HS conditions) and subperiods 2, 3, 4, and 5 (day 7 to 15, 16 to 29, 30 to 43, and 44 to 58, respectively). Growth performance (ADFI per metabolic BW and ADG) was calculated for each subperiod.

For the carcass traits, carcass dressing was calculated as the percentage of hot carcass to slaughter BW (sBW). Wholesale cut weights were expressed as a percentage of the cold right carcass side. Carcass lean meat content was calculated using the CGM measurements (G2 and M2) according to the equation by Daumas et al. (2010): Lean meat content (%) = $62.19 - 0.729 \text{ G2} + 0.144 \text{ M2}$. Average BFT was calculated as the mean of the measurements from the three different locations previously described. For the thermoregulation parameters, temperature gradient was calculated as the difference between rectal and skin temperatures. Data of enzymes CK and LDH were log-transformed to follow normal distribution.

Statistical analyses

According to the factorial design based on 2 postnatal growing environments (GE; GTN and GHS) and 2 prenatal environments (PE; PTN and PHS), there were 4 treatments (i.e., GTN-PTN, GTN-PHS, GHS-PTN, and GHS-PHS) with 4 pens per treatment, for a total of 12 pigs per treatment.

For the overall growth performance, the pen ($n = 16$) was considered as the experimental unit and data were analyzed using a repeated measure of the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) considering PE ($n = 2$), GE ($n = 2$), the two main periods ($n = 2$; $n = 3$ for live BW), their interactions, and sire ($n = 4$) as fixed effects. The average growth performance during the second main period was also analyzed using PROC MIXED model with the PE ($n = 2$), GE ($n = 2$), their interaction, and sire ($n = 4$) as fixed effects, and including the growth performance during the first main period as covariates. For growth performance calculated per subperiod (ADG and ADFI per metabolic BW), data were analyzed using a repeated measure of the PROC MIXED procedure considering the PE ($n = 2$), GE ($n = 2$), subperiod ($n = 5$), their interactions, and sire ($n = 4$) as fixed effects and with performance measured during the first main period as covariates.

For carcass and physiological parameters, the pig ($n = 48$) was used as the experimental unit. Individual pig data were analyzed using the PROC MIXED procedure with the PE ($n = 2$), GE ($n = 2$), their interaction, pen ($n = 16$), and sire ($n = 4$) as fixed effects. Slaughter BW was included in the model as a covariate for data analysis of carcass traits. Thermoregulation and blood parameters were subjected to a repeated measurement PROC MIXED procedure based on the days of measurement ($n = 8$ for rectal temperature, $n = 7$ for skin temperature and temperature gradient, and $n = 3$ for blood parameters) and the interactions

with PE and with GE. Thermoregulation responses were also subjected to another repeated measurement analysis but only considering measurements during the second main period (days 2, 3, 7, 29, 43, and 53 measurements).

Results

The average hourly temperature of the rooms (Figure 2) indicates that the actual average minimal temperatures (20 to 25 °C for PTN; 29 to 34 °C for PHS; 19 to 24 °C for GTN; 29 to 34 °C for GHS) were slightly higher than the targeted temperatures.

Thermoregulatory responses

The results for the skin and rectal temperatures of the sows during gestation and lactation are presented in Figure 3. For the overall duration of HS exposure, gestating PHS sows had higher skin temperature (36.40 vs. 33.96 °C on average; $P < 0.001$) and rectal temperature (38.51 vs. 38.35 °C on average; $P = 0.020$) than PTN sows. For growing pigs, thermoregulation data of two pigs from GTN-PHS group were removed because they were sick from day 0 to 2 of the second main period. The skin and rectal of the growing pigs are presented in Figure 4. Considering only the second main period, regardless of the PE, GHS pigs had higher skin temperature (37.55 vs. 35.94 °C on average; $P < 0.001$) and rectal temperature (39.57 vs. 39.27 °C on average; $P < 0.001$) compared with their GTN counterparts. On day 2 of the second main period, GHS pigs had higher skin temperature

(37.89 vs. 36.27 °C on average; $P < 0.001$) and rectal temperature (39.96 vs. 39.43 °C on average; $P < 0.001$) compared with GTN pigs. Thereafter, skin temperature remained significantly and constantly higher in GHS than in GTN pigs until day 53 (37.45 vs. 36.11 °C on average; $P < 0.001$), whereas rectal temperature gradually decreased and by day 53, the difference was less pronounced although still significant (39.30 vs. 39.15 °C on average; $P = 0.011$).

Regarding the interaction between PE \times GE, the rectal temperature of the two GHS groups was not different from those of GTN-PHS pigs ($P > 0.100$) but was higher than those of GTN-PTN pigs on days 7, 43, and 53 of HS ($P < 0.050$). Considering all repeated measures during the second main period, an overall significant PE \times GE interaction was also observed for skin temperature ($P = 0.005$) and for temperature gradient ($P = 0.018$; data not shown). In GTN conditions, PHS pigs had higher skin temperature (36.10 vs. 35.77 °C on average; $P < 0.001$) and narrower temperature gradient (3.20 vs. 3.42 °C on average; $P = 0.015$) than PTN pigs, but not in GHS conditions (37.55 °C on average, $P = 0.979$ for skin temperature; and 1.96 °C on average $P = 0.997$ for temperature gradient). In GTN conditions, the rectal temperature of PHS pigs was also higher than those of PTN pigs (39.32 vs. 39.22 °C on average; $P = 0.038$) but not in GHS conditions (39.57 °C on average; $P = 0.735$).

Growth performance

Table 1 shows the summary of the growth performance of the growing pigs starting at 35.9 ± 0.8 kg BW. The interaction

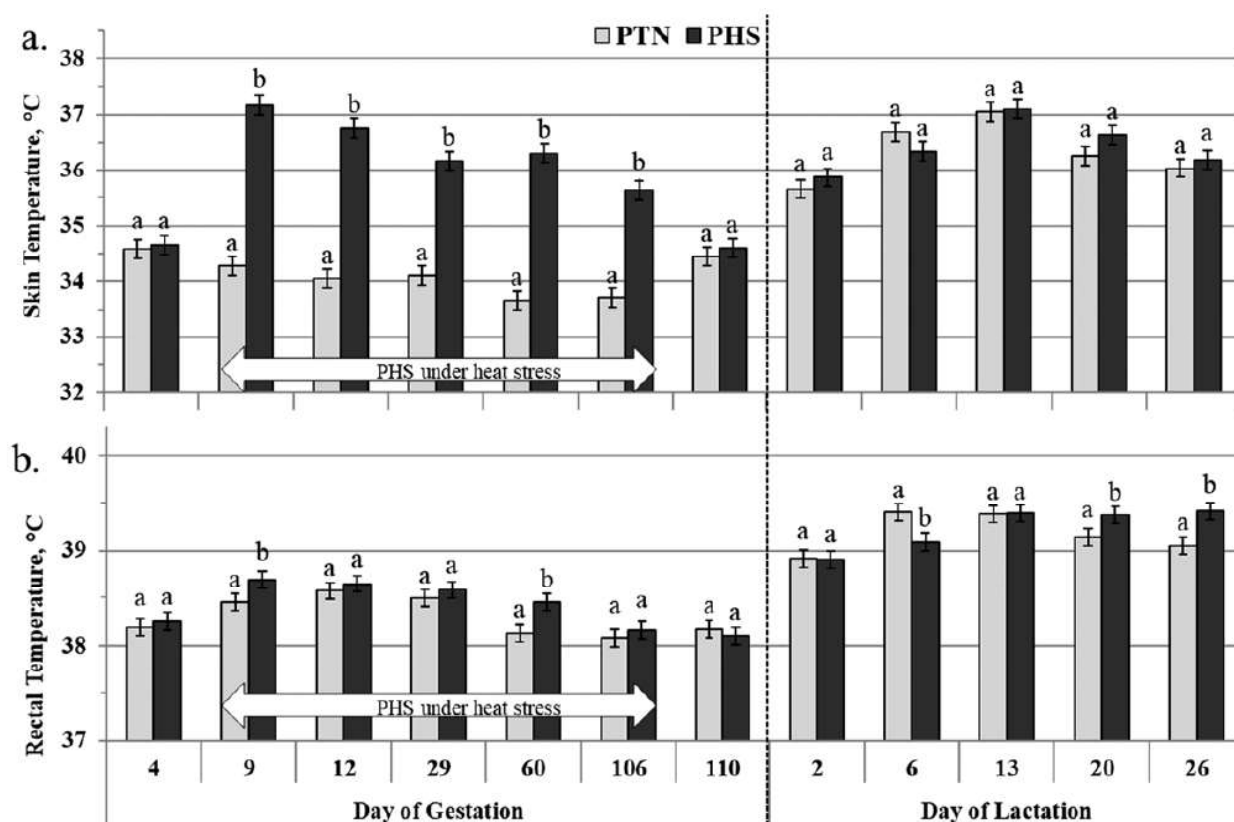


Figure 3. Effect of the climatic environment on the skin (a) and rectal (b) temperatures of mixed parity sows from gestation to lactation (LSmeans \pm SEM). From day 9 to 109 of pregnancy, sows ($n = 24$) were subjected to one of two environments: thermoneutral (PTN; 18 to 24 °C) and heat-stressed (PHS; 28 to 34 °C). During the whole lactation period, sows were kept at 25 °C. ^{a,b}Within each day, LSmeans with different superscript letters differ according to the experimental group ($P < 0.05$).

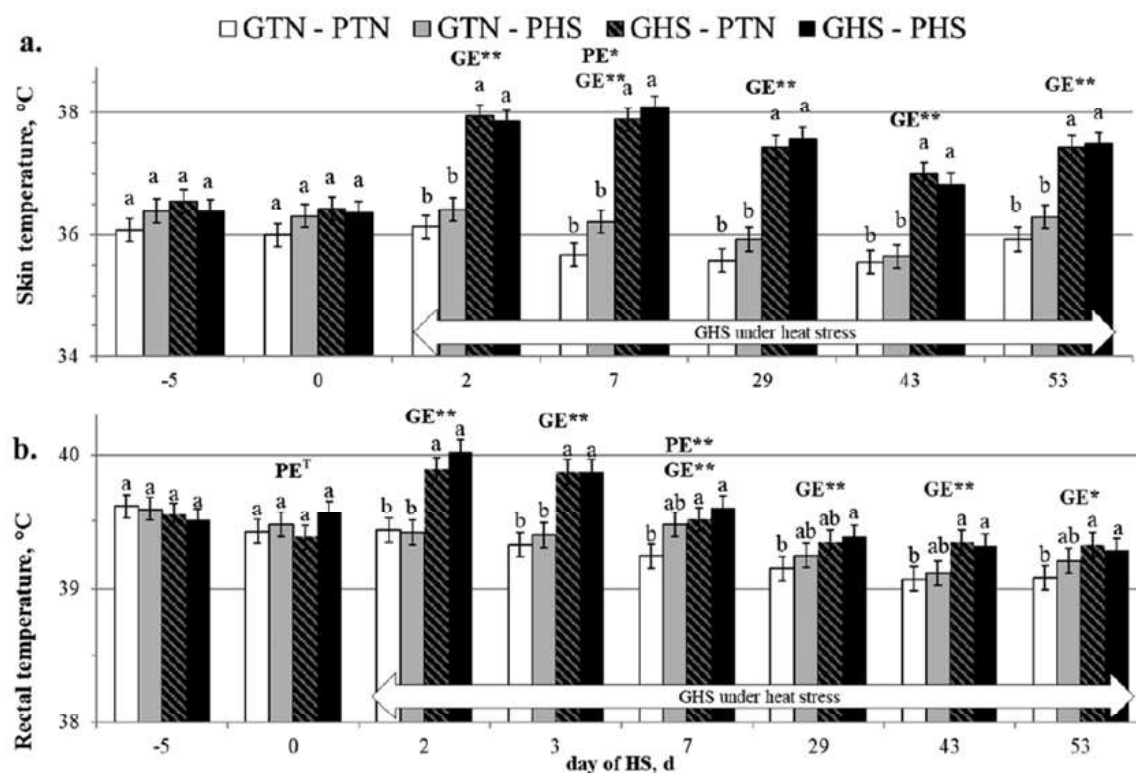


Figure 4. Effect of climatic environment (TN or HS) during prenatal development (PE; PTN vs. PHS) and during growing (GE; GTN vs. GHS) on the skin (a) and rectal (b) temperatures of growing pigs (LSMeans \pm SEM). ^{a,b}Within each day, LSmeans with different superscript letters differ according to the experimental group ($P < 0.05$). PE = effect of the prenatal environment, regardless of the growing environment. GE = effect of the growing environment regardless of the prenatal environment. * $P < 0.05$, ** $P < 0.01$.

Table 1. Effect of the prenatal and postnatal (growing) climatic environment on the growth performance of growing pigs¹

Items	GTN-PTN	GTN-PHS	GHS-PTN	GHS-PHS	RSD ²	Statistics ³
Live BW, kg						
75 d	35.8	36.1	36.6	35.2	1.7	GE**, P**, S*
82 d	42.3	42.6	43.7	41.6	1.69	PE \times GE†, GE \times P**
140 d	104.4 ^a	105.8 ^a	98.8 ^b	95.6 ^b		
ADG, g \cdot d ⁻¹						
75 to 81 d	859	888	997	943	70	P**, GE \times P**
82 to 140 d	1,147	1,114	1,017	989		
82 to 140 d ⁴	1,110 ^a	1,129 ^a	983 ^b	967 ^b	52	GE**
ADFI, g \cdot d ⁻¹						
75 to 81 d	1,729	1,884	1,996	1,901	168	P**, GE \times P**
82 to 140 d	2,635	2,683	2,347	2,307		
82 to 140 d ⁴	2,602 ^a	2,680 ^a	2,215 ^b	2,255 ^b	84	GE**, S*
ADFI per metabolic BW, g \cdot kg ^{-0.60} \cdot d ⁻¹						
75 to 81 d	198	208	218	210	11	P**, GE \times P*
82 to 140 d	196	202	180	181		
82 to 140 d ⁴	198 ^a	202 ^a	172 ^b	178 ^b	6	PE†, GE**, S*
FCR						
75 to 81 d	2.02	2.12	2.01	2.02	0.09	P**
82 to 140 d	2.30	2.41	2.31	2.33		
82 to 140 d ⁴	2.34	2.37	2.29	2.31	0.08	ns

¹A total of 48 female pigs (housed three pigs per pen) were distributed to a 2 \times 2 factorial design based on their prenatal environment (PE) and their postnatal growing environment (GE): TN = thermoneutral (18 to 24°C), HS = heat-stressed (28 to 34°C). First main period: Pigs were maintained in TN conditions from 75 to 81 d of age. Second main period: The GTN room was maintained in TN conditions until 140 d of age; in GHS room, temperature transition started at 82 d of age and full-blown thermal challenge was from 84 to 140 d of age.

²Residual standard deviation.

³The pen was considered as the experimental unit. Data were analyzed using PROC MIXED model with PE, GE, period (P), their interactions, and sire (S) as fixed effects. LS means with different superscript letters differ according to the experimental group * $P < 0.10$, * $P < 0.05$, ** $P < 0.01$.

⁴Adjusted performance based on the average value measured during first main period (944 g/d, 1,915 g/d, 212 g \cdot kg BW^{-0.60} \cdot d⁻¹, and 2.03 for ADG, ADFI, ADFI per metabolic BW, and FCR, respectively).

between PE and GE treatments was not significant except a trend ($P = 0.059$) for the live BW, with PHS pigs tending to be lighter at final BW than PTN pigs when raised in GHS conditions but not in GTN conditions. Regardless of PE treatment, GHS pigs had lower final BW than GTN pigs (97.2 vs. 105.1 kg; $P < 0.001$). Neither GE nor PE had significant effect on the FCR ($P = 0.221$ and $P = 0.549$, respectively). In the second main period (82 to 140 d of age), the GHS pigs had overall lower performance than GTN pigs in terms of ADG (1,003 vs. 1,130 g/d on average; $P = 0.017$), ADFI (2,327 vs. 2,659 g/d on average; $P = 0.008$), and ADFI per metabolic BW (199 vs. 181 g·kg^{-0.60}·d⁻¹ on average; $P = 0.045$). When corrected for the same performance during the first main period, PHS tended to have higher ADFI per metabolic BW than PTN pigs in the second main period (190 vs. 185 g·kg^{-0.60}·d⁻¹ on average; $P = 0.097$).

Looking at the subperiods during the second main period (Figure 5), there were no significant interactions between GE and subperiod for ADFI per metabolic BW ($P = 0.819$) and ADG ($P = 0.467$), because GHS pigs performed consistently lower than GTN pigs ($P < 0.050$). Meanwhile, there was a significant interaction between PE and subperiod for ADFI per metabolic BW ($P = 0.012$). Regardless of the GE group, PHS pigs ate more than PTN pigs starting only from the subperiod 3 with this difference being significant on subperiod 5 (186 vs. 175 g·kg^{-0.60}·d⁻¹ on average for PTN and PHS pigs, respectively; $P = 0.022$). There was also a tendency for a PE and subperiod interaction ($P = 0.061$) for

ADG but no significant difference was seen between PHS and PTN pigs in any of the subperiods.

Carcass and organ traits

The slaughter performance of the pigs is presented in Table 2. The sBW of GHS pigs was lower than those of GTN pigs ($P < 0.001$), while the hot carcass weight of GHS-PHS pigs was lower than those of the two GTN groups ($P < 0.006$) but not different from those of the GHS-PTN pigs ($P = 0.431$). Postnatal HS decreased carcass length ($P < 0.001$) and tended to increase carcass dressing ($P = 0.068$) but was not affected by the prenatal treatment. Expressed as a percentage of the sBW, PHS pigs tended to have lower head weight ($P = 0.066$) than PTN pigs, regardless of the GE.

Looking at carcass traits adjusted for the same sBW, lean meat content was decreased both by postnatal HS ($P = 0.028$) and prenatal HS ($P = 0.029$), with GHS-PHS pigs having the lowest lean meat content compared with the three other groups ($P < 0.040$). A PE × GE interaction was also observed for average BFT ($P = 0.003$) and for cold carcass proportions of backfat ($P = 0.016$) and of perirenal fat ($P = 0.017$), with PHS pigs being fatter than their PTN counterparts when they were submitted to postnatal HS. Irrespective of the prenatal treatment, postnatal HS also increased average BFT ($P < 0.001$) and ham percentage ($P = 0.001$) and decreased loin percentage ($P < 0.001$). Meanwhile, prenatal HS decreased ham percentage

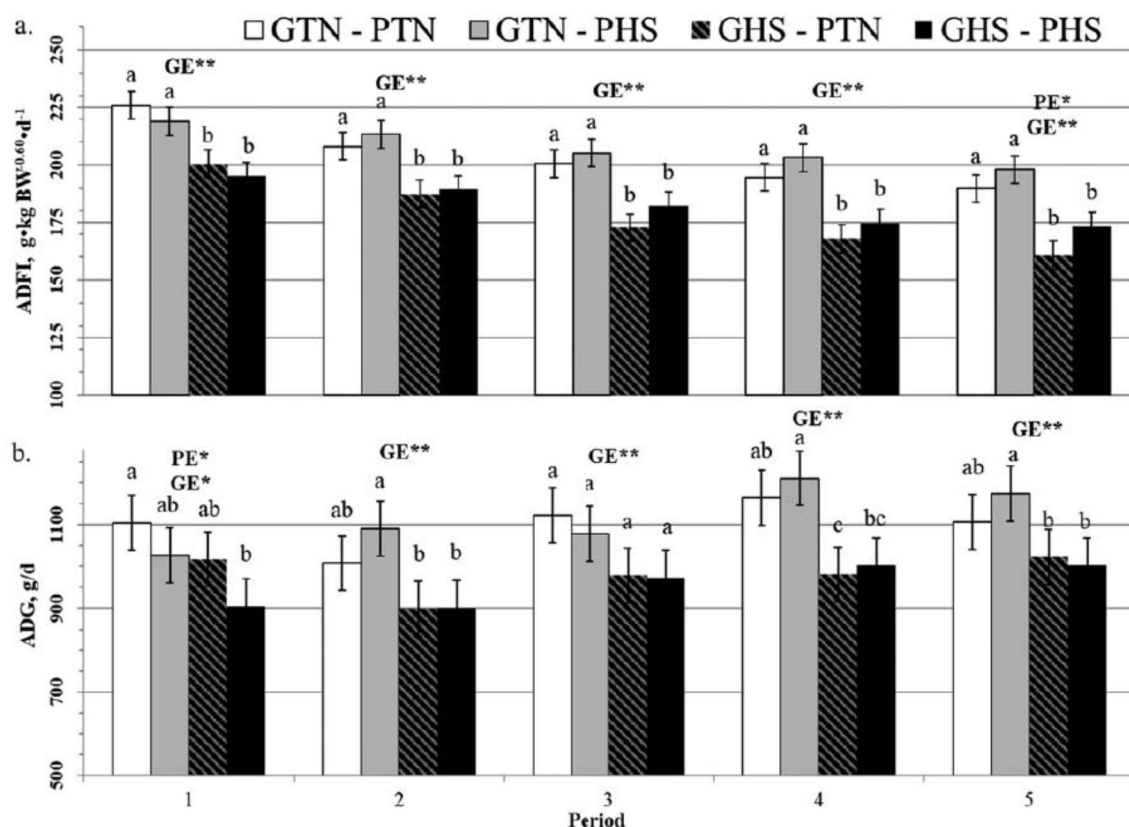


Figure 5. Effect of climatic environment (TN or HS) during prenatal development (PE; PTN vs. PHS) and during growing (GE; GTN vs. GHS) on ADFI per metabolic BW (a) and ADG (b) in growing pigs using TN performance as covariate (LSMeans ± SEM). Subperiod 1: day 0 to 6 of GHS; subperiod 2: day 7 to 15; subperiod 3: day 16 to 29; subperiod 4: day 30 to 43; and subperiod 5: day 44 to 58. ^{a,b,c}Within each subperiod, LSmeans with different superscript letters differ according to the experimental group ($P < 0.05$). PE = effect of the prenatal environment, regardless of the growing environment. GE = effect of the growing environment regardless of the prenatal environment. * $P < 0.05$, ** $P < 0.01$.

Table 2. Effect of the prenatal and postnatal (growing) climatic environments on the carcass and organ traits of growing pigs¹

Items	GTN-PTN	GTN-PHS	GHS-PTN	GHS-PHS	RSD ²	Statistics ³
No. of pigs	12	12	12	12		
Slaughter BW (sBW), kg	102.3 ^{ab}	103.6 ^a	96.8 ^{bc}	93.7 ^c	5.6	GE**
Hot carcass weight, kg	80.8 ^a	81.7 ^a	77.0 ^{ab}	74.2 ^b	4.5	GE**
Carcass dressing, %	79.1	78.8	79.6	79.2	0.9	GE ^T
Carcass length, cm	95.7 ^{ab}	97.4 ^a	94.9 ^{bc}	93.4 ^c	1.9	GE**, PE × GE**
Head, % sBW	4.47 ^{ab}	4.31 ^b	4.59 ^a	4.49 ^{ab}	0.24	PE ^T , GE*
Carcass composition ⁴						
Lean meat, %	63.5 ^a	63.4 ^a	63.2 ^a	61.8 ^b	1.2	PE*, GE*, PE × GE ^T , sBW**
Average BFT, mm	18.7 ^{bc}	17.0 ^c	19.3 ^{ab}	20.9 ^a	1.7	GE**, PE × GE**, sBW**
Perirenal fat, g/kg sBW	7.6 ^{ab}	7.0 ^b	7.6 ^{ab}	9.2 ^a	1.5	PE × GE*
Carcass cuts ⁴ , % cold carcass wt						
Loin	29.0 ^a	29.2 ^a	27.8 ^b	27.9 ^b	0.9	GE**
Ham	26.8 ^{bc}	26.3 ^c	27.5 ^a	27.1 ^{ab}	0.6	PE*, GE**
Belly	12.1	12.0	12.2	12.5	0.6	sBW**
Backfat	4.9 ^{ab}	4.6 ^b	4.9 ^{ab}	5.4 ^a	0.6	GE ^T , PE × GE*, sBW**
Organ wt., mg/kg BW						
Hypothalamus	11.2 ^a	9.8 ^b	10.1 ^{ab}	10.8 ^{ab}	1.5	PE × GE*
Pituitary gland	2.8 ^{ab}	2.7 ^b	2.7 ^b	3.0 ^a	0.3	PE × GE*

¹A total of 48 female pigs (housed three pigs per pen) were distributed to a 2 × 2 factorial design based on their prenatal environment (PE) and their postnatal growing environment (GE): TN – thermoneutral (18 to 24°C), HS = heat-stressed (28 to 34°C).

²Residual standard deviation.

³The pig was considered as the experimental unit. Data were analyzed using PROC MIXED model with PE, GE, their interaction, pen, and sire as fixed effects. ^TP < 0.10, *P < 0.05, **P < 0.01. LS means with different superscript letters differ according to the experimental group.

⁴Slaughter BW was used as covariate (adjusted sBW = 99.1 kg).

($P = 0.007$) regardless of the GE treatment. Neither postnatal nor prenatal HS affected belly percentage ($P = 0.171$ and $P = 0.514$, respectively).

For the brain parts, a PE and GE interaction was observed for the weight of the hypothalamus ($P = 0.022$) and of the pituitary gland ($P = 0.038$) expressed per kg BW. Hypothalamus of GTN-PTN pigs were heavier ($P = 0.027$) than that of GTN-PHS pigs but were not different from the two GHS groups ($P > 0.050$). Meanwhile, pituitary gland of GHS-PHS pigs was heavier ($P < 0.050$) than those of GTN-PHS and GHS-PTN pigs but not different ($P = 0.223$) to that of GTN-PTN pigs.

Plasma parameters

Whatever the parameter, plasma concentration was similar among all four treatments on day –4 (Figure 6). The PE × GE interaction was significant for some blood parameters at some specific days. Plasma creatinine concentration was higher in GHS pigs (GHS-PTN and GHS-PHS) compared with GTN-PHS pigs on day 3 ($P = 0.049$ and $P = 0.026$, respectively) and was highest in GHS-PTN pigs among all groups on day 49 ($P < 0.030$). Plasma glucose of the GTN pigs (GTN-PTN and GTN-PHS) was higher than the GHS-PTN pigs on day 49 ($P = 0.008$ and $P = 0.036$, respectively). Plasma NEFA of the GHS pigs (GHS-PTN and GHS-PHS) were lower only when compared with GTN-PHS pigs ($P = 0.001$ and $P = 0.008$, respectively) and only on day 49. The GTN groups (GTN-PTN and GTN-PHS) had higher SOD plasma levels on day 3 ($P = 0.004$ and $P = 0.031$, respectively) and higher plasma T3 levels on day 49 ($P = 0.006$ and $P = 0.025$, respectively) compared with GHS-PHS pigs. And on day 49, plasma T4 was higher in GTN-PTN pigs than in GHS-PHS pigs ($P = 0.003$).

Regardless of PE treatment, acute and chronic postnatal HS (on days 3 and 49, respectively) increased creatinine ($P = 0.012$ on day 3; $P < 0.001$ on day 49) and decreased glucose

($P < 0.001$ on day 3; $P = 0.002$ on day 49), T3 ($P < 0.001$ on days 3 and 49), and T4 ($P < 0.001$ on day 3; $P = 0.020$ on day 49) plasma concentrations. Compared with GTN pigs, plasma NEFA levels of GHS pigs were lower on day 3 (trend at $P = 0.074$) and on day 49 ($P < 0.001$). Higher activities of CK and LDH ($P < 0.001$) were observed in GHS pigs but only on day 3. Postnatal HS also decreased plasma SOD levels on day 3 ($P = 0.004$) and BAP levels on day 49 ($P < 0.001$). Regardless of the postnatal treatment, plasma SOD level tended to be lower ($P = 0.082$) in PHS pigs than in PTN pigs on day 3. Prenatal HS also decreased plasma T4 on day 49 ($P = 0.007$) without a significant decrease in plasma T3 ($P = 0.305$).

Discussion

In this study, we investigated the effects of both prenatal and postnatal thermal environments on performance and physiological responses in growing pigs. The cyclic HS treatments applied in the experiment were enough to elicit responses from both the PHS sows and GHS pigs as they exhibited biphasic acclimation HS responses similar to previous studies (Black et al., 1993; Renaudeau et al., 2010). This response, as described by Renaudeau et al. (2010), is characterized by a short-term heat acclimation where pigs experience rapid physiological changes as shown by the spike in rectal temperature and followed by a long-term heat acclimation where animals show improved heat tolerance by increasing the ability to dissipate heat and to decrease HP. The higher skin temperature of PHS sows and GHS pigs throughout the thermal challenge can be related to the shift of the blood flow more toward the peripheral tissues and away from internal tissues (Collin et al., 2001a). The GHS pigs in our study were able to adapt to chronic HS as reflected in their decreasing rectal temperatures. Exposure to HS has

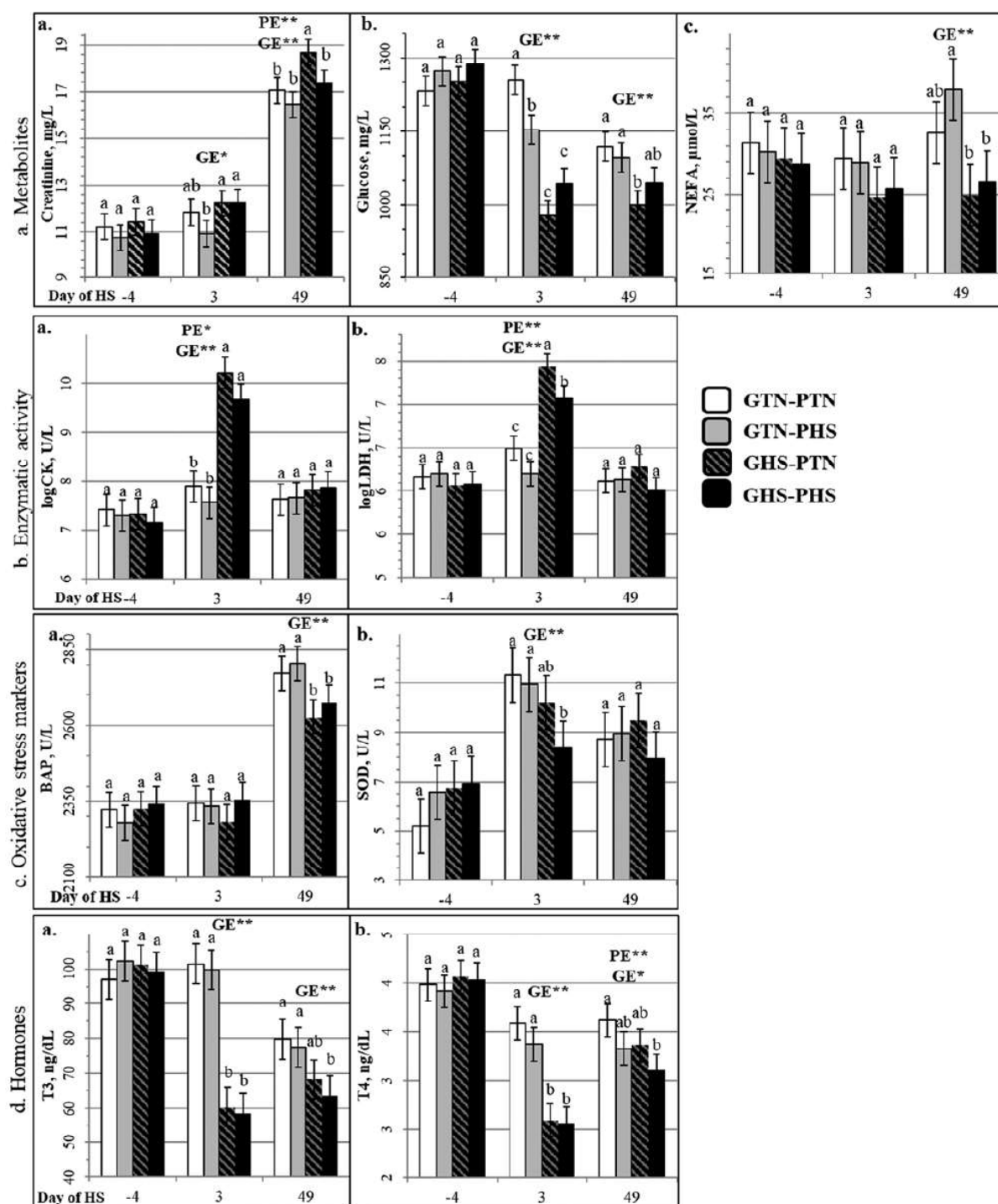


Figure 6. Effect of climatic environment (TN or HS) during prenatal development (PE; PTN vs. PHS) and during growing (GE; GTN vs. GHS) on plasma parameters of growing pigs (LSMeans \pm SEM). ^{a,b}Within each day, LSMeans with different superscript letters differ according to the experimental group ($P < 0.05$). PE = effect of the prenatal environment, regardless of the growing environment. GE = effect of the growing environment regardless of the prenatal environment. * $P < 0.05$, ** $P < 0.01$.

been shown to decrease FI and to decrease metabolic HP due to a reduction in maintenance requirements as adaptation responses (Quiniou et al., 2000; Renaudeau et al., 2013). The FI reduction was not observed in gestating sows submitted to HS

(data not shown) because a large part of the ration was given at 0800 hours when the ambient temperature was low and because they had a strict daily feed allowance, thus leaving no room for refusals.

Effects of postnatal HS on growth performance and metabolism

In the present study, postnatal HS decreased ADFI per metabolic BW of growing pigs by $39 \text{ g} \cdot \text{kg}^{-0.60} \cdot \text{d}^{-1}$ on average, which is slightly lower than the values reported by Renaudeau et al. (2008) in younger pigs (-40 , -65 , and $-104 \text{ g} \cdot \text{kg}^{-0.60} \cdot \text{d}^{-1}$ at constant temperatures of 28, 32, and 36 °C, respectively, compared with controls). It is possible that implementing cyclic rather than constant HS conditions allowed the pigs in the present study to compensate by eating more feed during the colder parts of the day (decrease in ADFI is lower at 28 °C than at 32 °C). Nevertheless, the reduction in ADFI had negative consequences on the growth rate due to a reduction in nutrient intake similar to a previous study (Collin et al., 2001b).

Carcass traits were adjusted for the same sBW in our study to evaluate the strict effect of HS on carcass composition and to take into account the study limitations of not having the pigs slaughtered at the same live BW. Based on the fact that maximum protein deposition can be reached at 80 kg and maximum fat deposition is not reached even in the range of 110 to 130 kg BW (Van Milgen and Noblet, 2003), it can be assumed that GHS pigs (average of 95.3 kg at slaughter) would deposit more fat than lean if they had been slaughtered at the same BW (but older age) as GTN pigs (average of 103.0 kg at slaughter). In our study, the GHS pigs were fatter than GTN pigs which is in contrast to other studies with ad libitum-fed pigs subjected to constant high ambient temperature (32 to 33 °C) being leaner and having less lipid deposition than pigs in TN conditions (Collin et al., 2001b; Cruzen et al., 2015). Differences in results could be related to the previously discussed higher level of ADFI reduction in pigs under constant HS conditions compared with the cyclic HS conditions implemented in our study. Kouba et al. (2001) reported increased triglyceride uptake and storage in HS pigs compared with their pair-fed TN counterparts and the “additional” FI in our study could have been deposited as fat since pigs decrease their metabolic HP during chronic postnatal HS and it is more energy efficient to deposit fat (Van Milgen and Noblet, 2003). The fatter carcasses of GHS pigs are logically associated to lower loin (lean cut) percentage, whereas the increased ham percentage of GHS pigs may be explained by the changes in conformation as shown by their shorter carcass.

The drop in plasma thyroid hormone levels of GHS pigs can be linked to the reduction of their ADFI and to the lower growth and HP (Collin et al., 2002). Since thyroid hormones stimulate dietary and endogenous fat breakdown (Sinha et al., 2018), the lower T3 and T4 levels found in postnatal HS pigs in our study and in agreement with Sanz Fernandez et al. (2015) could contribute to the increased body fat content of GHS compared with GTN pigs, as also shown in rats (Iossa et al., 2001). Plasma NEFA of GHS pigs were also lower which can be due to lower amounts of lipid ingested because of the reduced FI or to a limited ability of HS pigs to mobilize fat as demonstrated by Pearce et al. (2013). Indeed, it has been previously suggested that pigs subjected to postnatal HS had lower metabolic flexibility with a lower fatty acid oxidation in their skeletal muscles than pair-fed TN pigs (Baumgard and Rhoads, 2013; Zhao et al., 2018). The elevated plasma creatinine level and CK activity in GHS pigs can also suggest increased muscle protein catabolism instead of fat catabolism for energy production (Clarkson et al., 2006). Lower plasma SOD (in acute HS) and plasma BAP (in chronic HS) levels measured in GHS pigs could be indications of reduced antioxidant capacities similar to previous reports in pigs (Yang et al., 2014; Liu et al., 2018) which, in humans, could be markers

of metabolic syndrome such as lower adiponectin and increased insulin (Kim et al., 2014). Altogether, the results of our study thus validate the effects of postnatal HS as reported in existing literature, such as reduction in growth performance, increased carcass adiposity, and altered physiology and metabolism.

Effects of prenatal HS on growth performance and metabolism in growing pigs raised in postnatal TN conditions

The effects of prenatal HS on growth performance, metabolism, and physiology in swine have been studied in the past few years as reviewed by Johnson and Baumgard (2018). According to Johnson et al. (2015c), prenatal HS increased the core temperature of growing pigs, which is comparable to our results. Prenatal stress and increased cytokines in the fetal environment have both been reported to alter set point in the hypothalamic-pituitary-adrenal (HPA) axis (Glover et al., 2010; Dreier et al., 2014), which plays a role in the body temperature control of mammals including pigs (Bligh, 1966; Baldwin and Ingram, 1968). In our study, there were indeed differences in the relative weight of hypothalamus and pituitary gland among the treatments. The anterior and posterior parts of these glands have different functions which could explain why the glands did not have the same pattern of increase or decrease in weight. However, the gland parts were not weighed separately in our study, so this remains to be theoretical. Nevertheless, pituitary gland size itself does not signify a lower or higher hormone activity; it can only be suggested that these observed changes could be related to morphological changes and/or abnormalities that, in humans, are related to altered pituitary function (Maghnie et al., 1991; Cooper et al., 2017). These changes could also be related to smaller head size of GHS pigs observed in our and in other studies (Cruzen et al., 2015; Johnson et al., 2015b). It can thus be hypothesized that the higher rectal temperature of prenatal heat-stressed pigs observed at many stages during the growing phase could be a consequence of an altered HPA set point due to their fetal environment.

Chapel et al. (2017) suggested that the elevated core temperature of pigs submitted to in utero HS pigs during mid-gestation could be associated to higher fasting HP and circulating T3 levels. Meanwhile, Johnson et al. (2015c) found that although in utero HS pigs in their study also exhibited increased body temperature, they found no effect in plasma levels of T4 (a pro-hormone) nor of T3 (active form of T4) regardless of the timing (first or second half of gestation) and the length of gestational HS. In our study, however, PHS pigs subjected to whole-gestation HS had lower levels of plasma T4 with no significant change in plasma T3 levels. Nevertheless, these results suggest that prenatal HS, depending on the timing and duration, can alter thyroid functions which to some extent could also be associated to the previously discussed hypothesis of an altered HPA set point. Between the two GTN groups in our study, those subjected to prenatal HS also had higher skin temperature denoting increased heat dissipation activity (possibly from the increased rectal temperature) and had a narrower temperature gradient implying a lower capacity to further dissipate heat (Cuddy et al., 2014).

The increased core temperature could also be partly attributed to a higher thermic effect of feeding linked to the age-dependent ADFI increase of PHS pigs in our study. This higher ADFI of prenatally heat-stressed pigs, however, failed to translate to a significant ADG increase in agreement to previous studies (Cruzen et al., 2015; Safranski et al., 2015; Wilmoth et al., 2015). There is also an age-dependent decrease in feed efficiency

in in utero HS pigs as it was observed during the finishing phase (Johnson et al., 2015b) but not in the growing phase (Johnson et al., 2015a). These results suggest the limited ability of PHS pigs to convert increased intake to gain which can be an effect of intrauterine growth restriction due to fetal undernutrition (Ji et al., 2017). This can be related to reports of prenatal HS causing placental insufficiency or inefficiency (Galan et al., 1999; Zhao et al., 2019) perhaps due to blood redirection away from the placenta in heat-stressed sows. According to Boddicker et al. (2014), the positive effect of prenatal HS on subsequent pig FI is dependent on the HS timing, and pigs from sows heat-stressed in the first half of gestation ate more than pigs from sows heat-stressed in the second half of gestation. Altogether, this suggests that prenatal HS can indeed affect postnatal pig performance. Whether this effect is neutral, positive, or negative seems to depend, among other things, on the age of the offspring and the timing and duration of the prenatal HS.

In utero heat-stressed pigs reared in TN conditions have been described to have increased fat deposition in some studies (Boddicker et al., 2014; Johnson et al., 2015b); however, other studies found no effect (Cruzen et al., 2015; Johnson et al., 2015a) comparable to our results. Differences between studies could be due to several factors, such as pig physiological stage considered or sex: fatter carcasses of prenatal HS in TN-reared pigs have been observed in mixed-sex (barrows and females) finishing pigs (Johnson et al., 2015b), but not in mixed-sex young and growing pigs (Boddicker et al., 2014; Johnson et al., 2015a) nor in finishing barrows (Cruzen et al., 2015). These discrepancies may be at least partly explained by differential effects of prenatal HS on pig FI between studies, as the increased FI generally observed with increasing pig age and BW is associated to increased fat and decreased protein deposition (Van Milgen and Noblet, 2003). In our study, the increased FI of PHS pigs was significant only at the end of the experimental growth period, which was probably insufficient to significantly influence body fatness of PHS pigs in postnatal TN conditions. Finally, as mentioned previously, the prenatal HS effect can depend on the timing and duration. For example, pigs exposed to prenatal HS only in the first half of gestation had thicker subcutaneous fat than control pigs, but pigs exposed during the entire gestation exhibited no significant response (Boddicker et al., 2014), in agreement with present results. Overall, the results from our study and from existing literature show that in pigs in postnatal TN conditions, exposure to prenatal HS can increase body temperature attributed to a possible alteration in the HPA axis and in thyroid functions. Depending on the timing and duration, prenatal HS can also increase carcass adiposity but this is not proven for TN-raised pigs exposed to a whole-gestation prenatal HS.

Effects of prenatal HS on growth performance and metabolism in growing pigs subjected to postnatal HS

Despite the increasing literature in prenatal HS in pigs, there is still little known on the effect of prenatal HS on the ability of pigs to cope with postnatal HS. In our study, prenatal HS tended to reduce plasma SOD during acute postnatal HS. This result is in agreement with previous studies (Lista et al., 2010; Yin et al., 2018), which suggests a decreased anti-oxidative capacity previously reported in the offspring of stressed mammals. This decreased oxidative stress tolerance and other prenatal HS effects discussed so far can influence the pig's postnatal life and production efficiency. Indeed, prenatal HS seemed to aggravate the production performance of GHS

pigs in our study as GHS-PHS pigs had slightly lower ADG and final BW than GHS-PTN pigs even with similar overall ADFI. Under postnatal HS, both GHS groups still had higher rectal temperature than GTN-PTN pigs even after 53 d of chronic HS suggesting they were unable to dissipate all heat necessary to lower body temperature to a similar level with pigs kept in prenatal and postnatal thermoneutrality. In the previous section, the decreased ability of pigs to dissipate heat as a consequence of prenatal HS was discussed. At 28 to 34 °C, pigs in our study may have already reached the maximum heat dissipation level through the skin as GHS-PHS and GHS-PTN had similar skin temperature and temperature gradient. With a reduced anti-oxidative capacity, one could hypothesize a decreased heat tolerance in PHS pigs as they were subjected to postnatal HS wherein heat dissipation has already been maximized.

In the present study, the GHS-PHS pigs had lower carcass lean meat content than GHS-PTN pigs and tended to exhibit higher fatness at both subcutaneous and internal levels. Reasons for this decrease in leanness are still unclear but one theory could be related to the hypothesized fetal growth restriction that, in other mammals, is linked to impaired glucose and insulin metabolism (Thureen et al., 1992; Lesage et al., 2004). Indeed, Johnson et al. (2015b) also reported that insulin per unit of glucose or per kg of FI was higher in in utero HS pigs but only if raised in the postnatal HS environment. Boddicker et al. (2014) reported that it is the exposure to prenatal HS during the first half of the gestation that increases circulation insulin levels regardless of the environment during the second half of gestation. A whole-gestation prenatal HS has also been reported to increase postnatal stress response of pigs (Merlot et al., 2018) possibly linked to the hypothesized altered HPA set point. Hypersecretion of chronic stress hormones in humans is attributed to increased visceral fat accumulation and muscle loss (Pervanidou and Chrousos, 2012) and could be related to the increased visceral adiposity of pigs subjected to both prenatal and postnatal HS in our study.

With effects such as heightened postnatal stress responses, altered thyroid functions, decreased heat dissipation ability, and reduced anti-oxidative capacity, chronic prenatal HS seems to impair long-term heat tolerance of pigs in our study, as indicated by their reduced growth, increased body adiposity, and overall decreased productive efficiency. However, it must be noted that the present study considered only a chronic whole-gestation HS effect. Existing literature in pigs and other mammals suggests that impacts on pig performance and carcass composition could differ depending on the timing, duration, and intensity of the prenatal HS applied, and this still remains to be further investigated.

Conclusion

Studies about prenatal HS on growing pigs are still limited. Chronic prenatal HS in our study decreased heat tolerance of growing pigs due to increased body temperature and altered thyroid functions and physiological stress responses. With the increasing impact of climate change, this implies a decreased global swine production efficiency. Existing literature and present data suggest that multiple factors can influence prenatal HS effects, such as duration and timing during gestation, postnatal thermal environment, and physiological stage of pigs. More studies are needed to further elucidate its effects and biological mechanism in pigs.

Acknowledgments

We want to thank F. Le-Gouevrec, A. Chauvin, M. Genissel, J. Georges, J. Delamarre, H. Demay, D. Boutin, F. Guerin, Y. Surel, and P. Touanel from the UEPR, INRAE, 35590 Saint-Gilles, France for animal care and sample collection, A. Constantin, P. Ganier, A. Marchais, C. Mustière, C. Perrier, G. Robin, and C. Trefeu for lab analyses. We also want to thank Lallemand SAS for the PhD grant of A. M. Serviento. This study was funded by INRAE, France AgriMer and to the French Ministry of Agriculture (CASDAR financial support; SCANALI project).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Chapter 2. Effect of heat stress and feeding management on growth performance and physiological responses of finishing pigs

The summary of the growth performance of the eight experimental groups of Experiment 2 is presented in *Annex table 1*. This chapter presents the results of Experiment 2 focusing only on the non-supplemented groups (4 out of 8 experimental groups: TN-AL, TN-PF8, HS-AL, and HS-PF8). This decision was taken because LY supplementation did not have any significant effect on the growth parameters measured and due to the unprecedented effect of the experimental design on the feeding behavior especially of the *ad libitum* groups (*Annex figure 2*). Since each of the two rooms according to the temperature treatment housed all the three feeding managements together (*ad libitum*, PF2, and PF8), there was a stimulation of feeding activity in the other groups (especially apparent in the *ad libitum* groups) each time there was a feed distribution for the PF8 pigs.

The results of Experiment 2 dealing with the non-supplemented groups were initially presented in the 71st Annual Meeting of the European Federation of Animal Science (EAAP) in 2020 as shown in *Annex 2* and were afterwards published in the Journal of Animal Science in 2020 as presented in the following pages.



ENDOCRINOLOGY AND PHYSIOLOGY

Effect of heat stress and feeding management on growth performance and physiological responses of finishing pigs

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Abstract

This study aimed to determine whether pig responses to heat stress (HS) were directly due to heat exposure (regardless of feeding level and pattern) or were indirectly due to the reduction of feed intake (FI) and to determine if increasing feeding frequency (splitting heat increments) can improve pig response to HS. A total of 48 pigs (66.1 ± 1.7 kg) were allocated to four groups in three replicates. After 7 d in thermoneutral (TN) conditions (22 °C; period 1 [P1; day -7 to -1]), pigs were placed in either TN or HS (32 °C) conditions for 20 d (period 2 [P2; day 0 to 19]). The diet was provided either ad libitum (AL; 2 distributions/d) or pair-fed (PF8; 8 distributions/d) using HS-AL pigs as the reference group. Thus, the four experimental groups were TN-AL, HS-AL, TN-PF8, and HS-PF8. The daily ration of PF8 pigs was distributed at every 90-min intervals from 0900 to 1930 hours. Data were analyzed using the PROC MIXED procedure with replicate ($n = 3$), experimental group ($n = 4$), and their interactions as fixed effects, and the REPEATED statement was used for repeated measures data. Pigs had a similar average daily feed intake (ADFI) during P1 ($P > 0.05$). In P2, HS-AL and PF8 pigs had lower ADFI (-19%), average daily gain (-25%), and final body weight (-6.1 kg) than TN-AL pigs ($P < 0.01$). TN-AL pigs had thicker backfat than TN-PF8 pigs ($P < 0.05$), while the HS pigs had intermediate results. HS pigs had a higher perirenal fat percentage based on the contrast analysis between PF8 pigs ($P < 0.05$). Thermoregulatory responses of pigs increased with HS exposure but did not differ between HS or between TN groups ($P > 0.05$). For TN pigs, variation in muscle temperature (T_{muscle}) depended on feeding and physical activity, while for HS pigs, T_{muscle} gradually increased throughout the day. The T_{muscle} of PF8 pigs increased with each additional meal but plateaued earlier for HS-PF8 than TN-PF8 pigs; an increase in T_{muscle} per meal was also lower in HS-PF8 than TN-PF8 ($P < 0.05$). Exposure to HS decreased plasma T3 and T4 ($P < 0.05$) and increased plasma creatinine ($P < 0.05$). Between the PF8 groups, HS pigs also had a transient increase in plasma insulin on day 8 ($P < 0.05$). The effect of HS on FI decreased the growth rate of pigs but there are heat-induced effects, such as altered physiological responses, which might explain the direct HS effects seen in other literature especially in terms of increased adiposity. The increased feed provision frequency in the present study did not improve the HS response of pigs.

Key words: feeding behavior, heat stress, meal frequency, pair-feeding, pig, thermoregulation

Abbreviations

ADG	average daily gain
ADFI	average daily feed intake
AL	ad libitum
BFT	backfat thickness
BW	body weight
FCR	feed conversion ratio
FI	feed intake
HP	heat production
HS	heat stress or heat-stressed
NEFA	nonesterified fatty acids
pCO ₂	partial pressure of carbon dioxide
RR	respiratory rate
sBW	slaughter body weight
TEF	thermic effect of feeding
T _{muscle}	muscle temperature
TN	thermoneutral
T _{rectal}	rectal temperature
T _{skin}	skin temperature

Introduction

With increasing global temperature and more frequent summer heat waves (Perkins-Kirkpatrick and Gibson, 2017), the climatic environment remains to be a major limiting factor in swine production in many countries. Reduction of pig performance during heat stress (HS) is mainly related to the reduced feeding level (Collin et al., 2001a) as a way to reduce the thermic effect of feeding (TEF) and thereby of heat production (HP) (Quiniou et al., 2001). However, pair-feeding (PF) models demonstrate that heat-induced reduction of feed intake (FI) does not fully explain the negative consequences of HS (Baumgard and Rhoads, 2013; Ross et al., 2017). Using PF studies, HS exposure has been shown to limit lipid mobilization or increase body adiposity in ruminants (Wheelock et al., 2010), rats (Katsumata et al., 1990) poultry (Zeferino et al., 2016), and pigs (Kouba et al., 2001; Pearce et al., 2013; Sanz Fernandez et al., 2015a; Kellner et al., 2016). These reported changes are theorized to be adaptive responses to help the animals to better cope with HS.

Using PF as a nutrient intake control is a widely accepted experimental method but has its limitations, since it imposes a different type of stress on animals (e.g., prolonged fasting and alteration of feeding behavior), which can increase stress responses and plasma nonesterified fatty acids (NEFA) release (Russell et al., 2008). PF pigs may also be subjected to the additional stress of an abrupt shift from being fed ad libitum (AL) to suddenly receive 2 to 3 joule-deficit meals per day. Furthermore, being trained to a fixed meal pattern may alter ghrelin responses (Drazen et al., 2006), which could then have consequences on other metabolic responses (Pradhan et al., 2013). It is thus difficult to isolate and determine the direct effects of HS especially if confounded with the effects of changing the feeding pattern and of the feeding pattern itself. Therefore, in designing the PF model to evaluate direct HS effects, the present study aimed to remove these confounding variables by having a PF group in both TN and HS conditions with the same feed distribution frequency and size and adapting these two groups to the set feeding schedule even before the experimental period.

Modifying feeding behavior via feeding frequency has already been shown to alter growth performance and metabolic response even in pigs housed in the same thermoneutral (TN) conditions (Schneider et al., 2011; Le Naou et al., 2014).

Meanwhile, exposure to high ambient temperature itself alters feeding behavior in pigs such as decreased meal size and/or frequency in lactating sows (Quiniou et al., 2000) and in younger pigs (Collin et al., 2001b). Increasing meal frequency has also been linked to improved performance during HS exposure in dairy cows (West, 1999) and in pigs (Labussière et al., 2016a), which can be linked to the lower TEF per meal and thus lower instantaneous heat load for the animals. This study, therefore, aims to evaluate and to confirm indirect HS effects (caused by nutrient intake reduction) and direct HS effects (regardless of nutrient intake and meal patterns) on finishing pig performance and to determine if increasing meal frequency can help in mitigating these effects.

Materials and Methods

The experiment was conducted in accordance with the French legislation on animal experimentation and was approved by a Committee for Consideration of Ethics in Animal Experimentation (authorization: APAFIS#18973-2019020622003043).

Animals and treatments

The experiment was conducted from June to October 2019 in the INRAE experimental facilities at the Unité Expérimentale Physiologie et Phénotypage des Porcs (UE 3P) located in Saint-Gilles, France. The experimental design consists of two environmental treatments (TN vs. HS) and, within each environment, two feeding management treatments in which the diet (Table 1) was provided either AL (2 distributions/d) or PF (PF8; 8 distributions/day) with AL-fed HS pigs as the reference group. Thus, there were four experimental groups: TN–PF8, HS–AL, and HS–PF8.

A total of 48 Pietrain × (Large White × Landrace) female finishing pigs (66.1 ± 1.7 kg live body weight [BW]) in three successive replicates were used in the experiment and placed in individual pens (1 × 2 m on iron-slatted floor). Within each replicate, pigs were allotted to the four experimental groups according to live BW and litter origin (same sire). Lights were switched on from 0730 to 1930 hours in both rooms. All pigs were maintained at a constant temperature of 22 °C (actual: 22.1 ± 1.0 °C) during 11 d of adaptation and then for another 7-d period (period 1 [P1]: day –7 to –1). Pigs of the TN group were then maintained at 22 °C (actual: 21.41 ± 0.25 °C) until the end of the experiment (period 2 [P2]: day 0 to 19). For pigs of the HS group, the ambient temperature was gradually changed at a rate of 2 °C/h from 1200 to 1800 hours on day 0 and was thereafter maintained at a constant temperature of 32 °C (actual: 32.6 ± 1.8 °C) until day 21. The decision to use 22 °C as the TN room temperature and 32 °C as room temperature to induce HS was based on previous literature (Brown-Brandl et al., 2001; Renaudeau et al., 2011).

For the PF8 pigs, PF was based on the pig's predicted BW and on the 3-d average FI per kilogram BW of the HS–AL group. The HS–AL group was exceptionally given feed allowance of –20% and –10% of their pre-challenge average daily feed intake (ADFI) on days 1 and 2 of P2, respectively, instead of feeding them AL to remove the risk of inaccurate PF on the first days of the thermal challenge. The values used (–20% and –10% ADFI reduction on the first 2 days) were based on the results from a previously conducted experiment in HS pigs (Serviento et al., 2020). In AL groups, feed was distributed at 0900 and at 1530 hours, while for PF8 groups, the calculated daily ration was divided into eight feed provisions (around 300 to 400 g

Table 1. Composition of the experimental diet¹

Ingredients, % as-fed	Finisher diet
Wheat	34.00
Barley	25.00
Corn	15.00
Soybean meal	5.06
Rapeseed meal	9.00
Soft wheat bran	8.00
Rapeseed oil	1.00
L-Lysine HCl	0.65
L-Threonine	0.09
L-Tryptophan	0.05
DL-Methionine	0.04
Calcium carbonate	1.10
Vitamin-mineral premix ²	0.50
Salt	0.40
Acidifier	0.10
Phytase	0.01
Analyzed chemical composition, % dry matter basis	
Dry matter	88.72
Crude protein	14.80
Crude ash	4.53
Crude fat	3.10
Neutral detergent fiber	16.46
Acid detergent fiber	5.68
Acid detergent lignin	1.73
Starch	45.8
Calculated values, % dry matter basis	
Net energy, MJ/kg	10.97
SID lysine, g/ kg	9.18

¹Diet fed in pellet form.

²Provided per kilogram of complete diet: vitamin A, 1,000,000 IU; vitamin D3, 200,000 IU; vitamin E, 4,000 mg; vitamin B1, 400 mg; vitamin B2, 800 mg; calcium pantothenate, 2,170 mg; niacin, 3,000 mg; vitamin B12, 4 mg; vitamin B6, 200 mg; vitamin K3, 400 mg; folic acid, 200 mg; biotin, 40 mg; choline chloride, 100,000 mg; iron (sulfate), 11,200 mg; iron (carbonate), 4,800 mg; copper (sulfate), 2,000 mg; zinc (oxide), 20,000 mg; manganese (oxide), 8,000 mg; iodine (iodate), 40 mg; cobalt (carbonate), 20 mg; and selenium (selenite), 30 mg.

each depending on feed allowance), which were distributed at every 90-min interval from 0900 to 1930 hours. Pigs had free access to water during the whole experimental period. The day prior to slaughter (i.e., day 20), feed was removed from the feeders at 1800 hours. In order to standardize the digestive tract content, each pig was given a 500-g feed ration 2 h before slaughter.

Measurements

Growth, slaughter, and feeding behavior

The pigs were individually weighed on days -7, 0, and 20 of the experimental period and on the day of slaughter. The individual daily FI of the pigs was measured as the difference between offered and refused feed. Refusals and spillages were collected daily at 0800 hours before the first feed distribution of the day. In case of wet refusals, dry matter was determined (in a ventilated oven at 103 °C for 24 h) and used to correct FI on an as-fed basis. An infrared barrier system was installed in each of the individual feed trough to record time, duration, and number of feeding bouts. A visit was recorded each time the infrared barrier was interrupted by the presence of the pig; the duration

of each visit was the elapsed time between the recorded time the pig was detected by the infrared barrier and the time the pig left.

Pigs were slaughtered in the experimental slaughterhouse of INRAE-UE3P by electrical stunning and exsanguination and in compliance with the current EU regulations ([Commission Delegated Regulation, 2017](#)). Hot carcass, perirenal fat, and visceral organs (liver, kidneys, and heart) were weighed just after slaughter. Backfat (G2) and muscle (M2) depths were measured on one dorsal spot between the third and fourth last ribs at 6 cm of spinal canal axis, using a Capteur Gras Maigre (Lean Fat Sensor) (CGM) device (Fives Syleps, Lorient, France). Backfat thickness (BFT) was measured directly on the carcass using a caliper square at three different locations: at the level of the first and last ribs and of *Gluteus medius* (minimum fat thickness). The day after slaughter, cold carcass weight was measured and recorded.

Physiological parameters

Thermoregulation parameters were measured on the pigs at 1000 hours on days -4, 0, 1, 2, 3, 6, 10, 13, 17, and 20. To evaluate the acute thermoregulatory response, additional measurements were done on day 0 (transition day of the HS room to high ambient temperature) at 1200, 1400, 1600, and 1800 hours. Rectal temperature (T_{rectal}) was measured using a digital thermometer (Microlife Corporation, Paris, France; accuracy ± 0.1 °C) and skin temperature (T_{skin}) by a Type K thermocouple probe (HH-21 model, Omega, Stamford, CT, USA; accuracy ± 0.1 °C). Respiratory rate (RR) was determined by counting flank movements for 30 s and was expressed in breath per min (bpm) only on resting animals (total observations $n = 619$). At the beginning of the adaptation period, a measurement probe for measuring internal body temperature (Anipill, Body Cap, Hérouville Saint-Clair, France) was implanted on eight pigs per experimental group (in two pigs per group for replicates 1 and 2 and in four pigs for replicate 3). The capsule was implanted 2 to 3 cm deep into the brachiocephalic muscle at the neck of the animal similar to the method described by [Renaudeau \(2016\)](#). The capsules recorded the body temperature at the muscular level (T_{muscle}) of the pigs every 2 min.

Blood was collected on restrained animals via the jugular vein in two heparin tubes (one 8 mL and one 2 mL; Vacutest Kimia, Arzegrade, Italy) on days -1, 1, 8, and 20 at 1330 hours. Blood carbon dioxide (partial pressure or $p\text{CO}_2$ and concentration or $[\text{CO}_2]$), bicarbonate, and pH were measured in the 2-mL tubes using a blood gas analyzer (ABL80 FLEX, Radiometer SAS, France) within 30 min following the sampling. The blood samples in the 8-mL tubes were centrifuged ($3,000 \times g$; 10 min; 4 °C), and plasma was stored at -20 °C until analysis. Commercially available kits were used to measure plasma levels of NEFA (FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany), creatinine (Creatinine [Jaffe], Thermo Fisher Scientific Oy, Vantaa, Finland), glucose (Glucose [HK], Thermo Fisher Scientific Oy, Vantaa, Finland), and urea (Urea, Thermo Fisher Scientific Oy, Vantaa, Finland). Intra-assay coefficient of variation (CV) of NEFA, creatinine, glucose, and urea was 0.4%, 3.7%, 2.0%, and 1.5%, respectively; inter-assay CV was 9.8%, 4.8%, 8.7%, and 7.0%, respectively. Plasma levels of insulin (ST AIA-PACK IRI, Tosoh Corporation, Tokyo, Japan), total triiodothyronine or T3 (ST AIA-PACK T3, Tosoh Corporation, Tokyo, Japan), and total thyroxin or T4 (ST AIA-PACK T4, Tosoh Corporation, Tokyo, Japan) were also determined, and their intra-assay CV was 2.3%, 3.8%, and 3.9%, respectively.

Calculations and statistical analysis

There were four experimental groups based on the climatic environment (TN vs. HS) and based on the feeding management (AL vs. PF8): TN-AL, TN-PF8, HS-AL, and HS-PF8. For each of the three replicates, there were four pigs per experimental group. Three pigs (one TN-AL, one TN-PF8, and one HS-AL) were removed from the final calculation and statistical analysis because of sickness or causes unrelated to feeding management and/or environmental treatments. For growth, feeding behavior, slaughter, and blood parameters, the pig ($n = 45$) was considered as the experimental unit. In looking at the relationship between the T_{muscle} and the corresponding feeding behavior (presence at feeder), only data from pigs implanted with an internal temperature probe that worked properly were included ($n = 26$ wherein TN-AL, $n = 6$; TN-PF8, $n = 8$; HS-AL, $n = 5$; and HS-PF8, $n = 7$).

Growth, slaughter, and physiological parameters

For growth and feeding behavior data, the trial was divided into two periods: P1 (day -7 to -1) and P2 (day 0 to 19). Growth parameters such as average daily gain (ADG; g/d), ADFI (g/d or g·kg BW⁻¹·d⁻¹), and feed conversion ratio (FCR) were calculated per period. For the feeding behavior data, visits to the feeder that lasted for <5 s were removed from the dataset. Two consecutive visits separated by a time interval not longer than a given meal criterion were considered to belong to one meal. The calculated meal criterion of the present study was 15 min. These data were based on the first derivative of the polynomial function estimating the number of meals based on different meal criteria (Renaudeau et al., 2002). This adopted meal criterion was used for further calculation of feeding behavior parameters per period such as meal frequency (number of meals/day) and meal duration (min·meal⁻¹ and min·meal⁻¹). Growth and feeding behavior data were analyzed using the REPEATED measure of the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) with replicate ($n = 3$), experimental group ($n = 4$), period ($n = 2$) or day for live BW ($n = 3$), and their interactions as fixed effects, and pen and sire as random effects.

For carcass traits, carcass dressing was calculated as the percentage of hot carcass to slaughter BW (sBW), while carcass lean meat content was calculated using the CGM measurements (G2 and M2) according to the equation published by Dumas et al. (2010): Lean meat content (%) = $62.19 - 0.729 \times G2 + 0.144 \times M2$. Slaughter data were analyzed using a PROC MIXED model with replicate ($n = 3$), experimental group ($n = 4$), and their interactions as fixed effects, and pen and sire as random effects.

Thermoregulation traits measured on day 0 were used for the acute HS responses. Daily means of T_{muscle} and thermoregulatory responses measured at 1000 hours were used to assess chronic HS responses. Physiological parameters were analyzed using the PROC MIXED procedure with replicate ($n = 3$), experimental group ($n = 4$), day ($n = 4$ for blood and $n = 10$ for chronic thermoregulation responses) or time of measurement ($n = 6$ for acute thermoregulation and $n = 13$ for acute T_{muscle} responses), and their interactions as fixed effects. Pen and sire were included as random effects, and the REPEATED statement was used to specify covariance structures for repeated measures data.

Orthogonal contrast statements were used to evaluate the overall effect of feeding management (TN-AL and HS-AL) vs. (TN-PF8 and HS-PF8), of climatic environment in AL-fed groups: (TN-AL) vs. (HS-AL), and of climatic environment in PF8-fed groups: (TN-PF8) vs. (HS-PF8). $P < 0.05$ was considered as significant, and $P < 0.10$ was considered as a trend.

Pattern of body temperature and presence at the feeder

Data from day 6 (the day in which T_{rectal} of HS groups stabilized) until day 19 were considered, and data from day 8 (blood sampling day) were removed. Data averaged every 10 min were used to look at the nycthemeral pattern of the pigs. Data ($n = 144$ for every 10 min) were analyzed using the REPEATED statement of the PROC MIXED procedure with replicate ($n = 3$), experimental group ($n = 4$), and their interactions as fixed effects, and pen and sire as random effects.

For the AL-fed groups, data of T_{muscle} and presence at the feeder were averaged per hour, and a reference value was considered from the hour at which feeder activity is the lowest for each respective group: hour 11 for TN-AL and hour 0 for HS-AL. The description of the kinetics was then based on the contrast comparison of hourly values to the reference. As meal provision was similar for both PF8 groups, the corresponding data were used to evaluate the effect of ambient temperature on the relationship between body temperature and feeding behavior traits. For each meal, each pig, and each day, the median T_{muscle} value measured 10 min before the meal (meal_n) distribution was determined (baseline T_{muscle} or parameter A in Figure 1) and the same was done for the following meal (meal_{n+1}) (B parameter). The slope (S) between points A and B was calculated; for the eighth meal, the slope was calculated using its baseline T_{muscle} (i.e., median T_{muscle} from 1920 to 1930 hours) and the median T_{muscle} after 90 min (2050 to 2100 hours) to have the similar time range with the first seven meals. The threshold point at which T_{muscle} started to increase after the meal distribution (C) corresponded to the time at which T_{muscle} was significantly different from A, and, finally, parameter D is the maximum T_{muscle} after the meal provision. The effect of meal distribution and temperature on the T_{muscle} was evaluated by calculating the surface under the curve as defined in Figure 1. The described parameters were analyzed using the repeated measures of the PROC MIXED model with replicate ($n = 3$), experimental group ($n = 2$), meal ($n = 8$), and their interactions as fixed effects, and with pen and sire as random effects. The SLICE option was used to determine contrasts between the two PF8 groups per meal and among the meals per experimental group.

Results

Growth and slaughter performance

Table 2 provides the summary of the growth performance and feeding behavior traits of the finishing pigs according to the experimental treatment. There was no significant difference among all groups in any of the parameters during P1. In P2, the reference group HS-AL and both PF8 groups had lower ADFI (-547 g·d⁻¹ or -5.1 g·kg BW⁻¹·d⁻¹ on average; $P < 0.01$) and ADG (-279 g·d⁻¹ on average; $P < 0.01$) compared with the TN-AL group. FCR of HS-PF8 pigs was higher than the value measured for TN-AL pigs ($+0.37$; $P = 0.04$) and tended to be higher than FCR of HS-AL ($+0.29$; $P = 0.09$) but did not differ from FCR of TN-PF8 pigs ($P = 0.38$). Based on the contrast analysis between the environmental treatments on P2, exposure to HS decreased total meal duration in both the AL and the PF8 groups (-22 min on average; $P < 0.03$). Regarding the contrast between the two feeding managements, pigs fed AL had fewer meals per day than the PF8 pigs in P2 (-2.9 meals·d⁻¹ on average; $P < 0.01$) but their meals had a longer duration ($+1.3$ min·meal⁻¹ on average; $P = 0.04$).

Looking at the slaughter performance (Table 3), the HS-AL, TN-PF8, and HS-PF8 pigs had lower sBW (-6.6 kg on average; P

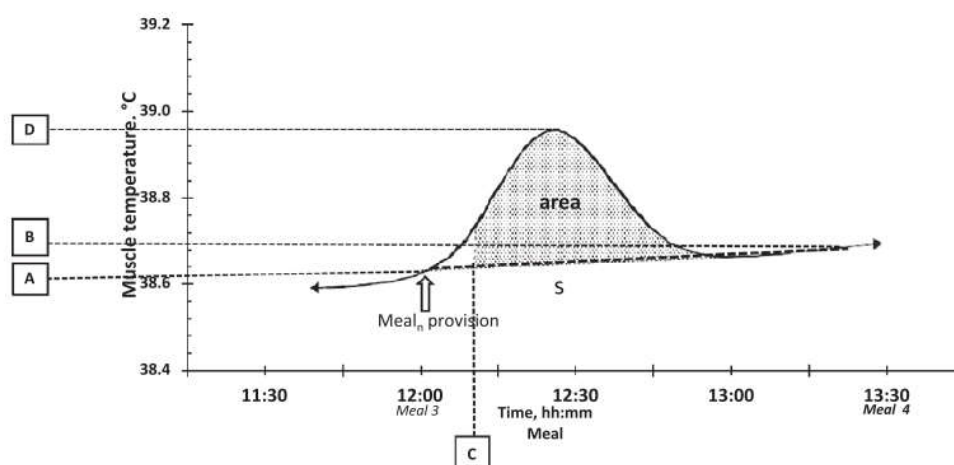


Figure 1. Graphical illustration of how the area under the curve was calculated for a specific meal using the T_{muscle} ($^{\circ}\text{C}$) of the PF8 pigs and the time passed (min) after each meal provision. A = baseline T_{muscle} of meal_n; B = baseline T_{muscle} of meal_{n+1}; C = starting point of peak in T_{muscle} ($P < 0.05$ vs. point A); D = maximum T_{muscle} of meal_n; S = slope between points A and B; area = area under the T_{muscle} curve.

Table 2. Effect of HS and feeding management on growth performance and feeding behavior of finishing pigs¹

Items	TN-AL	TN-PF8	HS-AL	HS-PF8	RSD ²	Statistics ³	Contrasts P-value		
							AL vs. PF8 ⁴	AL: TN vs. HS ⁵	PF8: TN vs. HS ⁶
Live BW, kg									
Day -7	66.2	66.0	65.8	67.0	1.3	R ^{**} , D ^{**} , G × D ^{**}	0.65	0.81	0.52
Day 0	74.6	73.8	73.5	74.6			0.89	0.51	0.60
Day 20	97.7 ^a	91.7 ^b	91.6 ^b	90.9 ^b			<0.01	<0.01	0.61
ADFI, g·d ⁻¹									
P1	2,605	2,527	2,655	2,649	135	G ^{**} , P ^{**} , G × P ^{**}	0.45	0.54	0.15
P2	2,929 ^a	2,415 ^b	2,388 ^b	2,343 ^b			<0.01	<0.01	0.40
ADFI per BW, g·kg BW ⁻¹ ·d ⁻¹									
P1	37.1	36.2	38.1	37.7	1.9	R ^{**} , G ^{**} , P ^{**} , G × P ^{**}	0.29	0.30	0.13
P2	34.1 ^a	29.2 ^b	28.9 ^b	28.6 ^b			<0.01	<0.01	0.54
ADG, g·d ⁻¹									
P1	1,195	1,122	1,100	1,085	134	G ^{**} , P ^{**} , G × P [*]	0.18	0.14	0.56
P2	1,154 ^a	903 ^b	911 ^b	811 ^b			<0.01	<0.01	0.14
FCR									
P1	2.19	2.29	2.45	2.49	0.29	R [*] , G [*] , P ^{**}	0.43	0.06	0.14
P2	2.55 ^b	2.70 ^{ab}	2.63 ^{ab}	2.92 ^a			<0.01	0.53	0.11
Total meal duration/day, min·d ⁻¹									
P1	62	63	69	60	17	G × P [*]	0.60	0.45	0.74
P2	73	72	51	50			0.87	0.03	0.03
No. of meals/day, n·d ⁻¹									
P1	12.2	10.9	11.3	13.0	1.6	P ^{**} , G × P ^{**} , R × G × P [*]	0.76	0.41	0.08
P2	11.8 ^b	15.1 ^a	11.8 ^b	14.2 ^{ab}			<0.01	0.95	0.44
Meal duration, min·meal ⁻¹									
P1	5.2	6.2	6.4	4.8	1.5	G × P [*]	0.59	0.17	0.17
P2	6.5 ^a	4.9 ^{ab}	4.8 ^{ab}	3.9 ^b			0.04	0.05	0.33

¹A total of 48 pigs were allocated to four experimental groups in three replicates. All pigs were housed at thermoneutrality (P1: day -7 to -1) before being subjected to either thermoneutral (TN) or heat stress (HS) conditions (P2: day 0 to 19). In each environment, diet was provided either ad libitum (AL) (2 feed provisions/day) or pair fed (PF8) (8 feed provisions/day) with the HS pigs fed AL as the reference group.

²Residual standard deviation.

³The pig was considered as the experimental unit. Data were analyzed using PROC MIXED model with replicate (R), experimental group (G), day (D), or period (P), and their interactions as fixed effects.

⁴Contrast statement: [TN-AL, HS-AL] vs. [TN-PF8, HS-PF8].

⁵Contrast statement: [TN-AL] vs. [HS-AL].

⁶Contrast statement: [TN-PF8] vs. [HS-PF8].

^{a-c}LSmeans within a row with different superscripts letters differ ($P < 0.05$) according to the experimental group.

^{*} $P < 0.05$; ^{**} $P < 0.01$.

Table 3. Effect of HS and feeding management on slaughter traits of finishing pigs¹

Items	TN-AL	TN-PF8	HS-AL	HS-PF8	RSD ²	Statistics ³	Contrasts P-value		
							AL vs. PF8 ⁵	AL: TN vs. HS ⁶	PF8: TN vs. HS ⁷
Slaughter BW, kg	96.7 ^a	90.6 ^b	90.2 ^b	89.6 ^b	3.8	R*, G**	0.02	<0.01	0.57
Hot carcass wt., kg	75.0 ^a	70.8 ^b	70.4 ^b	70.8 ^b	3.5	R**, G*	0.10	<0.01	0.98
Carcass dressing, %	77.6	78.2	78.1	78.9	1.3	R*	0.13	0.42	0.28
Lean meat, %	62.8	62.5	62.1	62.8	1.6		0.71	0.35	0.73
Ave BFT, mm ⁴									
BFT first rib	34.0 ^a	31.1 ^{ab}	30.0 ^b	32.5 ^{ab}	3.2	G*, R × L*	0.85	<0.01	0.34
BFT last rib	19.3	16.9	17.9	18.5			0.38	0.32	0.26
BFT gluteus	12.6	11.0	11.4	11.3			0.39	0.40	0.88
Average	22.0 ^a	19.7 ^b	19.8 ^{ab}	20.8 ^{ab}			0.29	0.02	0.27
Total viscera, g·kg ⁻¹ sBW ⁸	224	218	219	211	13	R*	0.13	0.42	0.28
Kidneys	3.8 ^a	3.3 ^b	3.4 ^{ab}	3.3 ^b	0.4	G*	0.03	0.05	0.90
Liver	20.3 ^a	18.5 ^b	19.3 ^{ab}	18.6 ^b	1.4	R**, G*	0.02	0.15	0.90
Perirenal fat	8.0	7.8	8.4	9.4	1.6	R*	0.37	0.61	0.04
Heart	3.7	3.7	3.5	3.6	0.4	R*	0.37	0.25	0.45
Others	188	184	184	176	13	R*	0.17	0.50	0.16

¹A total of 48 pigs were allocated to four experimental groups in three replicates. All pigs were housed at thermoneutrality for 7 d (P1) before being subjected to either thermoneutral (TN) or heat stress (HS) conditions for 20 d (P2). In each environment, diet was provided either ad libitum (AL) (2 feed provisions/day) or fed (PF8) (8 feed provisions/day) with the HS pigs fed AL as the reference group.

²Residual standard deviation.

³The pig was considered as the experimental unit. Data were analyzed using PROC MIXED model with replicate (R), experimental group (G), and their interactions as fixed effects.

⁴The pig was considered as the experimental unit. Data were analyzed using PROC MIXED model with replicate (R), experimental group (G), measurement location (L), and their interactions as fixed effects.

⁵Contrast statement: [TN-AL, HS-AL] vs. [TN-PF8, HS-PF8].

⁶Contrast statement: [TN-AL] vs. [HS-AL].

⁷Contrast statement: [TN-PF8] vs. [HS-PF8].

⁸Not emptied: including total weight of heart and lungs, digestive tract, kidneys, and liver.

^{a-c}LSmeans within a row with different superscripts letters differ ($P < 0.05$) according to the experimental group.

* $P < 0.05$; ** $P < 0.01$.

< 0.01) and hot carcass weight (−4.3 kg on average; $P = 0.02$) than the TN-AL pigs, while lean meat content was similar among all groups (62.6% on average; $P > 0.10$). The feeding management significantly affected kidneys and liver proportions relative to sBW (higher in AL than in PF8 pigs; $P = 0.03$ and $P = 0.02$, respectively). The TN-PF8 pigs had a lower average BFT than TN-AL pigs ($P < 0.05$), while the HS groups had intermediate results. Meanwhile, based on the contrast analysis between the two PF8 groups, average BFT did not significantly differ ($P = 0.27$) but HS increased the perirenal fat percentage of PF8 pigs ($P < 0.04$).

Thermoregulatory responses

Figure 2 shows the acute thermoregulatory responses for the HS groups during the transition period. The two HS groups had similar T_{skin} throughout all the experiment ($P > 0.10$) and started to be significantly higher than the two TN groups just 2 h after the temperature rise ($P < 0.01$). RR of the HS groups also increased steadily with the room temperature and started to be significantly different from the one of TN groups after 4 h of temperature rise ($P < 0.01$). The TN groups had similar RR throughout the transition period ($P > 0.10$). However, HS-AL pigs had faster RR than the HS-PF8 pigs after 6 and 8 h of room temperature increase ($P < 0.01$). During the transition of the HS room temperature, HS-AL pigs started to have significantly higher T_{rectal} than the TN pigs just after 4 h ($P < 0.02$), while it was later for HS-PF8 (higher T_{rectal} than TN-PF8 and TN-AL from 6 and 22 h, respectively; $P < 0.01$). The two HS groups had higher T_{muscle} than the TN-AL pigs starting at 4 h after the transition ($P < 0.01$).

Compared with the TN-PF8 pigs, T_{muscle} started to be significantly higher in HS-AL and HS-PF8 pigs after 2 and 4 h, respectively ($P < 0.05$).

Chronic thermoregulatory responses of the pigs are shown in Figure 3. All parameters were similar among the four experimental groups on days −4 and 0 ($P > 0.10$) but, upon exposure to high ambient temperature, were elevated in the two HS groups starting on day 1 ($P < 0.01$). Pigs under HS had constantly higher T_{skin} and RR than TN pigs from the onset until the end of HS exposure ($P < 0.01$). Between the two TN-raised groups, T_{skin} of pigs fed AL was significantly higher on day 17 ($P < 0.01$) and day 20 ($P = 0.01$) than the PF8 pigs. Meanwhile, between the HS-raised groups, the T_{skin} of AL-fed pigs tended to be higher on day 3 ($P = 0.06$) and day 10 ($P = 0.08$) than the T_{skin} of PF8 pigs. RR of HS-AL pigs was also higher than RR of HS-PF8 pigs on day 3 ($P = 0.04$), day 6 ($P < 0.01$), day 10 (trend at $P = 0.05$), day 17 ($P = 0.01$), and day 20 ($P < 0.01$). Rectal temperature of the HS groups slowly declined and seemed to stabilize 6 d after the onset of HS but was still generally higher than that of TN groups. These differences were significant on days 10, 17, and 20, when T_{rectal} of HS-AL pigs was higher than T_{rectal} of the two TN groups ($P < 0.04$). Meanwhile, T_{rectal} of HS-PF8 pigs was higher than TN-PF8 pigs only on day 17 ($P < 0.01$). The HS groups had higher daily T_{muscle} than TN-PF8 pigs from day 0 until day 20 ($P < 0.03$) and TN-AL pigs from day 1 until 17 ($P < 0.05$), while this was only a tendency on day 20 ($P \leq 0.08$). In TN conditions, pigs fed AL also had higher T_{muscle} than PF8 pigs on days 10, 17, and 20 ($P \leq 0.04$).

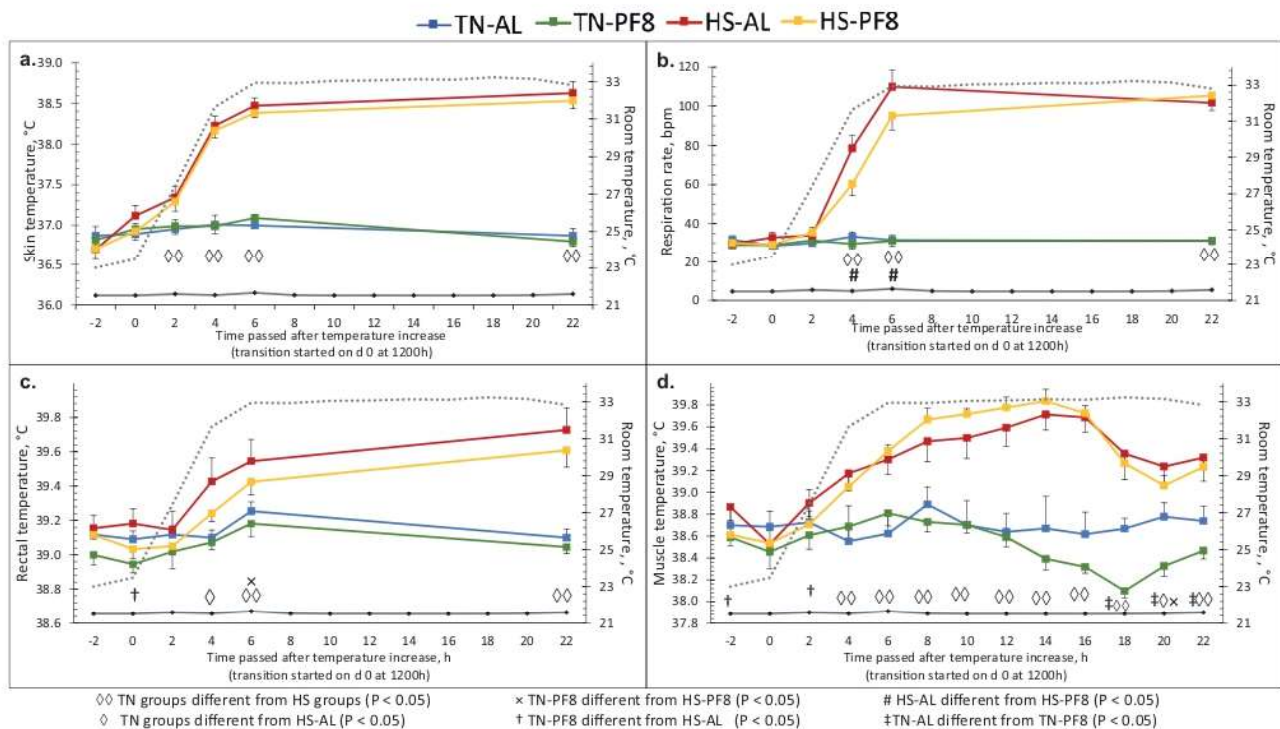


Figure 2. Effect of the climatic environment and feeding management on acute response of finishing pigs in terms of T_{skin} (a), respiration rate (b), T_{rectal} (c), and T_{muscle} (d) (means \pm SE). From day -7 to -1, pigs were housed at thermoneutrality (TN; 22 °C). On day 0, the ambient temperature of HS room (.....) was increased at a rate of 2 °C/h from 1200 to 1800 hours and was maintained at a constant temperature of 32 °C thereafter, while the TN room (—) remained at 22 °C. In each environment, the diet was provided either AL (2 distributions/d) or PF8 (8 distributions/day) with HS pigs fed AL as the reference group.

Plasma and blood gas parameters

The results on the plasma parameters are shown in Figure 4. On day -1 of the experiment, plasma concentrations of metabolites measured at 1300 hours were similar among the four groups ($P > 0.05$). Plasma creatinine was lower in the TN-AL pigs groups compared with the two HS groups ($P < 0.01$ on days 1, 8, and 20) and TN-PF8 ($P < 0.01$ on days 8 and 20). The level of plasma NEFA was not significantly different among all groups regardless of the day, while that of glucose was only different on day 8 between TN-AL and TN-PF8 ($P = 0.04$). On day 8, TN-PF8 pigs had lower plasma insulin than the HS-PF8 ($P < 0.01$) and TN-AL pigs ($P = 0.04$). Meanwhile, plasma insulin:glucose ratio was higher in the HS-PF8 pigs than both TN-PF8 ($P < 0.01$) and HS-AL pigs ($P < 0.02$) on day 8. In hot conditions, increasing feeding frequency tended to increase plasma insulin ($P = 0.07$) and significantly increased the plasma insulin:glucose ratio ($P = 0.02$) with higher values for HS-PF8 than for HS-AL pigs. Exposure to HS decreased plasma T3 whatever the feeding management treatment ($P < 0.01$ on days 1 and 8; $P < 0.03$ on day 20). Plasma T4 concentration was lower in HS-AL pigs compared with HS-PF8 pigs on day 1 ($P < 0.02$), but both HS groups had lower levels than TN-AL pigs on days 1, 8, and 20 ($P < 0.03$) and TN-PF8 pigs but only on day 1 ($P \leq 0.03$). Plasma T3:T4 ratio of TN-PF8 pigs was higher than HS-PF8 on days 1, 8, and 20 ($P < 0.02$) and HS-AL pigs only on day 1 of HS ($P < 0.01$).

Still, in Figure 4, the contrast analysis of the environmental treatment within each feeding management (TN-AL vs. HS-AL or TN-PF8 vs. HS-PF8) shows that HS exposure decreased plasma T3 ($P < 0.01$ on days 1, 8, and 20) and increased plasma creatinine levels (contrast at $P < 0.01$ on days 1, 8, and 20 between AL groups; $P < 0.03$ on days 8 and 20 between PF8 groups). Plasma T4 levels were also decreased by HS exposure on days 1, 8, and 20 within

AL pigs ($P < 0.01$) but only on day 1 within PF8 pigs ($P < 0.01$). HS also decreased the ratio of plasma T3:T4 between the PF8 groups ($P < 0.01$ on days 1, 8, and 20) but not within the AL groups ($P > 0.05$). On day 8 of the experiment, HS tended to decrease plasma insulin and insulin:glucose ratio of HS pigs when comparing the two AL-fed groups ($P = 0.07$ and $P = 0.09$, respectively), but these parameters were increased in HS pigs when comparing the two PF8 groups ($P < 0.01$ for both parameters).

Looking at the blood gas parameters in Table 4, blood $p\text{CO}_2$ levels were higher in TN groups than HS-PF8 on day 8 ($P < 0.01$) and in TN-PF8 than HS-PF8 on day 20 ($P < 0.01$). Compared with TN pigs, blood (CO_2) was lower in HS-PF8 pigs on day 1 ($P = 0.01$) and in both HS groups on day 8 ($P < 0.04$). Blood pH of HS-PF8 pigs was higher than HS-AL pigs and TN-PF8 pigs on days 1 and 8 ($P < 0.05$) and on days 8 and 20 ($P < 0.01$), respectively. The blood pH of the two AL groups did not significantly differ regardless of the environment ($P > 0.10$). From the contrast analysis, HS exposure decreased blood (CO_2) and bicarbonate on days 1 and 8 ($P < 0.04$) in AL-fed groups and significantly decreased blood $p\text{CO}_2$ on days 1, 8, and 20 ($P < 0.03$) and (CO_2) levels on days 1 and 8 ($P < 0.01$) in PF8 pigs.

Pattern of body temperature and feeding behavior

Figure 5 shows the nycthemeral variations of feeding activity and T_{muscle} of the finishing pigs. Looking at the T_{muscle} every 10 min, HS groups had higher T_{muscle} than TN-PF8 pigs throughout the day ($P < 0.05$) except from 1600 to 1700 hours (trend at $P < 0.10$). Compared with TN-AL pigs, HS pigs also had generally higher T_{muscle} ($P < 0.05$). Regardless of feeding management, pigs raised in HS conditions had similar T_{muscle} throughout the day ($P > 0.10$), while TN-PF8 pigs had significantly lower T_{muscle} than TN-AL pigs from 0130 to 1040 hours ($P < 0.05$).

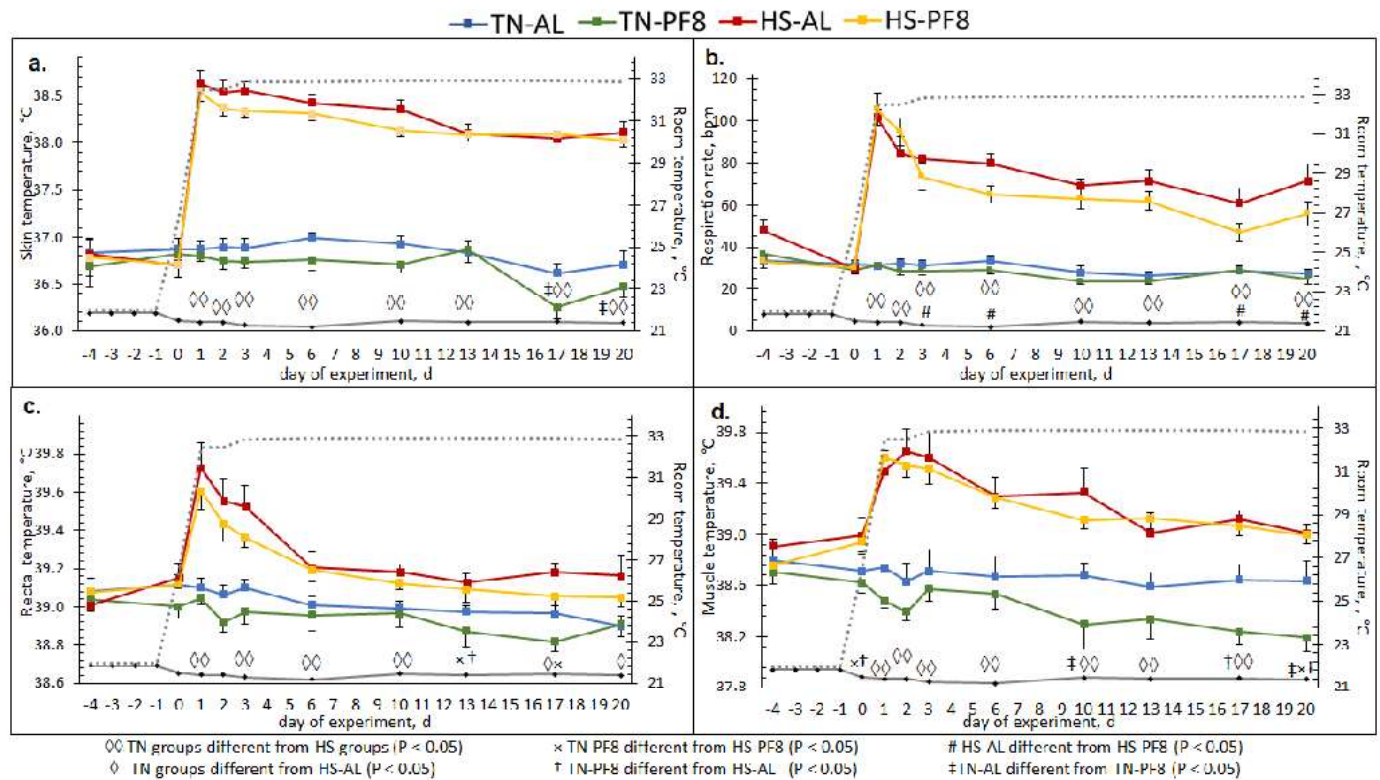


Figure 3. Effect of the climatic environment and feeding management on the T_{sk} (a), respiration rate (b), T_{rectal} (c), and T_{muscle} (d) of finishing pigs (means \pm SE). From day -7 to -1, pigs were housed at thermoneutrality and then from day 0 to 21, either in TN (22 $^{\circ}\text{C}$) or in HS (32 $^{\circ}\text{C}$) conditions. In each environment, the diet was provided either AL (2 distributions/day) or PF8 (8 distributions/day) with HS pigs fed AL as the reference group.

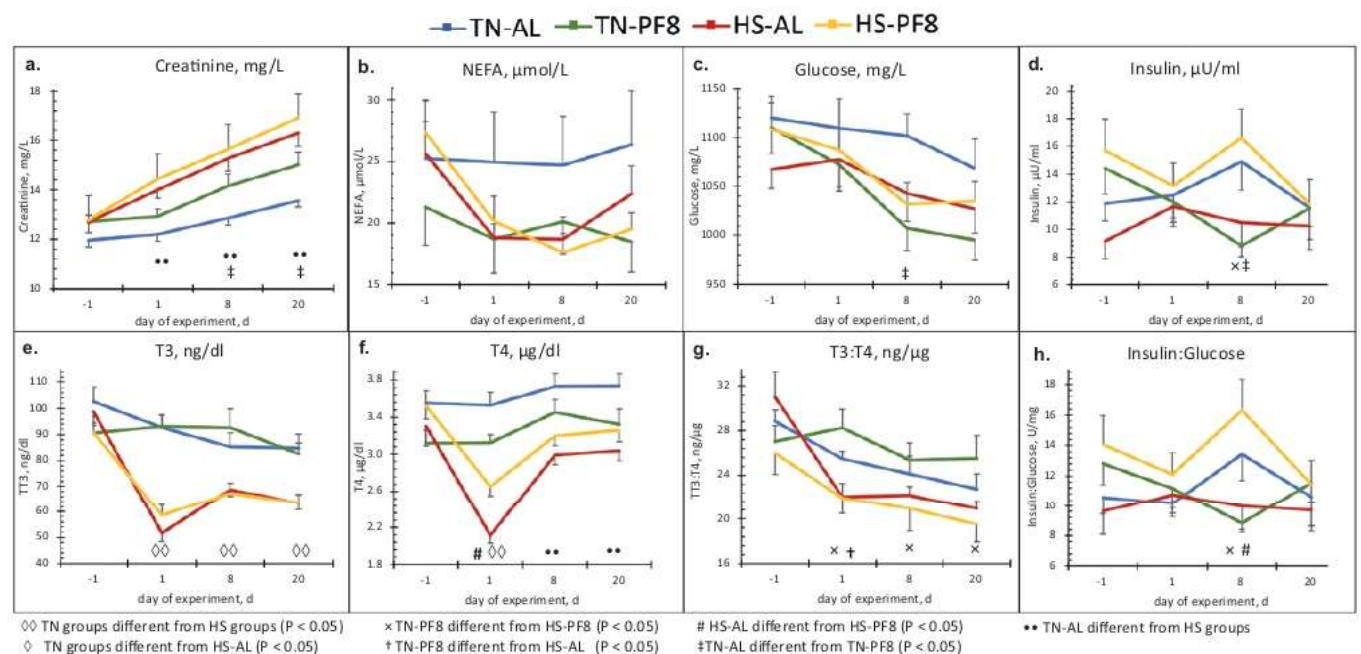


Figure 4. Effect of the climatic environment and feeding management on plasma metabolites [creatinine (a), NEFA (b), glucose (c)] and plasma hormones [insulin (d), T3 (e), T4 (f), T3:T4 (g), and insulin:glucose (h)] of finishing pigs: (means \pm SE). From day -7 to d-1, pigs were housed at thermoneutrality and then from day 0 to 21, either in TN (22 $^{\circ}\text{C}$) or in HS (32 $^{\circ}\text{C}$) conditions. In each environment, the diet was provided either AL (2 distributions/day) or PF8 (8 distributions/day) with HS pigs fed AL as the reference group.

Table 4. Effect of HS and feeding management on the blood gas parameters of finishing pigs¹

Items	TN-AL	TN-PF8	HS-AL	HS-PF8	RSD ²	Statistics ³	Contrasts P-value		
							AL vs. PF8 ⁴	AL: TN vs. HS ⁵	PF8: TN vs. HS ⁶
pCO ₂ , mmHg									
Day -1	62.3	73.4	72.4	68.7	9.6	G**, D*, G × D*	0.21	0.02	0.26
Day 1	64.8	67.1	66.8	58.1			0.28	0.63	0.03
Day 8	73.8 ^a	76.1 ^a	66.7 ^{ab}	59.5 ^b			0.41	0.09	<0.01
Day -20	68.5 ^{ab}	77.0 ^a	69.8 ^{ab}	62.8 ^b			0.82	0.77	<0.01
[CO ₂], mmol/L									
Day -1	28.8 ^b	32.1 ^a	30.1 ^{ab}	30.5 ^{ab}	2.3	R**, G**, G × D**	<0.01	0.19	0.13
Day 1	32.0 ^a	31.9 ^a	29.5 ^{ab}	28.6 ^b			0.51	0.03	<0.01
Day 8	32.6 ^a	32.5 ^a	29.6 ^b	29.4 ^b			0.80	<0.01	<0.01
Day -20	31.1	31.8	29.7	30.0			0.46	0.19	0.09
pH									
Day -1	7.33	7.31	7.29	7.31	0.05	G**	0.79	0.06	0.89
Day 1	7.33 ^{ab}	7.33 ^{ab}	7.31 ^b	7.36 ^a			0.08	0.31	0.08
Day 8	7.31 ^{ab}	7.29 ^b	7.30 ^b	7.36 ^a			0.19	0.69	<0.01
Day -20	7.32 ^{ab}	7.28 ^b	7.31 ^{ab}	7.34 ^b			0.72	0.62	<0.01
HCO ₃ ⁻ , mmol/L									
Day -1	31.3 ^b	34.6 ^a	32.7 ^{ab}	33.1 ^{ab}		R*, G*, D*	0.02	0.24	0.22
Day 1	34.9 ^a	35.0 ^a	31.9 ^{ab}	31.6 ^b			0.85	0.02	0.01
Day 8	34.5	35.0	32.0	32.3			0.64	0.04	0.02
Day -20	34.2	34.8	33.8	32.8			0.80	0.74	0.09

¹A total of 48 pigs were allocated to 4 experimental groups in 3 replicates. All pigs were housed at thermoneutrality (P1: day -7 to -1) before being subjected to either thermoneutral (TN) or heat stress (HS) conditions (P2: day 0 to 19). In each environment, diet was provided either ad libitum (AL) (2 feed provisions/day) or pair fed 8 (PF8) (8 feed provisions/day) with the HS pigs fed AL as the reference group.

²Residual standard deviation.

³The pig was considered as the experimental unit. Data were analyzed using PROC MIXED model with replicate (R), experimental group (G), day (D), and their interactions as fixed effects.

⁴Contrast statement: [TN-AL, HS-AL] vs. [TN-PF8, HS-PF8].

⁵Contrast statement: [TN-AL] vs. [HS-AL].

⁶Contrast statement: [TN-PF8] vs. [HS-PF8].

^a-^cLSmeans within a row with different superscripts letters differ ($P < 0.05$) according to the experimental group.

* $P < 0.05$, ** $P < 0.01$.

To describe feeding activity and T_{muscle} of each AL-fed group, averaged hourly data were compared with the reference value or to the data at the hour at which feeder activity was lowest. In the TN-AL group and in comparison to the reference value, feeder activity was significantly higher at 0900, 1500, 1600, and 1800 hours ($P < 0.03$) and tended to be higher at 1700 hours ($P = 0.06$) than the reference value. The pattern of T_{muscle} was similar and was significantly higher from 0800 to 1000 hours, 1400 to 1900 hours ($P < 0.02$), and tended to be higher at 1300 hours ($P = 0.06$) than the T_{muscle} than the reference value. For the HS-AL group, feeder activity was higher at 0900, 1500, 1600, 1800, and 2000 hours ($P < 0.02$) and tended to be higher at 0600, 0800, and 1900 hours ($P < 0.10$) than the reference value. In contrast, T_{muscle} of the HS-AL group at the hour of lowest feeder activity (at 0000 hours) was higher than T_{muscle} from 0400 to 2100 hours ($P < 0.05$).

The results regarding the effect of environmental temperature on T_{muscle} patterns in PF8 groups are shown in Figure 6. Baseline T_{muscle} seemed to increase with each meal with the fourth meal having higher values than the first two meals in HS-PF8 pigs ($P < 0.05$) and with the sixth meal having higher values than the previous meals in TN-PF8 ($P < 0.05$). For each PF8 group, maximum T_{muscle} also increased with each meal until the fifth meal, which had higher values than the first to second meals and the first to third meals for HS-PF8 and TN-PF8 pigs, respectively ($P < 0.05$). Feeder presence was the highest in the first meal compared with the other seven meals for the TN-PF8 pigs ($P < 0.05$), while for the HS-PF8 pigs, it was only higher compared with the second ($P = 0.03$) and not for the other

meals ($P \geq 0.08$). In terms of the body temperature increase, HS pigs had overall less area under the T_{muscle} curve regardless of the meal (-4.7 °C·min on average; $P < 0.01$), but this difference was more apparent in the first four meals ($P < 0.02$ in meals 1, 2, and 4; $P = 0.10$ in meal 3). The time (or order) of the meal tended to affect the area under the curve for the TN-PF8 pigs ($P = 0.07$) but not for the HS-PF8 pigs ($P = 0.99$). In both PF8 groups, T_{muscle} increased after each meal provision with a delay of around 11 min whatever the meal order ($P = 0.77$) or the ambient temperature ($P = 0.50$) (data not shown).

Discussion

The study aimed to confirm direct HS effects as described in the literature and to determine if, with the same feeding level, increasing feed provision frequency in hot conditions could help pigs to better adapt to HS.

Body temperature pattern and feed restriction in TN conditions

In order to facilitate discussion on pig responses of HS, this subsection briefly establishes first the normal body temperature pattern and the effects of feed restriction in pigs under TN conditions. For both TN groups, T_{muscle} peaks coincided with peaks of feeder activity. This close relationship between body core temperature and feeding behavior could be related to the HP from the TEF and from the concomitant variation in physical

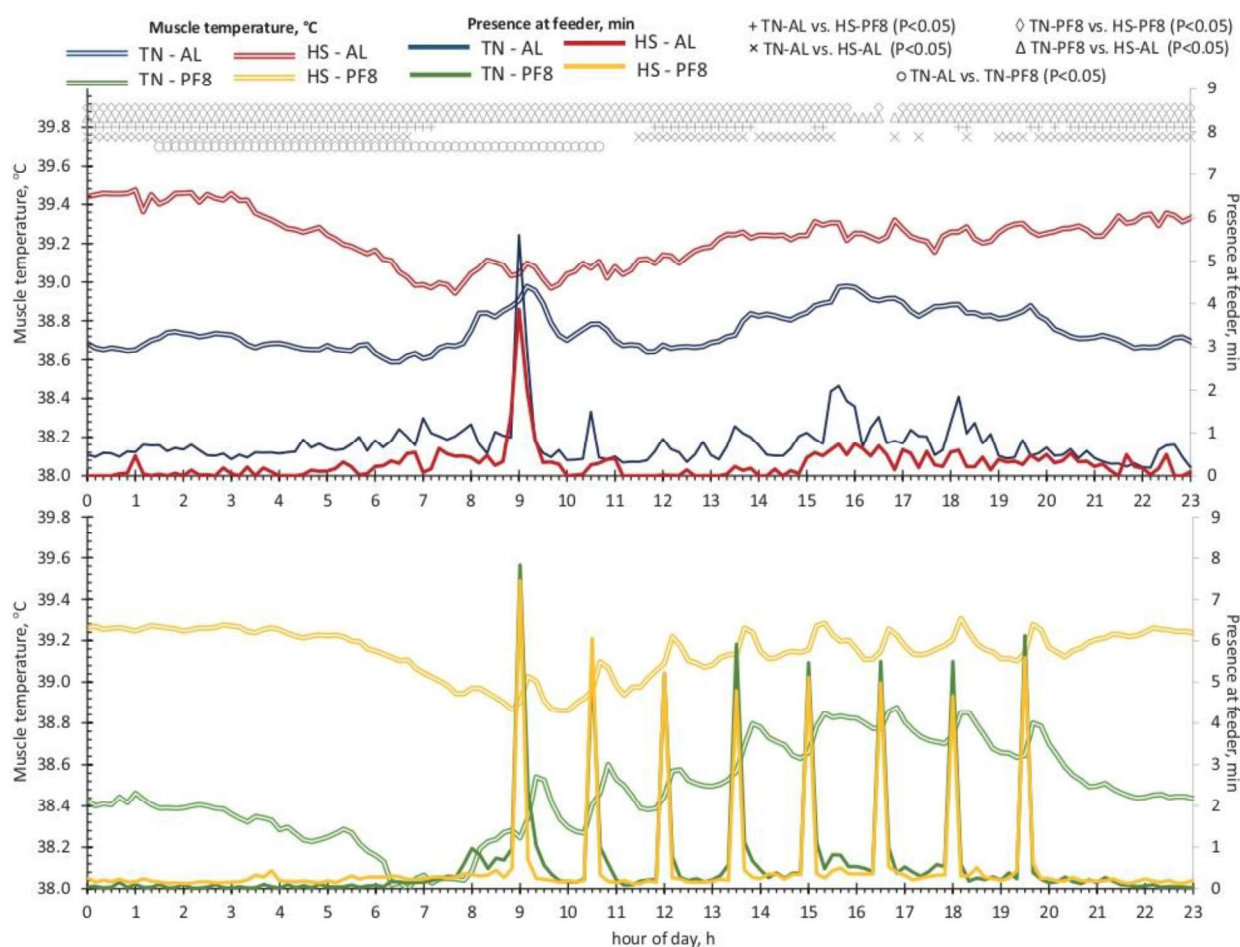


Figure 5. Effect of the environmental treatment and feeding management on the nycthemeral pattern of T_{muscle} ($^{\circ}\text{C}$) and presence at the feeder (min) of finishing pigs. From day -7 to -1, pigs were housed at thermoneutrality and then from day 0 to 21, either in TN (22°C) or in HS (32°C) conditions. In each environment, the diet was provided either AL (2 distributions/day) or PF8 (8 distributions/day) with HS pigs fed AL as the reference group. Data included are from day 6 to 19 of the experimental period with day 0 as the transition period of HS groups from TN to HS conditions.

activity from standing up and walking to the feeder (van Milgen et al., 1998). This observation is similar to those described in the literature (Ingram and Dauncey, 1985; Morales et al., 2018) wherein body temperature pattern depends largely on feeding (level and behavior) and on the related physical activity. In the TN groups, the total duration of daily presence at the feeder was not different despite dissimilar ADFI. This result is probably linked with a feed anticipation behavior phenomenon in TN-PF8 pigs similar to observations by Collin et al. (2001c) in restricted-fed pigs. During feed restriction, a higher proportion of the total energy intake goes to maintenance, and protein deposition is prioritized instead of lipid (Quiniou et al., 1996), which explains why restricted-fed TN pigs in this study were lighter and thinner. Restricted feeding also decreased visceral organ weights, which may have reduced maintenance energy (Koong et al., 1982), but PF8 pigs were also more active due to more feeding distributions, thus energy costs for physical activity may explain the numerical increase of FCR and the accentuated effect on ADG.

Effect of exposure to high ambient temperature

In the present study, pigs exposed to hot conditions tried to maintain their body temperature first by dissipating heat using sensible pathways as indicated by their increased T_{skin} (Collin

et al., 2001a) and then through the evaporative pathway as indicated by faster RR. Rise in T_{rectal} and in T_{muscle} indicates that, at 32°C , pigs are no longer able to lose more body heat to maintain their homeothermic status. There was afterward a gradual decrease in the thermoregulation responses as the pigs adapted to HS, comparable to the biphasic acclimation HS response previously reported by Renaudeau et al. (2010). Similar to the results of Liu et al. (2016), panting of HS-AL pigs decreased their blood (CO_2) and, due to reduced carbonic acid formation, also their blood bicarbonate. This was a transient response in HS-AL pigs as levels returned to normal by day 20, which may be due to the slower RR once the pigs have acclimated to HS.

According to Collin et al. (2001c), adaptation to HS is mainly related to HP reduction caused by a lower FI (lower TEF) and decreased maintenance requirements. Between the two AL-fed groups (TN-AL vs. HS-AL), HS exposure decreased ADFI by $541 \text{ g}\cdot\text{d}^{-1}$ close to an estimated value of $-550 \text{ g}\cdot\text{d}^{-1}$ for 75-kg pigs (Renaudeau et al., 2011). This 18% decrease in ADFI consequently reduced BW gain by 21% and was linked to a shorter time spent at the feeder, which is comparable to previous reports of lower consumption time in hot conditions (Quiniou et al., 2000; Collin et al., 2001a). It can be assumed that the meal size of the AL-fed pigs in the present study decreased with heat exposure since

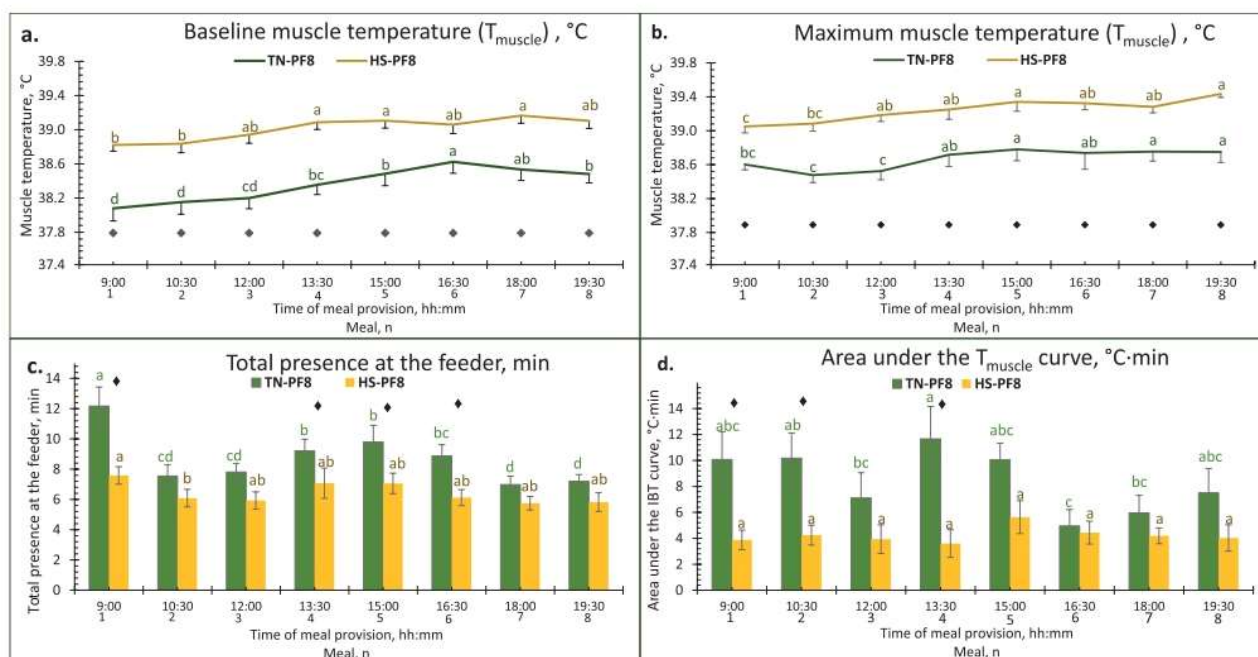


Figure 6. Effect of the environmental treatment and order of meal in terms of T_{muscle} [baseline (a) and maximum (b)], total presence at the feeder (c), and area under the T_{muscle} curve (calculated based on Figure 1) of PF8 finishing pigs. From day -7 to -1, pigs were housed at thermoneutrality and then from day 0 to 21, either in TN (22 °C) or in HS (32 °C) environment. Pigs were PF to HS pigs fed AL, and the diet was provided in 8 distributions/day. Data included are from day 6 to 19 of the experimental period with day 0 as the transition period of HS groups from TN to HS conditions. ^{a-c}Within each feeding management, means with different superscript letters differ according to the meal ($P < 0.05$). ♦Groups are different within each meal ($P < 0.05$).

the number of visits to the feeder was not affected but ADFI was reduced. The observed shorter duration of standing at the feeder and the smaller meal sizes may be considered as adaptation responses to HS (Collin et al., 2001b) as they reduce energy from HP associated with physical activity and TEF. Reduction in ADFI in HS animals is usually linked with a decrease in fasting HP (Labussière et al., 2016b) and, though this was not measured in our study, it may explain the lower levels of plasma thyroid hormones in HS-AL pigs as they are also indicative of lower metabolic rate (Yavuz et al., 2019).

In this study, exposure to a high ambient temperature not only altered the pattern of feeding behavior of the AL pigs but also of their body temperature. In TN pigs, the T_{muscle} pattern increased with feeding and physical activity but in HS-AL pigs, T_{muscle} increased progressively during the day (which corresponds with the HP associated with TEF) but remained high until the early morning of the following day (until 0300 hours in the present study). The T_{muscle} of HS-AL pigs at the hour of lowest feeder activity (hour 0) was even higher than the T_{muscle} during the day in which hours of highest feeder presence were observed. This observation is similar to the results of Cervantes et al. (2018) on ileal temperatures of HS pigs even though the pigs in their study were subjected to a natural diurnal pattern of high ambient temperature compared with the constant, controlled temperature of the current study. This suggests that the body temperature pattern of HS pigs does not generally depend on the type of HS challenge pattern they are subjected to. As reviewed by Gonzalez-Rivas et al. (2020), the saturated heat loss pathways of the HS pigs during the day could have slowed down gastric emptying and motility rates as observed in animals under HS since these processes are known to contribute to HP. This somewhat explains higher T_{muscle} during the night despite the low feeder activity and suggests that body

temperature differences of TN and HS pigs are more apparent at night, thus only considering measurements during the day may underestimate thermoregulatory responses of HS pigs (Renaudeau, 2020).

Exposure to hot conditions resulted in lighter pigs with slightly lower proportions of kidneys and liver, which could be due to the feed reduction effect of HS as this was also observed in TN-PF8 pigs. However, FI reduction does not seem to fully explain other physiological responses of HS pigs. Comparing HS-AL and TN-PF8 responses, HS-AL pigs had a higher increase in plasma creatinine and greater reduction in plasma T4 than TN-PF8 pigs despite similar ADFI. Furthermore, plasma T3 responses were maintained in TN-PF8 but were greatly reduced in HS-AL pigs. It can be argued that HS-AL pigs should have been fatter than the TN-PF8 pigs since they had higher creatinine levels (higher protein catabolism) and lower plasma thyroid hormones (lower lipolysis and metabolic rate). Indeed, reduced feeding level significantly reduced BFT in TN-PF8 pigs but not in HS pigs, and, compared with TN-AL, there was a numerical increase in the perirenal fat in HS-AL pigs not in TN-PF8 pigs. Along with the observed physiological responses, it could also be possible that slower gastric emptying in HS pigs "shortened" their fasting period during the night and thus decreased the need to break down fat for energy.

The present study thus confirms that when pigs can no longer maintain a homeothermic status in hot conditions, one of the ways that they reduce HP or heat load indirectly is by decreasing ADFI consequentially altering feeding behavior. Exposure to high ambient temperature also changes nycthemeral pattern and other physiological responses of pigs, which is mostly related to feeding and the HP associated with it. However, HS effects do not seem to be explained by merely feed reduction, and these heat-induced effects unrelated to ADFI are tackled more in detail in the next subsection.

Direct and indirect effects of HS at the animal level

In distinguishing the direct effects of HS, the present study aimed to remove or at least minimize the confounding effects of different feeding levels and pattern (indirect HS effect) and of pair-feeding itself including feed restriction and imposed meal patterns. Hence, the TN-PF8 and HS-PF8 pigs in this study were not only PF to the HS-AL pigs, but both groups were also adapted to the same feeding schedule even before the experimental period. Regardless of feeding management, exposure to high ambient temperature increased the thermoregulatory responses of the pigs as described in the previous section. Comparing the T_{muscle} pattern of the two PF8 groups, the ambient temperature did not affect the average lag time between meal distribution and the rise in T_{muscle} , which is around 11 min, similar to the delay of <15 min reported by Morales et al. (2018). In both PF8 groups, T_{muscle} increased with each additional meal provision, which agrees with the pattern of HP related to TEF. However, baseline and maximum T_{muscle} plateaued earlier for the HS-PF8 pigs (both at the third meal) than for the TN-PF8 (at the sixth and fourth meal, respectively) although both groups had similar intake per kg BW and thus theoretically should have had the same HP due to TEF (de Lange et al., 2006). Less heat was also dissipated per meal by the HS-PF8 than the TN-PF8 pigs as indicated by the smaller area under T_{muscle} curve, suggesting that the heat loss capacity of HS pigs may have been maximized or compromised. This is consistent with the fact that body temperature increase depends on the heat exchange between the animal and the environment (Renaudeau et al., 2012). It is also in line with observations of Cervantes et al. (2018) who indicated that the body temperature of pigs in hot conditions depends on both ambient temperature and FI, and that heat load of the pigs before a meal limits their capacity to dissipate TEF-related heat.

The reduction in ADFI in TN-PF8 pigs resulted in an ADG reduction of 22% similar to HS-AL pigs (21% for HS-AL) but slightly lower than the reduction in HS-PF8 pigs (30%). Comparing the ADG of the two PF8 groups, those exposed to HS seemed to have an additional 8% BW loss, which is not explained by merely feed restriction. In dairy cows, Baumgard and Rhoads (2013) reported 35% to 50% production losses, which are unrelated to FI. Our results are in contrast to those of Collin et al. (2001c) and Pearce et al. (2013), wherein the HS pigs in their studies gained more weight than PF TN counterparts despite the ADFI reduction (around 9 and 19 g/kg BW, respectively, vs. 5 g/kg BW in our study). Differences in results may be due to implemented PF design (e.g., PF TN counterparts were compared with AL-fed HS pigs in their studies) or to their shorter experimental challenge duration (7 d) which may not have been enough for their PF TN pigs to adapt to the sudden meal size reduction.

Between the PF8 groups, those exposed to HS had a greater proportion of perirenal fat (internal level) similar to the results of Kouba et al. (2001) but only numerically higher BFT. Seibert et al. (2018) also reported higher abdominal adipocyte size in HS pigs than their TN-PF counterpart. According to Le Bellego et al. (2002), maximal protein deposition is considered to be limited in hot conditions due to its high energetic cost. From that, it can be assumed that the extra energy was redirected for lipid deposition. The redirection of fat storage internally (perirenal fat) rather than externally (backfat) could be an adaptation response, which is hypothesized to reduce body insulation and facilitate heat dissipation during long-term heat exposure (Le Dividich et al., 1998). The higher fat deposition of HS-PF8 than TN-PF8 pigs might also be related to HS pigs possibly having a “shortened” fasting period as discussed previously or to their

increased insulin and insulin:glucose responses, similar to the findings of other PF studies in pigs (Pearce et al., 2013; Sanz Fernandez et al., 2015b). Baumgard and Rhoads (2013) reviewed in detail possible theories on the increased insulin response of HS pigs. In our study, this insulin response appears to be a direct effect of HS as it was only observed in the contrast between PF8 pigs in response to the environmental conditions. Higher insulin levels should have theoretically increased protein deposition since it is also an anabolic hormone for protein synthesis (Davis et al., 2001). However, it was only a transient response in HS-PF8 pigs (only on day 8), while their plasma creatinine, an indicator of muscle protein catabolism (Muller et al., 2017), was constantly higher than in TN-PF8 pigs, which perhaps negated the insulin effect on protein deposition and thus on lean meat content. In our study, plasma creatinine seemed to be a function of both FI reduction (since levels in TN-PF8 decreased compared with TN-AL) and also a direct effect of heat exposure (since HS pigs had higher levels than TN-PF8 pigs despite similar feeding level).

High ambient temperature also reduced the levels of plasma thyroid hormones and T3:T4 ratio (i.e., conversion rate of T4 to T3) in PF8 pigs, which are known indicators of lipolysis and increased metabolic rate (Silva, 2006). This is similar to the results of Sanz Fernandez et al. (2015a) and supports previously discussed claims that HS increases fatness and decreases maintenance energy requirements. Other studies also reported that increased fatness could be related to a decreased plasma NEFA response of HS pigs (Pearce et al., 2013; Sanz Fernandez et al., 2015a) but this was not the case in our study since TN-PF8 and HS-PF8 pigs had similar levels. The higher plasma NEFA exhibited by TN-raised PF compared with AL-fed HS pigs in the aforementioned studies may be unrelated to direct HS effects but could be an effect of PF itself and the prolonged fasting period (Russell et al., 2008). Nutritional status of the pigs during blood sampling could also explain the differences of results across studies since, according to Kouba et al. (2001), HS pigs have higher plasma NEFA than PF TN pigs in an unfed state but not in a fed state which was the case in our study. Nevertheless, our study agrees with previous pig studies on the direct HS effects such as lower thyroid activity and increased (transient) insulin response, which may have contributed to their altered body composition. Other effects of heat exposure demonstrated in this study also include increased plasma creatinine and the inability to export heat efficiently to the environment with a consequence on the body temperature pattern.

Increasing feed provision frequency in HS pigs

This subsection focuses on the two HS groups (HS-AL and HS-PF8) with the same feeding levels but different feed provision frequencies. Aside from fasting HP, heat from the utilization of nutrients contributes significantly to heat load of pigs under HS so the idea was to avoid overwhelming their heat loss pathways by splitting HP into smaller increments via the provision of feed into smaller portions. Between the two HS groups, the meal frequency of the HS-PF8 pigs was effectively increased by design consequently decreasing their meal sizes and time they spent per meal. Although only numerical, the HS-PF8 pigs had a slightly lower BW gain despite the similar ADFI with HS-AL. It is possible that the feeding strategy for the HS-PF8 pigs did not allow them as many opportunities as the HS-AL pigs to adjust their feeding behavior which is, in fact, also an adaptation response to heat exposure. Increasing feeding frequency seemed to improve RR in the present study as HS-PF8 pigs had generally slower breathing than the HS-AL pigs. However, this does not

indicate an overall better HS response since the measurement was done only in the morning when HS-PF8 pigs have only eaten one meal and thermoregulatory responses of HS pigs increase throughout the day as previously demonstrated. In the present study, thermoregulation response did not improve with increasing feeding frequency, and even the nycthemeral pattern of the T_{muscle} during chronic HS was similar between the two HS groups regardless of the time of day. These results might be related to the discussed HS effect of slowing down gastric emptying and/or digestion rate, which also somewhat explains why body temperature progressively increased during the day and is maintained during the night despite the low feeder activity for both HS groups.

Hence, increasing feeding frequency during hot conditions did not improve pig performance or thermoregulatory responses in the present study. It is possible, though, that it is not the increased feeding frequency per se that had no effect on pig response to HS but the specific way in which it was implemented in our study. In poultry studies, the timing of feeding such as the provision of 80% to 100% of diets in the afternoon improved turkey performance during HS (Farghly et al., 2018), and feed withdrawal in times of high heat load help mitigate HS effects in meat-type poultry (Syafwan et al., 2011). It might be interesting to evaluate other feeding management strategies (e.g., longer feeding intervals, timing of feed provision later during the day, or frequent feeding but during the night) to improve the ability of pigs to better cope with HS.

Conclusions

In the present study, HS pigs reduce FI, which changes their normal feeding pattern and reduces their growth performance. Using a pair-feeding design that removes the effects of the feeding level and pattern, HS pigs are shown to export heat less efficiently than their TN counterpart. Thus, the need to lower heat increment may help explain changes in their body temperature pattern (possibly linked to a slowed-down digestion process) and altered body composition (could be related to limited protein deposition). Other direct effects of heat exposure in pigs also include decreased thyroid hormone activity, increased plasma creatinine, and transient insulin responses. With the same feeding level, increasing feeding frequency did not improve pig response to HS and this may be related to the compromised heat dissipation ability of pigs and its consequence on the digestion process.

Acknowledgments

The authors want to thank F. Le-Gouevic, A. Chauvin, M. Genissel, J. Georges, J. Delamarre, and H. Demay from UE3P, INRAE, 35590 Saint-Gilles, France for animal care and sample collection, A. Constantin, P. Ganier, A. Marchais, C. Mustière, C. Perrier, G. Robin, and R. Comte for lab analyses. This study and the PhD grant of A.M. Serviento were funded by Lallemand Animal Nutrition (Blagnac, France) and French Technology Research National Agency.

Conflict of interest statement

The authors declare that there is no conflict of interest

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Chapter 3. Effect of live yeast supplementation and feeding frequency in male finishing pigs subjected to heat stress

This chapter presents the results of Experiment 3 wherein the objective was to evaluate the effects of LY supplementation and feeding frequency in HS pigs. The manuscript containing the results of this experiment was accepted in the British Journal of Nutrition. The submitted version of the manuscript is presented in the following pages and the supplementary information is presented in **Annex 5**.

Effect of live yeast supplementation and feeding frequency in male finishing pigs subjected to heat stress¹

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Key words: heat stress, energy metabolism, live yeast, meal frequency, pig, thermoregulation

Abstract

In growing pigs, reduced growth during heat stress (HS) is mainly related to decreased feed intake (FI). The study aimed to determine whether the reported positive effects of live yeast (LY) supplementation in HS pigs were due to a modified feeding behaviour or energy metabolism, and if these can be replicated by imposing an increased meal frequency. The effect of LY supplementation [0 (N) vs. 100 g/ton of feed (Y)], and of feeding window (FW) [unlimited, 2FW of 1 h each, and 8FW of 15 min each] were measured in entire male finishing pigs (n=36). Ambient temperature was at 22°C during period 1 (5 d) and at HS conditions (28°C) during period 2 (5 d). Heat exposure decreased FI and retained energy (RE) of pigs (-627 and -460 kJ/kg BW^{0.60}·d, respectively; P<0.01). During HS, LY-supplementation in Unli pigs decreased inter-meal intervals (P=0.02) attenuating HS effect on FI which tended to improve RE (P= 0.09). N—8FW had higher FI and RE than N—2FW (P<0.05) but protein deposition (PD) were similar. Supplemented pigs had higher PD during HS regardless of FW (+18 g·d⁻¹; P = 0.03). Comparing the 2FW groups, improved heat tolerance of LY-supplemented pigs were due to improved insulin sensitivity (P<0.05) and latent heat loss capacity after a meal (P<0.05) allowing them to increase their FI (via an increased number of meals) and thus their energy efficiency. Imposing an increased meal frequency improved FI in HS pigs but did not replicate positive effects of LY on protein deposition.

Introduction

Climatic environment is a major problem in pig production in many countries especially with global warming and more frequent heat waves. Pigs exposed to high ambient temperature enter a state of heat stress (**HS**) when their heat load exceeds their ability to dissipate heat and consequently they are no longer able to maintain a constant core body temperature ⁽¹⁾. Genetic selection for higher feed intake and faster lean growth resulted to higher metabolic HP in modern pigs ⁽²⁾ making them more susceptible to heat stress especially entire male pigs due to higher basal heat production (**HP**) ⁽³⁾ and higher protein deposition associated with higher heat increment ⁽⁴⁾. The reduced growth during HS is mainly due to the reduced feed intake as an adaptation to reduce HP related to the thermic effect of feed (**TEF**) ⁽⁵⁾. Several nutritional strategies to decrease total heat load (e.g. to improve energy utilization and to reduce heat increment) have thus been studied to mitigate HS effects in livestock animals ^(6,7). Nevertheless, most of these solutions aim at reducing the daily HP produced rather than considering that instantaneous body temperature results from the dynamic equilibrium between heat production and dissipation.

Indeed, HP and body temperature are not constant during the day but are closely related to variations in feeding behavior and in physical activity ^(3,8) in addition to a biological diurnal pattern. It is possible that rather than reducing the daily heat load, strategies to avoid saturating heat loss pathways (to limit instantaneous HP peaks and/or to improved heat losses) could help the pigs in coping better with HS. Recent studies have shown that live yeast (**LY**) supplementation improved HS response of pigs ⁽⁹⁾ and of dairy cows ⁽¹⁰⁾ possibly mediated by a modified feeding behavior changes (higher number of meals or shorter inter-meal intervals) and by an improved energy efficiency. One theory is that this increased meal frequency might have helped by splitting the heat increment into several events of smaller amplitude rather than a small number of events of a larger amplitude. Meanwhile, reported changes in gut microbiota composition ⁽⁹⁾, and improvement in immune responses and intestinal integrity of yeast-supplemented animals ^(11,12) could also be linked to an improved energy efficiency.

The present study thus aimed to evaluate whether or not the improved HS response due to LY supplementation in pigs is linked to a modification of feeding behavior and/or of energy metabolism, and to determine if increasing the meal frequency can replicate the observed positive effects of LY supplementation in HS pigs.

Table 1. Composition of the experimental diets.¹

Ingredients, % as-fed	Control (N)	Supplemented (Y)
Wheat	26.732	26.728
Barley	26.732	26.728
Corn	26.732	26.728
Soybean meal	4.659	4.658
Rapeseed meal	4.621	4.624
Soybean hulls	1.709	1.709
Soft wheat bran	1.709	1.709
Sunflower oil	0.631	0.634
L-Lysine HCl	0.296	0.296
L-Threonine	0.067	0.067
Molasses	3.000	3.000
Salt	0.450	0.450
Calcium carbonate	0.993	0.993
Monocalcium phosphate	0.866	0.866
Vitamin-Mineral Premix ²	0.500	0.500
Stearin	0.005	-
LSB	-	0.010
Indigestible marker (TiO ₂)	0.300	0.300
Analyzed chemical composition, % as fed ³		
Actual dry matter	89.0	88.9
Dry Matter	89.0	89.0
Crude Protein	12.6	12.4
Crude Ash	4.4	4.4
Crude Fat	2.7	2.7
NDF	11.5	11.7
ADF	4.1	4.1
ADL	0.8	0.8
Starch	47.9	47.9
Formulated values, % as fed		
Net energy, MJ/kg	9.60	9.60
SID lysine : NE (g/MJ NE)	0.65	0.65

¹Diet fed in pellet form.²Provided per kilogram of complete diet: vitamin A, 1,000,000 IU; vitamin D3, 200,000 IU; vitamin E, 4,000 mg; vitamin B1, 400 mg; vitamin B2, 800 mg; calcium pantothenate, 2,170 mg; niacin, 3,000 mg; vitamin B12, 4 mg; vitamin B6, 200 mg; vitamin K3, 400 mg; folic acid, 200 mg; biotin, 40 mg; choline chloride, 100,000 mg; iron (sulfate), 11,200 mg; iron (carbonate), 4,800 mg; copper (sulfate), 2,000 mg; zinc (oxide), 20,000 mg; manganese (oxide), 8,000 mg; iodine (iodate), 40 mg; cobalt (carbonate), 20 mg; and selenium (selenite), 30 mg.³As-fed basis. Aside from actual dry matter content, values are calculated for the same dry matter content (89.0%).

Materials and methods

The experiment was conducted in accordance with the French legislation on animal experimentation and was approved by a Committee for Consideration of Ethics in Animal Experimentation (Authorization: APAFiS #18973-2019020622003043).

Animals and Treatments

The study was designed to investigate the effect of LY supplementation and of increasing meal frequency on finishing pigs exposed to hot conditions in a 2×3 experimental design with 2 levels of Levucell SB TITAN supplementation: 0 (diet **N**) vs. 100 g/ton of feed (diet **Y**; 1×10⁶ CFU *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079/g of feed), and 3 feeding management practices called feeding windows (**FW**): unlimited access (**Unli**; 0900h to 1545h and 1600h to 0700h), **2FW** of 1 h each (from 0900h to 1000h and from 1500h to 1600h), and **8FW** of 15 min each (at every 90-min interval from 0900h to 1930h). The experimental diets (9.60 MJ/kg; 0.65 SID Lys:NE; shown in Table 1) were formulated to be slightly limiting in protein in order to evaluate differences in protein deposition among the groups. Titanium dioxide (TiO₂) was added in the diets (3 g/kg feed) as indigestible marker. Feed was automatically distributed twice for groups with unlimited and 2 FW (at 0855h and at 1455h) and eight times for groups with 8 FW (5 min before each feeding window). There were thus 6 experimental groups (N—Unli, Y—Unli, N—2FW, Y—2FW, N—8FW, and Y—8FW) in which feeding behavior, energy metabolism, and thermoregulation responses were measured.

In order to have 6 pigs per experimental group, 36 individually housed and fed entire male finishing pigs [Pietrain × (Large White × Landrace); 62.2 ± 1.0 kg initial live BW] were used in the experiment using two similar respiration chambers. Since only two pigs can be measured at the same time and because pigs from the experimental herd were only available every 3 weeks, the experiment was conducted in 9 replicates with 4 pigs coming from the same litter per replicate. There were 2 blocks in each replicate spaced 10 d apart and group allotment was decided to have a balanced comparison between experimental groups. For each block, two pigs were individually housed in metabolic crates and were fed the corresponding experimental diet (Table 1) during a 17-d adaptation period (Fig. 1). Thereafter, pigs were moved in a 12-m³ open circuit respiration chamber (as described by Vermorel et al.¹³) for 10 d of measurements

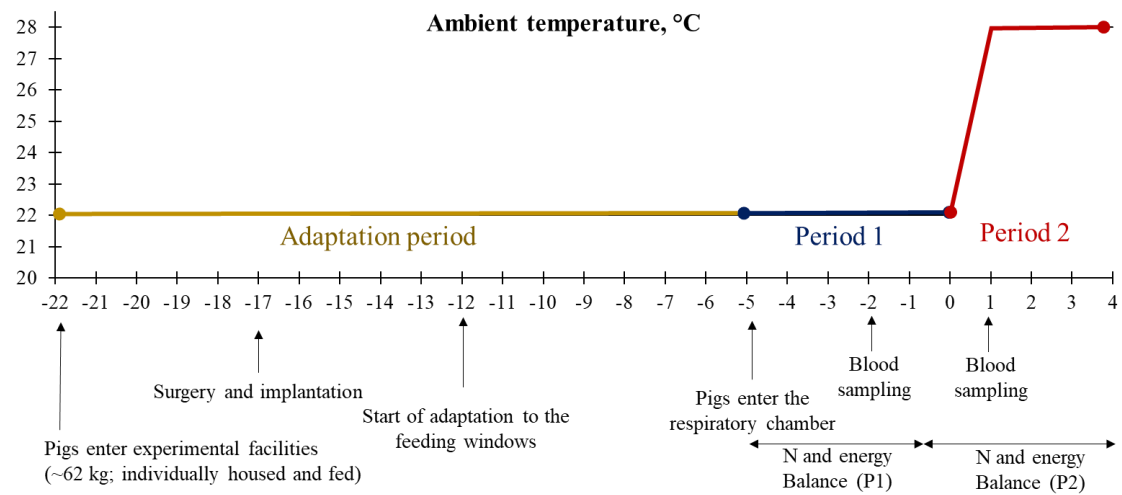


Figure 1. Description of experimental design and the timing of the measurements.

wherein ambient temperature was maintained at thermoneutral (**TN**) conditions of 22.0°C (actual 22.0±0.6°C) during the first 5 d of measurement (Period 1 or P1; d -5 to -1) and was increased for the last 5 d at HS conditions of 28.0°C (actual 27.4±0.5°C) (Period 2 or P2; d 0 to 4) with the transition on d 0 (fixed at 25°C at 0800h and an increase of 1°C/hour from 0800h to 1100h). The cage (3.3 m²) was equipped with an automatic feeder and an automatic weighing scale. At the beginning of adaptation period, a catheter was inserted in the external jugular vein through a collateral vein under general anesthesia (methods described by Melchior et al.¹⁴) allow for successive blood samplings and a temperature probe (Anipill, Body Cap, Hérouville Saint-Clair, France) was implanted 2 to 3 cm deep into the brachiocephalic muscle of the neck of the pig (method described by Renaudeau¹⁵). The surgery and implantation were then followed by a recovery period during which the animals were also adapted to their diet. For the first 10 d of adaptation, the pigs were adapted to the feed and feeding distribution schedule with unlimited feed access for the first 10 d and then the feeding windows were imposed in the last 7 d of adaptation. All throughout the experimental period, relative humidity (**RH**) was kept constant at 65% (actual 67.1 ± 2.7%), light was turned on from 0730h to 1930h, feeder was blocked from 0700h to 0900h for animal care and samplings, and water was provided ad libitum.

Measurements and samplings

The pigs were manually weighed twice at 0830h: before entering and after exiting the respiration chambers. Live BW data were also transmitted and registered each time the pig stepped in front of the feeding trough through a platform equipped with a set of load sensors calibrated to provide the live BW. Feed allowance was weighed daily and refusals were weighed per period. For each of the four batches of feed fabrication, diets were sampled during feed preparation and pooled samples were taken over the experimental period. Feed and water intake (time, duration, quantity) were continuously recorded through weight sensors placed below the feeding trough and the water storage tank (outside the chamber), respectively. Total fecal collection was done at the end of the 10-d measurement in the chamber and spot fecal sampling were done twice during each period between 0800h to 0900h (d -3 and d-1 for P1 and d 1 and d 4 for P2) in order to have separate digestibility coefficients per period. The spot fecal samples were pooled per period. Urine was collected in a bucket containing 250 ml of 10% H₂SO₄ to prevent from microbial fermentations resulting in ammonia losses. Urine was collected,

weighed, and sampled (2% of weight) daily, and the representative sample cumulated per period was stored at -20°C awaiting analyses. Nitrogen losses in the chamber recovered in the condensed water and outgoing air of the chamber were measured per period (methods as described by Noblet et al.¹⁶).

Gas concentrations of O₂, CO₂, and CH₄ of outgoing air were continuously measured with a paramagnetic differential analyzer (Oxymat 6, Siemens AG, Munich, Germany) for O₂ and with two infrared analyzers (Ultramat 6, Siemens AG, Munich, Germany) for the CO₂ and CH₄ concentration. Gas extraction rate of the air of the chamber was measured with a mass gas meter (Teledyne Brown Engineering, Hampton, VA) and corrected for humidity content. The cage in the respiration chamber was mounted on force sensors (9104A, Kistler, Winterthur, Switzerland) producing an electrical signal proportional to the physical activity of the pig⁽¹⁷⁾. Gas concentrations, the signals of the force sensors, the weight of the trough and water tank, gas flow rate, RH, ambient temperature, and atmospheric pressure in the respiration chamber were measured 60 times per second, averaged over 10-s intervals, and recorded for further calculations (as described by van Milgen et al.¹⁸). The recovery of known amounts of CO₂ and N₂ was measured before and after the experiment; it averaged 99.7 and 99.9 % for CO₂, and 100.2 and 99.5 % for N₂ for respiration chambers 1 and 2, respectively. Temperature of the drinking water was also continuously recorded.

The implanted temperature probes in the neck muscle captured body temperature data every minute. In order to evaluate postprandial metabolism differences among the groups and between the periods, blood sampling kinetics was done on d -2 (P1) and on d 1 (P2) in which the catheter was extended out of the respiration chamber to allow blood collection with the least disturbance to the animal. Blood (7 mL) was collected in 8-mL heparin tubes twice before the first meal at 0820h and 0840h (referred to as pre-prandial), at the beginning of the meal (0 min), and then at 20, 40, 60, 90, 120, 150, and 180 min after the beginning of the meal. The blood samples were placed in ice before centrifugation (3,000 g; 10 min; 4°C), and plasma was aliquoted and stored at -20°C until analysis. Hematocrit was measured at every sampling to correct for possible dilution of blood by saline solution that saturated the catheter line.

Laboratory analyses

The sampled feed per period and the feed refusals were oven-dried at 103°C overnight to subsequently determine their dry matter (**DM**) content. Representative samples of each

experimental diet per feed fabrication were analyzed for ash, starch, ether extract, and crude fiber contents according to Association of Official Analytical Chemists ⁽¹⁹⁾. Feed samples and total fecal samples (one per pig) were analyzed for DM, ash, nitrogen (N; Dumas method) and gross energy, and TiO₂ content ⁽²⁰⁾. Periodic fecal samples were also analyzed for DM and TiO₂ content. Urine was analyzed for N content using fresh samples and for gross energy content using urine (~30 mL) freeze-dried in polyethylene bags. Ammonia content in the solution where extracted air bubbled and in the condensed water was determined on fresh material using an enzymatic method (Enzytec fluid, Scil Diagnostics GmbH, Germany).

For plasma samples, commercially available kits were used to measure plasma levels of glucose [Glucose (HK), lactate (Lactic acid, ABX Pentra, Montpellier, France), NEFA (FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany), creatinine [Creatinine (Jaffe), Thermo Fisher Scientific Oy, Vantaa, Finland], triglyceride (Triglyceride, Thermo Fisher Scientific Oy, Vantaa, Finland) and urea (Urea, Thermo Fisher Scientific Oy, Vantaa, Finland). Inter-assay CV were 2.48%, 1.64%, 6.17%, 3.87%, 2.98%, and 2.87%, respectively. Intra-assay CV were 2.73%, 1.06%, 3.42%, 1.85%, 0.72%, and 2.32%, respectively. Plasma levels of α -amino N (Protéines-kit; bioMérieux, 182 Craonne, France), insulin (ST AIA-PACK IRI, Tosoh Corporation, Tokyo, Japan), total triiodothyronine or T₃ (ST AIA-PACK T₃, Tosoh Corporation, Tokyo, Japan), and total thyroxine or T₄ (ST AIA-PACK T₄, Tosoh Corporation, Tokyo, Japan) were also determined. Intra-assay CV were 5.20%, 2.3%, 3.8%, and 3.99%, respectively.

Calculations

Dry matter intake per period was measured as the difference between total DM feed offered per period (total feed offered \times % DM content) and the sum of the DM feed refused per period. Feeding behavior parameters were calculated from the continuous data collected from the feeding trough. The meal criterion is the maximal duration between 2 successive feeding bouts owing to the same meal. From preliminary analyses, increasing durations have been tested that resulted to different total number of meals. The meal criterion of the present study was determined to be 10 min because this was the minimal duration for which the resulting number of meals did not increase any longer ($P > 0.05$). Thus, two consecutive visits separated by a time interval shorter than 10 min were considered to belong to one meal. This adopted meal criterion was also used for calculation of daily feeding behavior parameters such

as number of meals, feeding duration, inter-meal interval, and rate of feed intake (**RFI**) (as described by Renaudeau et al. ²¹). Due to the imposed and prolonged feed access restriction in 2FW and 8FW groups, inter-meal intervals were considered from the first meal taken after 0900h (first feeding window of the day) and the last meal taken before 0900h of the following day.

Apparent fecal digestibility of DM, N, and energy was measured per pig and was split into 2 periods based on TiO₂ content in the periodic fecal samples. Nitrogen retention was calculated per period as the difference between N intake and N lost in the feces, urine, and as ammonia. The resulting N retention was multiplied by 6.25 to determine protein deposition (**PD**) per period. Digestible energy and metabolizable energy (**ME**) intake were computed according to standard methods and considering CH₄ production. Total HP was calculated from respiratory gas exchanges, CH₄, and urinary N (including evaporated N) according to the formula of Brouwer ⁽²²⁾. Energy retention (**RE**) was the difference between ME intake and HP. Fat deposition was then determined based on the energy balance, assuming that the RE was only deposited as protein ($\text{Rep} = \text{PD} \times 23.6 \text{ kJ}\cdot\text{g}^{-1}$) or as fat [$\text{lipid deposition} = (\text{RE} - \text{Rep}) / 39.5 \text{ kJ}\cdot\text{g}^{-1}$]. Components of HP were partitioned into activity heat production (**AHP**), thermic effect of feeding (**TEF**), and minimal heat production (**MHP**) calculated using simultaneous measurements of O₂ and CO₂ concentrations in the respiration chamber, of force sensor signals, and of physical characteristics of the gas in the chamber based on the modeling approach by van Milgen et al. ⁽¹⁸⁾ but with the modifications described by Quemeneur et al. ⁽²³⁾. The first day of P1 (considered as adaptation) and of P2 (ambient temperature varied during the transition on d 0 and thus it could not be determined when pigs were still in TN or already in HS conditions) were removed in the HP and feeding behavior calculations. Evaporated water and latent evaporative heat losses were calculated using the RH and temperature measurements in the chambers based on the principle described by Renaudeau et al. ⁽²⁴⁾. The energy used to warm feed and water intakes to the body temperature level was determined by multiplying the amount ingested (kg), the temperature difference of the body and ingesta (°C), and the specific heat of the ingesta (2.000 and 4.184 kJ·kg⁻¹·°C⁻¹, for feed and water, respectively). Since temperature of the feed was not measured, it was assumed to be 22°C for P1 and 28°C for P2. Sensible heat loss was assumed to be the difference between total HP and the sum of latent heat losses and the heat dissipated by warming the ingesta to body temperature level. Partitioning of HP were all expressed relative to the metabolic BW ($\text{BW}^{0.60}$) and the heat

dissipation parameters were afterwards expressed as %HP.

Body temperature data was averaged per hour within each period (removing adaptation, transition, and sick day/s if any) and per period. Results of plasma metabolites and hormones were adjusted in proportion to the hematocrit level measured in a particular sampling to the basal hematocrit (calculated as the mean of pre-prandial hematocrits of the pig). For postprandial plasma kinetics, the values at time 0 was considered to be the mean of the values at min -40, -20, and 0.

Statistical analyses

The number of animals (minimum 5 pigs per treatment) was determined using GLMPOWER⁽²⁵⁾ procedure (based on previous measurements of ME intake of pigs submitted to a HS challenge⁽²⁴⁾ to detect a reduction when the diet is supplemented with LY by half of the decreased ME intake under HS with a statistical power of 0.80 and a 0.05 alpha level. The experimental design allowed measuring each pig at two periods, at TN and under HS. For the statistical analyses, $P < 0.05$ was considered as significant, and $P < 0.10$ was considered as a trend.

During the experiment, one pig died (N—8FW) and two pigs got sick (1 N—8FW and 1 Y—2FW) with reasons unrelated to the treatments, and two pigs (1 N—Unli and 1 Y—Unli) also had erroneous data due to disturbances in the respiratory chamber and were thus removed from calculations and data analysis. The animal (n=31) was considered as the experimental unit. For plasma parameters, pigs with signs of fever were not included in the blood sampling thus only individuals with samples taken during both periods were included in the data analysis (n=28, and n=27 for postprandial kinetics) and for the body temperature measurement, only pigs whose probes worked and were secured properly in place throughout the experiment were included (n=29).

Growth performance, feeding behavior, fecal digestibility coefficients, components of N and energy balance, thermoregulation responses, and body temperature were summarized per period and were analyzed using the REPEATED statement of the PROC MIXED procedure⁽²⁵⁾ with diet (D), feeding window (FW), period (P), and their interactions as fixed effects, and replicate and block within replicate as random effects. For contrast analysis, the SLICE or the LSMESTIMATE statement of SAS was used. LSMESTIMATE statement was used to evaluate the diet effect in general [(N—Unli, N—2FW, N—8FW) vs. (Y—Unli, Y—

Table 2. Effect of live yeast supplementation and feeding window on feed and water intake, growth, and feeding behavior of male finishing pigs exposed to high ambient temperature.^{1,2,3}

Items	Unli		2FW		8FW		RSD ⁴	Statistics
	N	Y	N	Y	N	Y		
Animals, n	5	5	6	5	4	6		
Growth parameters								
Mean BW (kg)								
Period 1	81.4	80.6	81.7	82.6	80.9	81.1	1.2	P**
Period 2	85.2	85.0	84.2	87.0	85.4	85.2		
DMI (g DM·d ⁻¹)								
Period 1 [#]	2,567	2,566	2,229	2,365	2,345	2,394	246	D ^T , P**
Period 2* [†]	1,814	2,060	1,584	2,135	1,947	1,765		
Water intake (g·d ⁻¹)								P**, D×FW ^T , D×FW×P**
Period 1	7,045 ^{ab}	6,013 ^{ab}	5,200 ^b	5,242 ^b	5,126 ^b	5,149 ^b	3498	
Period 2* [‡]	8,637 ^{ab}	7,723 ^{ab}	7,075 ^{ab}	14,161 ^a	13,251 ^{ab}	6,015 ^{ab}		
ADG (g·d ⁻¹)								
Period 1	1221	1146	1082	1101	1150	1152	364	P**
Period 2 [†]	484	768	253	776	858	551		
Meal parameters								
Meal (n·d ⁻¹)								
Period 1 ^{# §}	5.6	7.1	2.0	2.4	5.1	4.2	0.8	FW**, P*, FW×P*
Period 2 ^{#§}	4.5	5.6	2.1	2.6	4.8	4.0		
Meal size (g·meal ⁻¹)								FW**, P**, FW×P ^T
Period 1 ^{#§}	694	409	1198	1124	543	659	152	
Period 2 ^{#§}	622	471	839	1008	495	560		
Total feeding duration (min·d ⁻¹)								FW*, P**, D×FW×P ^T
Period 1 [§]	61.8	59.2	52.1	53.4	72.3	63.8	5.0	
Period 2* ^{‡§}	42.2	42.6	37.3	42.5	59.4	42.0		
Inter-meal intervals within a day (min·d ⁻¹)								FW**, P**, FW×P*
Period 1	244	160	314	277	151	181	52	
Period 2* [×]	341	220	310	266	184	233		
Rate of feed intake (g·min ⁻¹)								D ^T , FW**, P**
Period 1 [§]	45.7	51.0	51.1	51.2	35.7	41.2	3.2	
Period 2* [§]	48.6	54.7	54.1	57.8	38.8	48.1		
Meal size at day of blood sampling (g) ⁵								
d -2 (P1) ^{#§}	693	396	1208	1190	544	554	161	d**
d 1 (P2) ^{†#§}	616	329	948	1283	558	533		

¹A total of 31 pigs were allocated to 6 experimental groups in 9 replicates with 2 blocks per replicate. All pigs were housed at 22°C from d -5 to -1 (Period 1 and then at 28°C from d 0 to 4 (Period 2). FW: feeding window; N: non-supplemented diet; Y: live yeast supplemented diet.

²Data was analyzed using PROC MIXED model with feeding window (FW), diet (D), period (P), and their interactions as fixed effects, with pig as a repeated unit per period. ^{a,b}LSmeans with different superscripts letters differ according to the experimental group and period according to D×FW×P interaction (P < 0.05). **P < 0.01, *P < 0.05, ^TP < 0.10.

³Contrast statements within period (P<0.05): * [N—Unli, N—2FW, N—8FW] vs. [Y—Unli, Y—2FW, Y—8FW]; [×] (N—Unli vs. Y—Unli); [†] (N—2FW vs. Y—2FW); and [‡] (N—8FW vs. Y—8FW) for diet effect. [#] [N—Unli, Y—Unli] vs. [N—2FW, Y—2FW]; ^{||} [N—Unli, Y—Unli] vs. [N—8FW, Y—8FW]; [§] [N—2FW, Y—2FW] vs. [N—8FW, Y—8FW] for FW effect.

⁴RSD: residual standard deviation.

⁵A total of 28 pigs out of the 33 pigs in the experiment. Data was analyzed using PROC MIXED model with feeding window (FW), diet (D), day of sampling (d), and their interactions as fixed effects, with pig as a repeated unit per period.

2FW, Y—8FW)] and within each FW [(N—Unli vs. Y—Unli), (N—2FW vs. Y—2FW), and (N—8FW vs. Y—8FW)], and to compare the FW: [(N—Unli, Y—Unli) vs. (N—2FW, Y—2FW)], [(N—Unli, Y—Unli) vs. (N—8FW, Y—8FW)], and [(N—2FW, Y—2FW) vs. (N—8FW, Y—8FW)].

Body temperature and postprandial plasma parameters were analyzed using the REPEATED statement of the PROC MIXED procedure⁽²⁵⁾ with D, FW, P, hour (h) or time of sampling (t) within period (depending on the variable), and their interactions as fixed effects. Meanwhile, hourly body temperature and evaporated water within period were also compared per experimental group using the REPEATED statement with P, h, and their interaction as fixed effects. The resulting Lsmeans were then compared to the maximum body temperature (Btmax) or evaporated water per group and per period. At P1, the Btmax was recorded at h 18 for Unli, at h 15 for 2FW, and at h 19 for 8FW groups. At P2, this was at h 2, 21, 19, 15, 21, and 20 for N—Unli, Y—Unli, N—2FW, Y—2FW, N—8FW, and Y—8FW, respectively. There was only one sampling point for plasmatic concentration of T3 and T4 therefore only D, FW, P, and their interactions were considered as fixed effects. The size of the meal at min 0 was not included in the statistical analysis of the plasma measures but was separately analysed to assess the effect of D, FW, P, and their interactions.

Postprandial plasma kinetics of insulin, glucose, lactate, α -amino nitrogen followed a bell-shaped curve and a modified Erlang model developed by van Milgen et al.⁽²⁶⁾ was adapted (Supplementary fig. 1) in order to describe their kinetics. This takes into account four parameters: initial or pre-prandial concentration in the plasma (Cinitial), the postprandial concentration after 180 min (Cfinal), the shape factor of the curve (λ), and the area under the curve above Cinitial and Cfinal (AUC). Maximum concentration (Cmax) and time at Cmax (Tmax in min) were calculated from the estimated parameters as described in the aforementioned paper. Data per pig per period was fitted into the model using PROC NLIN and the resulting estimates were subjected to REPEATED measure of the PROC MIXED procedure (25) with D, FW, P (based on the day of sampling), and their interactions as fixed effects.

Results

Growth performance and feeding behavior

Table 2 shows the summary of the growth performance and feeding behavior traits of the finishing pigs according to their experimental group. The effect of period on DMI and live

Table 3. Effect of feed access and live yeast supplementation on energy and nitrogen metabolism of male finishing pigs exposed to high ambient temperature.^{1,2,3}

Items	Unli		2FW		8FW		RSD ⁴	Statistics
	N	Y	N	Y	N	Y		
Fecal DM digestibility (%)								
Period 1	85.3	84.5	85.0	85.7	85.0	85.2	0.64	P**
Period 2	85.5	85.1	86.2	85.8	85.8	85.7		
Energy balance (kJ·kg BW ^{-0.60} ·d ⁻¹)								
ME intake								
Period 1 [#]	2765	2727	2398	2568	2524	2588	243	D ^T , P**
Period 2 ^{*†}	1890	2136	1658	2228	2027	1870		
Heat production (kJ kg BW ^{-0.60} ·d ⁻¹)								
Total HP								
Period 1	1484	1503	1401	1461	1426	1446	68	P**
Period 2 [†]	1257	1328	1225	1357	1295	1255		
MHP+TEF								
Period 1 [#]	1234	1230	1128	1179	1117	1141	56	FW ^T , P**
Period 2 [†]	964	1030	914	1018	932	930		
AHP								
Period 1	247	268	278	285	309	310	34	FW*, P**
Period 2	289	294	317	343	363	331		
RE								
Period 1 [#]	1265	1239	988	1122	1088	1126	182	D*, FW ^T , P**, D×FW*, D×FW×P ^T
Period 2 ^{*†}	617	821	423	884	722	599		
RE/ME intake (%)								
Period 1	38.0	38.5	33.3	36.8	36.0	35.5	6.0	D**, P**, D×FW* D×P ^T , D×FW×P ^T
Period 2 ^{*†}	27.1	32.6	15.8	33.2	29.6	25.7		
Nutrient deposition (g·d ⁻¹)								
Protein								
Period 1	160	158	135	146	150	150	24	D*, FW*, P**
Period 2*	83	109	70	91	82	90		
Fat								
Period 1 [#]	361	347	271	308	293	313	61	D*, P**, D×FW*, D×FW×P ^T
Period 2 ^{*†}	180	234	120	268	215	165		

¹A total of 36 pigs were allocated to 6 experimental groups in 9 replicates with 2 blocks per replicate. All pigs were housed at 22°C from d -5 to -1 (Period 1 and then at 28°C from d 0 to 4 (Period 2). FW: feeding window; N: non-supplemented diet; Y: live yeast supplemented diet.

²Data was analyzed using PROC MIXED model with feeding window (FW), diet (D), period (P), and their interactions as fixed effects, with pig as a repeated unit per period. **P < 0.01, * P < 0.05, ^TP < 0.10.

³Contrast statements within period (P<0.05): * [N—Unli, N—2FW, N—8FW] vs. [Y—Unli, Y—2FW, Y—8FW]; * (N—Unli vs. Y—Unli); † (N—2FW vs. Y—2FW); and ‡ (N—8FW vs. Y—8FW) for diet, and # [N—Unli, Y—Unli] vs. [N—2FW, Y—2FW]; || [N—Unli, Y—Unli] vs. [N—8FW, Y—8FW]; § [N—2FW, Y—2FW] vs. [N—8FW, Y—8FW] for FW.

⁴RSD: residual standard deviation.

BW was significant with lower DM intake ($-527 \text{ g DM}\cdot\text{d}^{-1}$; $P < 0.01$) and higher mean BW ($+4.0 \text{ kg BW}$; $P < 0.01$) in P2 than in P1. Supplementation of LY increased DMI during P2 ($+205 \text{ g DM}\cdot\text{d}^{-1}$; $P = 0.03$) especially for the 2FW group ($+551 \text{ g DM}\cdot\text{d}^{-1}$; contrast $P < 0.01$). For water intake, interaction between diet, FW, and period was significant. Water intake typically increased during P2 ($+3,848 \text{ g DM}\cdot\text{d}^{-1}$; $P < 0.01$) albeit being statistically significant only for the Y—2FW group ($+8,919 \text{ g}\cdot\text{d}^{-1}$; $P = 0.01$). For feeding behavior traits, heat exposure decreased feeding duration ($-16.4 \text{ min}\cdot\text{d}^{-1}$; $P < 0.01$) and increased RFI ($+4.3 \text{ g}\cdot\text{min}^{-1}$; $P < 0.01$). Interaction between FW and period was detected for the number of meals ($P = 0.02$) and meal size (trend at $P = 0.05$): exposure to HS reduced the number of meals of pigs in Unli groups ($-1.3 \text{ meals}\cdot\text{d}^{-1}$ on average; $P < 0.01$) but not in 2FW and 8FW groups, and also affected meal size for pigs in 2FW ($-237 \text{ g}\cdot\text{meal}^{-1}$; $P < 0.01$) but not in Unli and 8FW groups. For dietary treatment contrast comparison, supplemented pigs in the Unli group tended to have smaller meal size during P1 ($P = 0.07$). During P2, supplemented pigs had generally faster RFI ($P = 0.04$) regardless of FW. LY supplementation also decreased inter-meal intervals within the Unli group ($P = 0.02$) and decreased feeding duration within the 8FW group ($P = 0.02$) in P2.

N retention and energy utilization

As shown in Table 3, period significantly affected components of N retention and energy utilization ($P < 0.01$) with higher fecal digestibility coefficient ($+0.52$ points) and AHP ($+40 \text{ kJ kg BW}^{-0.60}\cdot\text{d}^{-1}$), and lower ME intake, HP, and RE (-627 , -166 , $-460 \text{ kJ kg BW}^{-0.60}\cdot\text{d}^{-1}$, respectively) in P2 than in P1. Regardless of period, FW significantly affected AHP ($P = 0.04$) with 8FW pigs having higher AHP than Unli pigs ($+54 \text{ kJ kg BW}^{-0.60}\cdot\text{d}^{-1}$ on average) while 2FW pigs had intermediate values. During P1, N—Unli and Y—Unli had higher ME intake ($P = 0.04$ and trend at $P = 0.06$, respectively) and RE ($P < 0.04$) than N—2FW. Unli groups also had higher MHP+TEF than both 2FW and 8FW groups (contrast at $P < 0.04$) during this period. During heat exposure in P2, supplemented pigs had generally higher ME intake ($P = 0.03$) with the difference most pronounced between the 2FW groups ($+570 \text{ kJ kg BW}^{-0.60}\cdot\text{d}^{-1}$; $P < 0.01$) but only numerical in the Unli pigs ($+245 \text{ kJ/kg BW}^{-0.60}\cdot\text{d}^{-1}$; $P = 0.16$). Supplemented pigs also had higher RE ($P = 0.01$) within the Unli ($+204 \text{ kJ kg BW}^{-0.60}\cdot\text{d}^{-1}$; trend at $P = 0.09$) and the 2FW group ($+461 \text{ kJ kg BW}^{-0.60}\cdot\text{d}^{-1}$; $P < 0.01$) but not in the 8FW group. LY supplementation also increased fat deposition ($P = 0.02$) but, like for ME intake, it was only significant in the 2FW groups ($+149 \text{ g}\cdot\text{d}^{-1}$; $P < 0.01$) and was only numerical in the Unli groups ($+54 \text{ g}\cdot\text{d}^{-1}$; $P =$

Table 4. Effect of feed access and live yeast supplementation on thermoregulation responses of male finishing pigs exposed to high ambient temperature.^{1,2,3}

Items	Unli		2FW		8FW		RSD ⁴	Statistics
	N	Y	N	Y	N	Y		
Evaporated latent water (g·d ⁻¹)								
Period 1	2659 ^{cde}	2676 ^{cde}	2249 ^d	2592 ^{bcd}	2628 ^{bcd}	2417 ^{cde}	592	P**, D×FW*, D×FW×P*
Period 2 ^{†‡}	4158 ^{ab}	4061 ^{ab}	3729 ^{abc}	5215 ^a	4864 ^a	3609 ^{abcd}		
Latent heat loss (% HP)								
Period 1	31.0 ^c	30.8 ^c	28.7 ^c	31.1 ^c	32.5 ^{bc}	29.6 ^c	5.1	P**, D×FW*, D×FW×P**
Period 2 ^{†‡}	55.6 ^a	51.8 ^a	51.9 ^a	65.1 ^a	63.3 ^a	49.0 ^{ab}		
Sensible heat loss (% HP)								
Period 1	66.3 ^{ab}	66.8 ^{ab}	69.0 ^a	66.7 ^{ab}	65.2 ^{abc}	68.2 ^a	5.7	P**, D×FW*, D×FW×P**
Period 2 ^{†‡}	42.0 ^{de}	46.0 ^{cde}	45.9 ^{cde}	30.9 ^e	32.7 ^{de}	49.2 ^{bcd}		
Heat loss due to water intake (% HP)								
Period 1	2.32	1.95	1.89	1.77	1.78	1.80	1.01	P*, D×FW ^T , D×FW×P*
Period 2 ^{†‡}	2.25	1.94	1.94	3.75	3.71	1.50		
Heat loss due to feed intake (% HP)								
Period 1 [#]	0.45	0.47	0.43	0.44	0.44	0.44	0.02	D*, FW ^T , P**
Period 2 ^{†‡}	0.24	0.27	0.22	0.27	0.25	0.24		
Body temperature, °C								
Period 1	38.58 ^{abcd}	38.70 ^{bcd}	38.42 ^{cd}	38.57 ^{abcd}	38.47 ^{cd}	38.39 ^d	0.18	P**, D×FW×P*
Period 2	38.89 ^{abcd}	39.11 ^{ab}	39.18 ^a	38.90 ^{abcd}	38.84 ^{abcd}	38.89 ^{abc}		

¹A total of 36 pigs were allocated to 6 experimental groups in 9 replicates with 2 blocks per replicate. All pigs were housed at 22°C from d -5 to -1 (Period 1 and then at 28°C from d 0 to 4 (Period 2). FW: feeding window; N: non-supplemented diet; Y: live yeast supplemented diet.

²Data was analyzed using PROC MIXED model with feeding window (FW), diet (D), period (P), and their interactions as fixed effects, with pig as a repeated unit per period. ***P* < 0.01, **P* < 0.05, ^T*P* < 0.10. ^{a,b,c,d,e,f}LSmeans with different superscripts letters differ according to the experimental group and period according to D×FW×P interaction (*P* < 0.05).

³Contrast statements within period (*P* < 0.05): **[N—Unli, N—2FW, N—8FW]* vs. *[Y—Unli, Y—2FW, Y—8FW]*; **(N—Unli vs. Y—Unli)*; **(N—2FW vs. Y—2FW)*; and **(N—8FW vs. Y—8FW)* for diet, and [#]*[N—Unli, Y—Unli]* vs. *[N—2FW, Y—2FW]*; ^{||}*[N—Unli, Y—Unli]* vs. *[N—8FW, Y—8FW]*; [§]*[N—2FW, Y—2FW]* vs. *[N—8FW, Y—8FW]* for FW.

⁴RSD: residual standard deviation.

0.16). Nevertheless, supplemented pigs had higher protein deposition regardless of FW (+18 g·d⁻¹; $P = 0.03$) and most notably for pigs in the Unli group (+28 g·d⁻¹; trend at $P = 0.06$).

Thermoregulation responses

Table 4 shows the thermoregulation responses related to heat dissipation (expressed as %HP) and the average body temperature of the pigs per period. Latent evaporative heat loss represented 30% of HP and body temperature averaged 38.5°C in TN conditions. When exposed to high ambient temperature in P2, these increased ($P < 0.01$) to 56% of HP and to 39.0°C, respectively. Under HS, proportion of heat dissipated via the sensible route and used to heat the ingested feed to body temperature level decreased ($P < 0.01$) from 67 to 41% and from 0.44 to 0.25% of HP, respectively, while proportion used to heat ingested water to body temperature level increased from 1.9 to 2.5% HP ($P < 0.01$). Latent evaporative water losses increased on average from 2,537 to 4,272 g·d⁻¹ ($P < 0.01$). Based on the contrast analyses in P2, LY-supplemented pigs had higher proportion of heat lost via evaporative route in the 2FW group ($P = 0.04$), but it was the opposite for those in the 8FW group ($P = 0.02$).

Figure 2 and 3 shows the pattern of hourly body temperature and evaporative water loss, respectively, for the experimental groups. There was a distinct diurnal variation of body temperature in Unli pigs (Fig. 2a) during P1 with peaks at around 0900h and 1400h, and this variation was more pronounced in the 2FW (Fig. 2b) and 8FW (Fig. 2c) groups. In the 2FW groups, during P1, body temperature peaked to similar level (BT_{\max}) at 0900h and at 1500h ($P > 0.10$), whereas in 8FW groups, BT_{\max} was measured in the evening with a significantly higher body temperature peak than the body temperature in the morning ($P < 0.05$). Meanwhile, latent evaporative water loss pattern was not correlated to the body temperature pattern and remained relatively constant throughout the day during P1 at around 131 g·h⁻¹.

Compared to P1, the diurnal variation of body temperature in Unli pigs was less apparent in P2 wherein body temperature was at BT_{\max} 80% of the day ($P > 0.10$). During this period the body temperature in the 2FW groups remained at BT_{\max} 5 h after the first meal in non-supplemented pigs and 2 h for the supplemented pigs, and both groups were at BT_{\max} for 7 to 8 h after the second meal. Body temperature of N—2FW pigs was higher in P2 than in P1 throughout the day, while that of Y—2FW pigs were similar across period except during non-feeding times when it was higher (at hours 0 to 9, 14, and 23 ($P < 0.05$)). For 8FW groups during P2, body temperature was at BT_{\max} for 11 h (1400h to 0000h) in non-supplemented pigs

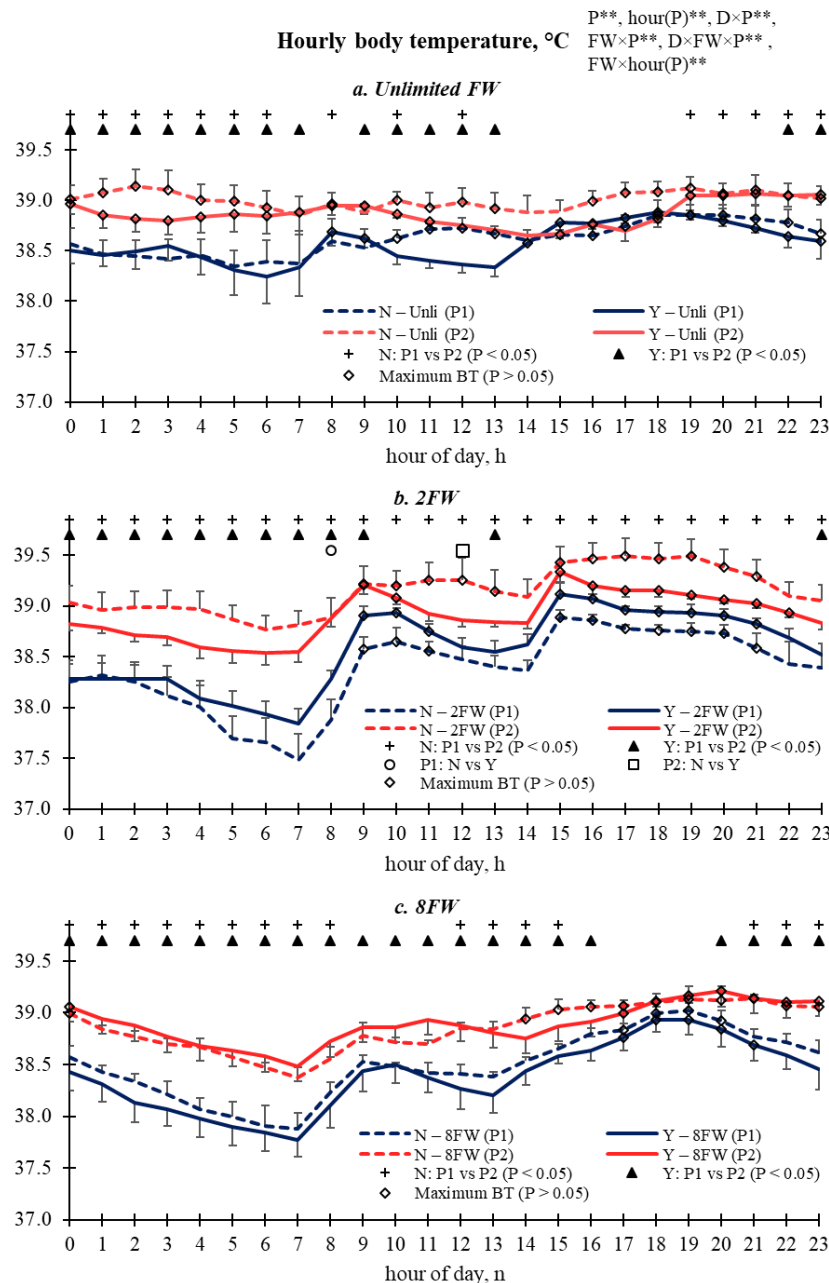


Figure 2. Effect of diet [0 (N) vs. 100 (Y) g/ton LY supplementation] within each feeding window [(a)Unli vs. (b) 2FW vs. (c) 8FW] on the hourly body temperature (°C) of heat-stressed entire male finishing pigs (means \pm SE). The broken line (---) represents pigs with N diet and the solid line (—) pigs fed with Y diet in TN (blue line) and HS (red line) conditions. Pigs were housed at thermoneutrality from d -5 to -1 (P1; 22°C) and then in heat-stressed conditions from d 0 to 4 (P2; 28°C). **P < 0.01.

while it was for 8 h (1700h to 0000h) for the supplemented pigs. In contrast to P1, pattern of evaporative water loss closely followed time of feeding and body temperature pattern during P2, except for the Y—8FW group for which it remained relatively constant compared to the other groups. Nevertheless, evaporative water loss increased for all groups in P2 ($P < 0.05$). In the 2FW groups, evaporative water losses was higher in the supplemented pigs starting from 0900h to 0000h ($P < 0.05$) while it was the opposite in the 8FW groups (higher in the non-supplemented pigs from 1100h to 2100h ($P < 0.05$)). The maximum evaporated water was recorded for N—8FW pigs for 9 h starting at 1500h (1 h after they attained BT_{max}), while it was recorded only for 5 h for the Y—8FW starting at 2000h (3 h after they attained BT_{max}).

Plasma metabolite and hormone concentrations

The average meal size at time 0 of blood sampling on d -2 (P1) and d 1 (P2), shown in Table 2, followed a similar trend as the respective meal size reported per period (described in a previous subsection). The meal size was significantly different only on d 1 (P2) for 2FW pigs (+335 g for Y pigs; $P = 0.04$), while the difference was only numerical for Unli pigs for P1 and P2 (-264 g on average for Y pigs; $P \leq 0.15$). Results of the postprandial insulin, glucose, lactate, and α -amino nitrogen are presented in Fig. 4 and the description of their kinetics is found in Supplementary table 1. Exposure to HS increased C_{final} (trend at $P = 0.06$) and C_{max} ($P < 0.01$) for plasma glucose, and decreased AUC (trend at $P = 0.06$) and C_{max} ($P = 0.04$) for plasma α -amino nitrogen, but did not have a significant effect on plasma insulin parameters. The effect of FW on plasma insulin kinetics was significant with 2FW pigs having higher AUC on average than Unli and 8FW pigs during P1 ($P \leq 0.04$), and than 8FW during P2 ($P = 0.04$), and higher C_{max} than 8FW regardless of the period ($P \leq 0.03$). Plasma insulin AUC and C_{max} were generally lower in LY-supplemented pigs within the Unli groups (trend at $P \leq 0.08$) during P1 and within the 2FW groups during P1 and P2 ($P \leq 0.04$). Supplemented pigs also showed lower C_{max} for plasma lactate ($P = 0.02$) and glucose ($P = 0.06$) than non-supplemented pigs in P1, especially for those in the 2FW group ($P = 0.02$). Between the 2FW groups, supplemented pigs also had lower AUC for lactate ($P = 0.03$) and lower C_{max} for α -amino nitrogen ($P = 0.03$) in P1.

Figure 5 shows the postprandial plasma concentrations of urea, creatinine, and NEFA. Regardless of the experimental group, there was an increase ($P < 0.01$) in plasma levels of creatinine and urea when exposed to hot conditions. The time of sampling was significant ($P < 0.01$) for the three metabolites with plasma level of urea increasing while those of creatinine

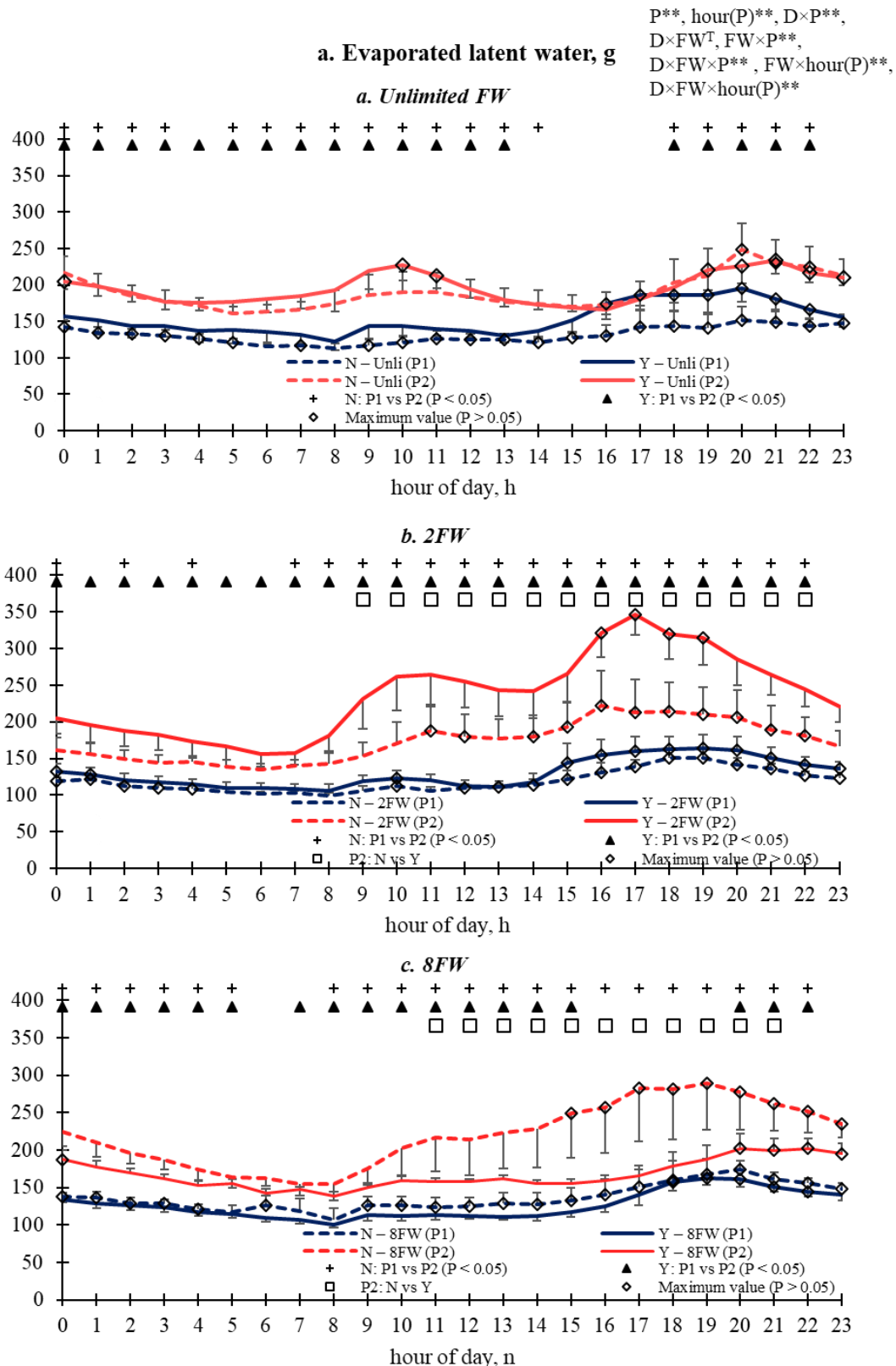


Figure 3. Effect of diet [0 (N) vs. 100 (Y) g/ton LY supplementation] within each feeding window [(a)Unli vs. (b) 2FW vs. (c) 8FW] on the hourly evaporated latent water (g) of heat-stressed entire male finishing pigs (means \pm SE). The broken line (---) represents pigs with N diet and the solid line (—) pigs fed with Y diet in TN (blue line) and HS (red line) conditions. Pigs were housed at thermoneutrality from d -5 to -1 (P1; 22°C) and then in heat-stressed conditions from d 0 to 4 (P2; 28°C). ^TP < 0.10, **P < 0.01.

and NEFA decreasing after a meal. There was an interaction between diet and FW wherein plasma levels of creatinine and NEFA were lower for supplemented pigs in 2FW group ($P < 0.05$) but not in other FW groups. The results of the analysis of plasma hormone T3 and pro-hormone T4 are presented in Fig. 6. Exposure to high ambient temperature resulted in a decrease in plasma T3 and T4 ($P < 0.01$). Regardless of the period, plasma levels of T3 tended to be affected by FW (higher in 2FW than Unli pigs; $P = 0.08$) while the diet significantly affected the level of plasma T4 (higher in supplemented diet; $P = 0.02$).

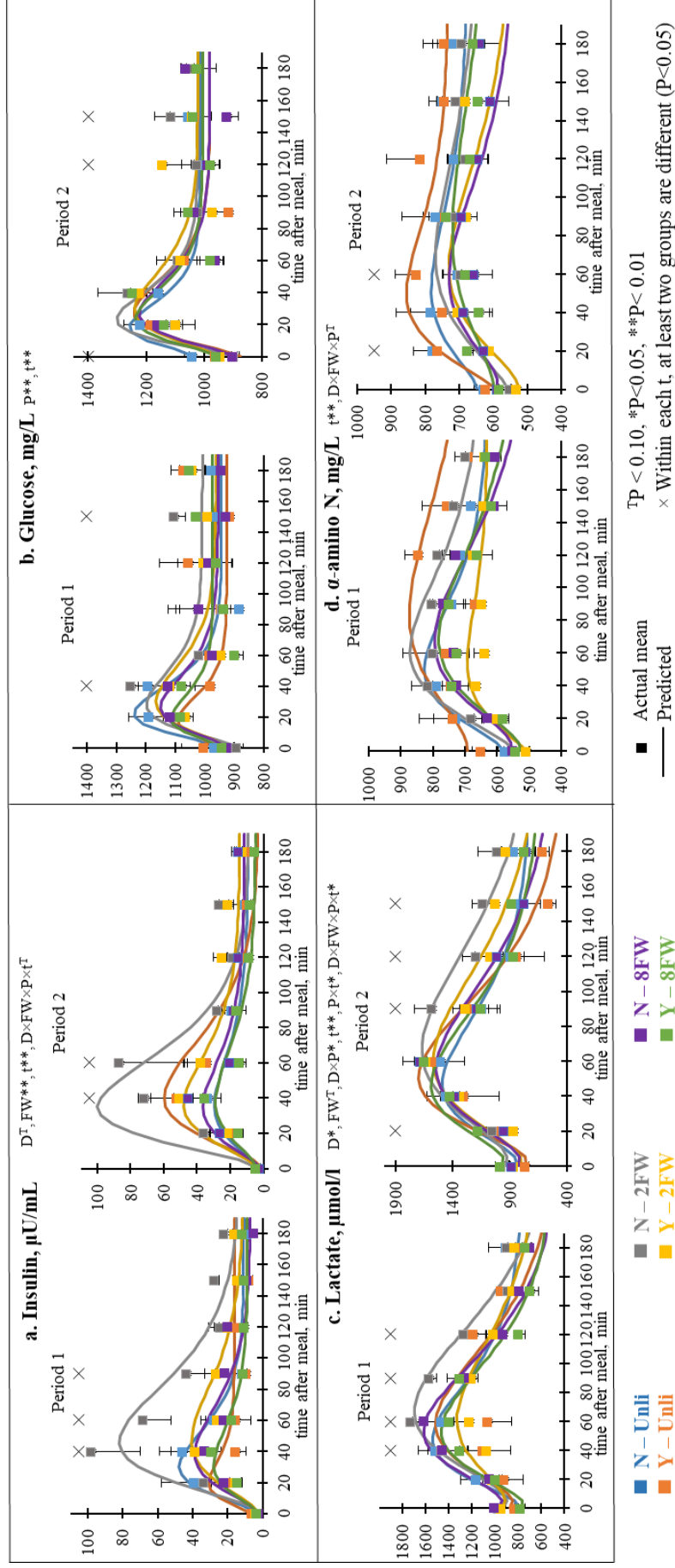


Figure 4. Effect of diet [0 (N) vs. 100 (Y) g/ton LY supplementation] and feeding window (Unli vs. 2FW vs. 8FW) on the postprandial plasma concentrations of (a) insulin, (b) glucose, (c) lactate, and (d) α -amino nitrogen of heat-stressed entire male finishing pigs (means \pm SE). Pigs were housed at thermoneutrality from d -5 to -1 (P1; 22°C) and then in heat-stressed conditions from d 0 to 4 (P2; 28°C). Blood sampling was done on d -2 for Period 1 and on d 1 for Period 2. The line (—) corresponds to the predicted values at minute (time; t) obtained with the non-linear model (Supplementary figure 1).

Discussion

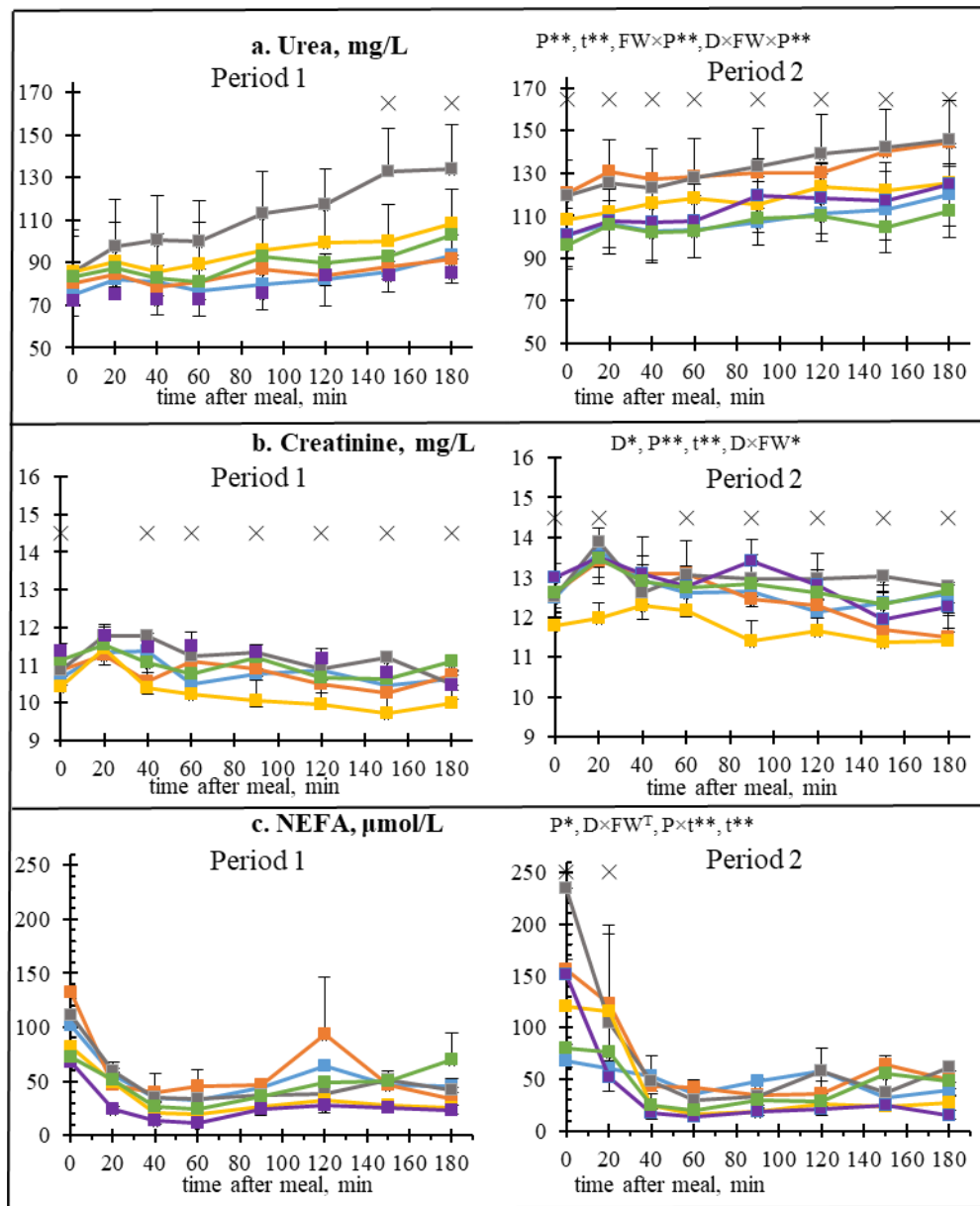
The present study aimed to evaluate whether the improved HS response of pigs supplemented with *S. cerevisiae var boulardii* results from a modified feeding behavior and/or energy metabolism and if these responses can be replicated by imposing an increased meal frequency.

Effect of high ambient temperature in non-supplemented pigs fed ad-libitum

As homeothermic animals, pigs strive to maintain a constant body temperature regardless of the environmental conditions ⁽²⁷⁾. In high ambient temperature, heat loss via sensible routes becomes limited due to the lower temperature gradient between the body surface and the environment, thus pigs relies more on the latent evaporative route for losing heat. In the present study, latent heat losses accounted for 56% of HP in N—Unli pigs kept in HS conditions in agreement with the 60% already reported by Renaudeau et al. ⁽²⁴⁾ for pigs kept at 33°C and 75% RH. Body water balance is vital for thermoregulation ⁽²⁸⁾ thus evaporative losses must be replenished explaining the increased water consumption during P2. The water intake which increased by 274% from P1 to P2 (if expressed as L water intake/kg DMI) also served other heat loss purposes in hot conditions as indicated by the higher HP proportion used to warm ingested water to body temperature level.

Whereas water intake increased, DMI decreased in the N—Unli pigs by -753 g·d⁻¹ (or -30%) to reduce metabolic HP related to nutrient utilization (TEF) ⁽²⁹⁾. This value is higher than the -400 g DM·d⁻¹ reported in the literature ⁽³⁰⁾ probably due to the pig's limited ability to adapt to the short duration of the heat challenge in the present study. This drop in feed intake was related to an altered feeding behavior such as less time spent in the feeder, faster RFI, and decreased number of meals ^(31,32) and resulted to lower HP, RE, and thus lower nutrient deposition. The reduced HP in P2 also accounts for the decreased thyroid hormones of HS pigs in the present and in previous studies ^(33,34). Heat exposure did not affect plasma insulin levels in this study in contrast to previous reports ^(8,35), but it slightly increased glucose levels suggesting reduced insulin sensitivity in agreement with previous studies ⁽³⁶⁾. It can be hypothesized that dietary glucose is a less preferred energy source in hot conditions ⁽³⁷⁾ which could partly explain the need for higher muscle breakdown during HS indicated by higher postprandial creatinine and urea levels in P2 ⁽³⁸⁾.

Effects of live yeast supplementation in pigs fed ad libitum and subjected to heat stress



$^{\text{T}}\text{P} < 0.10$, $^{\text{*}}\text{P} < 0.05$, $^{\text{**}}\text{P} < 0.01$ × Within each t, at least two groups are different ($\text{P} < 0.05$)

Figure 5. Effect of diet [0 (N) vs. 100 (Y) g/ton LY supplementation] and feeding window (Unli vs. 2FW vs. 8FW) on the postprandial plasma concentrations of (a) urea, (b) creatinine, and (c) NEFA of heat-stressed entire male finishing pigs (means \pm SE). Pigs were housed at thermoneutrality from d -5 to -1 (P1; 22°C) and then in heat-stressed conditions from d 0 to 4 (P2; 28°C). Blood sampling was done on d -2 for Period 1 and on d 1 for Period 2.

Between the two Unli groups, LY supplementation attenuated HS effects on DMI reduction (-20% in Y—Unli vs. -30% in N—Unli) that tended to improve energy efficiency (+5.5 points) and PD (+26 g·d⁻¹) in agreement with Labussière et al.⁽⁹⁾. Although effect of LY on meal frequency was not significant, shorter inter-meal intervals were observed in supplemented pigs in the present study similar to findings in dairy cows⁽³⁹⁾. Differences in body temperature and postprandial responses between the Unli groups were only numerical and not clearly differentiated, possibly due to indeterminate feeding times and the fact that body temperature and insulin levels are known to also depend on the characteristics of the previous meal (meal size and when the last meal was taken). Nevertheless, this study confirms improved feed intake and energy efficiency in LY supplemented pigs under HS but in contrast to the results published by Labussière et al.⁽⁹⁾, these improved performances were not clearly related to changes in feeding behavior.

The following subsections focuses on the FW-restricted groups with the aim to look at the effects related to different meal frequencies and to LY supplementation. As showed in the present study, HP and body temperature are not constant during the day hence, maintaining a thermal balance between heat production and loss must also be a dynamic process. Thus, this theory is further explored with a particular focus on the dynamic changes in the thermoregulation and physiological responses during the day of pigs subjected to high ambient temperature.

Effect of different meal frequencies in non-supplemented heat stressed pigs

In the present study, the 2FW strategy worked to drive meal frequency down to ~2 meals a day. However, the 8FW strategy did not achieve the expected increase in the number of meals (N—8FW and N—Unli had similar number of meals per day). As undigested nutrients in the ileum can trigger ileal brake which signals satiety, and inhibits gastric emptying and feed intake⁽⁴⁰⁾, the imposed 90-min meal interval may not have been enough time for satiety signals to end prompting the N—8FW to skip a meal or two. Nonetheless, N—8FW still had higher meal frequency than the N—2FW pigs in the present study.

In TN conditions, body temperature pattern closely followed feeding pattern, as highly evident in the 2FW and 8FW pigs. The immediate postprandial increase in body temperature was probably due to both the instantaneous AHP (feeding activity) and the TEF-related heat (energy needed for digestion, absorption, and from metabolic processes). Pre- and postprandial body temperature also increased with each additional meal (more gradual in N—8FW than in

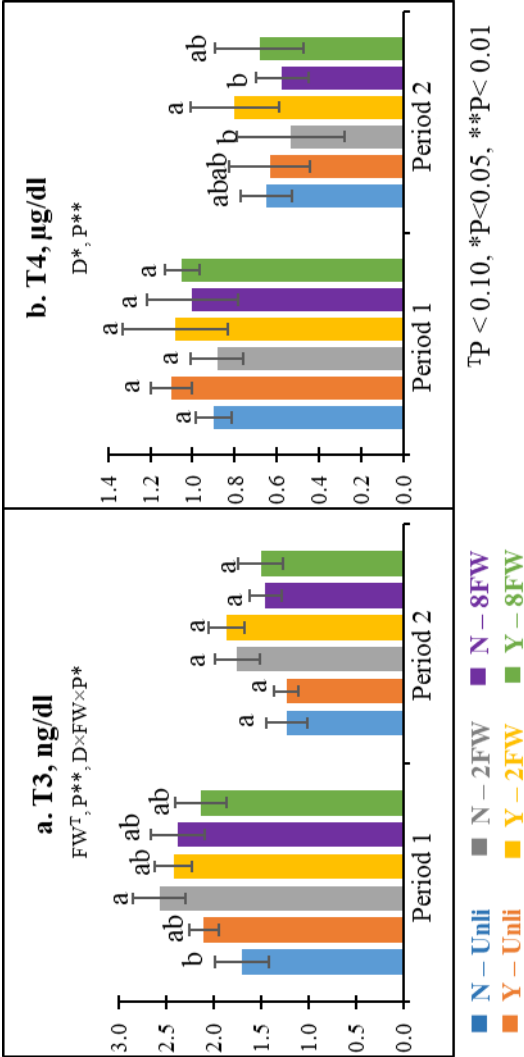


Figure 6. Effect of diet [0 (N) vs. 100 (Y) g/ton LY supplementation] and feeding window (Unli vs. 2FW vs. 8FW) on the plasma concentrations of (a) T3 and (b) T4 of heat-stressed entire male finishing pigs (means \pm SE). Pigs were housed at thermoneutrality from d -5 to -1 (P1; 22°C) and then in heat-stressed conditions from d 0 to 4 (P2; 28°C). Blood sampling was done on d -2 for Period 1 and on d 1 for Period 2. ^{a,b}LSmeans within a row with different superscripts letters differ ($P < 0.05$) according to the experimental group.

N—2FW) possibly from the cumulative heat increment following each meal. However, evaporative water loss remained relatively constant throughout the day and was clearly dissociated with body temperature, suggesting minimal need for latent heat losses as the sensible route was probably sufficient in TN conditions to maintain constant core body temperature immediately after a meal.

The observation that body temperature 1) remained at Btmax over a longer period after a meal in N—2FW pigs and 2) reached Btmax earlier in P2 than in P1 (1400h vs. 1700h) in N—8FW pigs, suggest that the ability to maintain thermal balance after a meal seemed to decrease upon heat exposure. Based on the higher pre-prandial body temperature of pigs in P2 than in P1, heat load was already high even before feeding. This result confirms previous data suggesting a reduced pig's ability to lose TEF-related heat in hot conditions ⁽⁴¹⁾. In fact, as evaporated water loss pattern closely followed the feeding time and the body temperature peaks during HS, sensible heat losses seemed to be no longer sufficient and consequently pigs relied more on the latent route in P2. It is unclear why Btmax was maintained longer in P2 even during non-feeding times. One hypothesis could be related to the reported decrease of gastric emptying rate in HS conditions ⁽⁴²⁾ possibly due to the restricted splanchnic blood flow during HS or because these processes entail energy and thermal load. Indeed, hyperthermia and its possible consequences on gastric emptying might have prolonged satiety signals which may have caused the observed longer inter-meal intervals in HS than in TN conditions in the present study. This can also help explain the exacerbated HS effects in the N—2FW pigs compared to the N—8FW pigs in terms of reduction of DMI and energy efficiency (-30% vs. -17% and -17.5 vs. -6.5 points, respectively). Large meal size and high insulin levels have been reported to delay not just gastric emptying but also carbohydrate absorption ^(43,44). Moreover, as insulin promotes glycolysis, it may have caused the prolonged and elevated lactate levels observed in N—2FW pigs from excess pyruvate production.

The N—8FW pigs in the present study seemed to have had better heat tolerance when compared to the other non-supplemented groups especially regarding ME intake and RE. This result did not seem to be related to the increased meal frequency per se but might be related to the imposed feeding management in this study. First, the imposed prolonged feed access restriction during the night (thus no further TEF-related HP) might have helped HS pigs to lower their body temperature. Indeed, a faster decrease in gastrointestinal temperature was reported when feed was withdrawn in pigs subjected to acute hyperthermia ⁽⁴⁵⁾. In addition, the

higher water intake and evaporative heat losses observed in the N—8FW pigs when compared to the N—2FW pigs would also explain their better heat tolerance. Between these two groups, the imposed increased meal frequency may have stimulated the pigs to drink more water with each meal since pigs are “prandial drinkers”, i.e. their drinking bouts are stimulated by feed ingestion ⁽⁴⁶⁾. As a result, the higher water intake may have contributed to the increased latent heat losses of N—8FW pigs since an animal’s hydration status is closely linked to its evaporative heat loss capacity in order to maintain body water balance ⁽⁴⁷⁾. These mechanisms might have helped the N—8FW pigs to maintain a lower body temperature during most parts of the day (which was not evident when looking only at the average daily body temperature) and thus allowed them to eat more in hot conditions compared to N—Unli and N—2FW pigs.

Despite the relatively higher RE measured in N—8FW compared to the other N groups during P2, their PD did not differ from N—Unli pigs and did not reach the highest PD observed for the Y-Unli group. The balance between protein synthesis and breakdown (protein turnover) regulates whole body PD. Even if protein breakdown is reduced during fasting, protein synthesis rate remains comparatively lower resulting to protein loss ⁽⁴⁸⁾ which is in line with the lower net PD observed in the present study for pigs subjected to extended feed withdrawal periods (2FW and 8FW) compared to the Unli pigs in TN or HS conditions. However, it does not explain the +35 g·d⁻¹ higher fat deposition of the N—8FW compared to N—Unli pigs. Protein deposition entails high thermal load thus capacity of pigs to reach their potential for PD is usually limited in hot conditions ⁽⁴⁹⁾. Since the N—8FW pigs already reached B_{max} early at h 14 during HS conditions, it can be assumed that additional ME intake from here onwards was deposited as fat since the animals needed to maintain homeostasis, and fat deposition has a lower thermal load than that of protein deposition.

This present study therefore demonstrates that the thermal balance is a dynamic process highly influenced by the feeding level and behavior because of the large contribution of TEF and feeding-related AHP to instantaneous increases in HP. Feeding large meals impairs ability of pigs to cope with HS possibly related to combined changes in gastric emptying, energy metabolism, and saturation of heat loss pathways. The results of the present study also demonstrates that pre-prandial body temperature is a contributing factor, together with the ability of the animal to lose heat after a meal, to improve feed intake and protein deposition in hot conditions.

Effect of live yeast supplementation in pigs subjected to different meal frequencies during heat stress

As discussed above the improvement in pig performance under hot conditions could not be associated to an increase in meal frequency in the present study. Comparing the N—8FW pigs to LY-supplemented pigs, the increased DMI during HS in the former group was not to the same extent as the latter (Y—Unli), neither did it result to an increase in PD. Furthermore, based on RE and PD measured in P2, supplemented pigs with low meal frequency (Y—2FW) tolerated heat better than N—8FW pigs. However, it remains unclear why Y—8FW pigs did not perform as well as the Y—Unli. As already mentioned, the feeding management employed in the present study may have been an oversimplified sense of “frequent feeding” in supplemented pigs. Duration of inter-meal intervals were similar among the supplemented groups in P2 (average 240 min), thus under hot conditions, FW did not affect the ability of supplemented pigs to start a new meal after the last one has ended. Nevertheless, Y—8FW still deposited more protein than the N—8FW despite having lower ME intake. The Y—8FW pigs attained Btmax at 1700h whether in TN or HS conditions, which means their reduced PD in P2 was not due to the effect of HS on PD potential, but rather due to lower feed intake. This inclination to prioritize and use higher proportion of ME intake for protein instead of lipid deposition has been observed in restricted-fed pigs in TN conditions ⁽⁵⁰⁾. One can thus hypothesize that Y—8FW pigs were experiencing a lower level of heat stress than N—8FW as supported by their relatively constant evaporative water loss in P2 suggesting that the sensible route was still their main route for maintaining a thermal balance. Hence, this raises the possibility that the positive effects of LY-supplementation might be related to improved thermoregulation responses either by reducing heat load or by dissipating heat more efficiently.

Pigs are reported to change feeding behavior with more meals taken during the cooler parts of the day in studies with cyclic or natural heat stress challenges ^(51,52). Thus, the idea behind imposing a low meal frequency (2FW groups) in an environment with a constantly high ambient temperature was to increase the severity of the heat challenge by preventing pigs to modify their feeding behavior as an adaptation response. As demonstrated in the previous subsection, this resulted to inability of non-supplemented pigs to cope with HS. However, this was not the case with the Y—2FW group that showed the least severe reduction in DMI and energy efficiency (-10% and -3.6 points, respectively) which also translated into higher RE and PD. The principal differences between the 2FW groups seem to be linked to energy metabolism

(linked to insulin response), and latent heat loss capacity. In terms of energy metabolism, supplemented 2FW pigs showed enhanced insulin sensitivity. A yeast extract from *S. cerevisiae* has already been reported to potentiate insulin action ⁽⁵³⁾ and *S. boulardii* has been demonstrated to attenuate diabetic-related complications such as hyperglycemia (due to impaired insulin activity or production) by microbiota modulation and improved immune responses ^(54–56). High insulin levels are associated with elevated lactate thus the improved insulin sensitivity in supplemented pigs may have contributed to their higher energy efficiency especially since lactate requires energy to be reconverted back to glucose before it can be used for nutrient deposition. Increased insulin sensitivity was also found to decrease de novo lipogenesis ⁽⁵⁷⁾ which may also partly explain the higher PD of supplemented pigs especially in hot conditions. Nevertheless, these are just few hypotheses since insulin is a key hormone in many metabolic processes not only in pigs but also in humans and in other livestock species. Thus, an improved insulin sensitivity may have caused many other cascades of reactions that contributed to the higher energy efficiency and thermoregulatory responses of supplemented pigs during HS.

As previously mentioned and as shown in the 2FW groups a low pre-prandial body temperature is important in alleviating effects of HS on feed intake. The lower pre-prandial body temperature and higher evaporative heat loss and water intake of Y—2FW pigs enabled them to decrease their body temperature immediately after a meal and maintain a relatively similar meal size in both periods, in contrast to the N—2FW pigs. The hypothesized impact of high insulin levels and high heat load on gastric emptying might have thus been mitigated by LY during hot conditions. This could further explain the shorter time spent at Btmax and their slightly shorter inter-meal intervals ($-44 \text{ min} \cdot \text{d}^{-1}$) compared to N—2FW pigs. This shorter inter-meal interval in Y—2FW pigs might have indirectly promoted water intake by prompting them to drink more often because of the prandial drinking behavior of pigs⁽⁴⁶⁾. Thus, the improved evaporative heat loss in the supplemented 2FW pigs may have been partly due to their higher water intake because an improved latent heat loss capacity is closely linked to a better hydration status. Another hypothesis on the improved evaporative heat loss capacity of supplemented pigs might be related to the gut-lung axis theory⁽⁵⁸⁾ and the modulatory effects of *S. boulardii* on gut microbiota especially in HS conditions⁽⁹⁾. *S. boulardii* supplementation during inflammatory challenges has been previously reported to alleviate lung injury and oxidative stress ^(59,60) but there are currently no studies on LY effect on respiratory health during heat

stress. Nevertheless, with a more efficient ability to dissipate heat via the latent pathway after a meal, one can assume that Y—2FW pigs had to rely less on a reduction in metabolic HP (alongside their T4 levels) to maintain homoeothermic status during hot conditions. Thus, the supplemented 2FW pigs were able to maintain higher DMI and potential for PD during HS which is also reflected in their lower plasma creatinine as there was less need to mobilize body protein for added energy. Finally, it is possible that the positive effects of LY during HS were most evident in the 2FW groups due to the increased severity of the heat challenge on these groups compared to other FW treatments.

Conclusion

To conclude, the present study demonstrates that improved heat tolerance of pigs lies in their ability to maintain the dynamic equilibrium between heat production and loss throughout the day. Live yeast supplementation increased insulin sensitivity and heat loss efficiency allowing the pigs to eat more (via shorter inter-meal intervals or increased meal frequency) and thus increasing their energy efficiency during heat stress. The lower thermal load in supplemented pigs helped them reach a higher maximum potential for protein deposition, which is otherwise limited in hot conditions. Imposing an increased meal frequency does not replicate the positive effects of LY supplementation during HS because the altered feeding behavior is not the cause but a mere consequence of the improved energy metabolism and thermoregulation responses of LY supplementation in pigs.

Acknowledgments

The authors want to thank G. Poupeau, F. Guerin, J. Fortin, J. Georges, M. Genissel, A. Chauvin, and F. Le-Gouevéc for animal care, J.F. Rouaud and S. Duteil for diet preparation, and C. Perrier, G. Le Roy, C. Mustière, A. Marchais, X. Arondel-Piekarz, P. Ganier, and R. Comte for lab analyses.

Financial support

This study and the PhD grant of A.M. Serviento were funded by Lallemand Animal Nutrition (Blagnac, France) and French National Association for Research and Technology with the contract number N°2018/1731 under the CIFRE PhD program (Conventions Industrielles de Formation par la Recherche). M.C. is a permanent employee and A.M.S. is a contractual employee of Lallemand SAS.

Conflict of interest

None.

Authorship

A.M.S., E. L., D.R., and M.C. designed the study. A.M.S. with direction from E. L. and D. R. was responsible for data collection and statistical analysis. E. L. assisted with study execution. A.M.S. wrote the manuscript. E. L., D. R., and M. C. reviewed the manuscript. All authors read and approved the final manuscript. E.L. is the corresponding author.

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Chapter 4. Effect of sex and live yeast supplementation in heat stressed pigs

This chapter presents the results of Experiment 4 that aimed to evaluate the effect of biological sex and LY supplementation in HS pigs.

Effect of sex and live yeast supplementation in heat stressed pigs

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Keywords: heat stress, pigs, live yeast, sex, growth

Introduction

Reduced growth performance of pigs during heat stress (**HS**) is mainly induced by a decrease in feed intake (**FI**). Live yeast (**LY**) supplementation was reported to improve HS responses by increasing FI and feed efficiency in entire male (**EM**) pigs (Labussière et al., 2021) but this has not been confirmed in females (**F**) (Chapter 2). Indeed, a sexual dimorphic effect of HS has been previously reported with barrows having lower feed efficiency than gilts during HS (Renaudeau et al., 2006) but no studies to date regarding the difference between EM and F. Thus, the objective of the study was to determine the effect of sex and LY supplementation in acclimated HS pigs.

Materials and methods

A total of 64 individually housed finishing pigs (32 EM and 32 F; 80.8±0.9 kg BW) were used to evaluate the effects of sex and LY supplementation in HS pigs with 2 successive replicates. There were 2 levels of LY supplementation: 0 (N) vs. 100 g/ton of feed (Y; 1×10⁶ CFU/g of feed of *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079) given to EM or F pigs. Thus, there were four experimental groups: N–EM, Y–EM, N–F, and Y–F. The pigs were equally distributed to two experimental rooms based on the treatments. Ambient temperature of both rooms were maintained at 22°C for 7 d and then at 32°C for 3 d during the adaptation period before the 27 d experimental period at 32°C. Feed and water were provided *ad libitum*. Growth and slaughter performance were measured. The pig was considered as the experimental unit and data was analyzed using PROC MIXED model with replicate I, sex (S), LY supplementation (D; diet), their interactions as fixed effects, and with litter origin as random effect.

Results and discussion

Table 1 shows the summary of the growth and slaughter performance. Replicate had a significant effect on live and slaughter BW wherein pigs in replicate 2 were heavier than pigs

Table 1. Effect of live yeast supplementation and sex on growth and slaughter performance of finishing pigs exposed to high ambient temperature.^{1,2}

Items	Rep 1				Rep 2				RSD ³	Statistics
	N – EM	Y – EM	N – F	Y – F	N – EM	Y – EM	N – F	Y – F		
Animals, n	8	8	8	8	8	8	8	8		
Growth										
Initial BW, kg	78.3	78.4	76.7	75.6	88.2	85.2	84.4	82.1	4.6	R*, S*
Final BW, kg	107.0	103.8	100.1	98.3	114.4	114.6	110.5	104.4	6.6	R**, S**
ADFI (g·kg ^{BW-0.60} ·d ⁻¹)	173 ^{ab}	158 ^b	170 ^{ab}	164 ^b	172 ^{ab}	190 ^a	180 ^{ab}	164 ^b	19	R×D×S*
ADFI (g·d ⁻¹)	2628 ^{abc}	2377 ^c	2502 ^{bc}	2387 ^c	2761 ^{abc}	3007 ^a	2826 ^{ab}	2491 ^{bc}	346	R*, R×D×S*
ADG (g·d ⁻¹)	1060 ^a	942 ^{abc}	866 ^{bc}	839 ^{bc}	973 ^{ab}	1083 ^a	959 ^{abc}	827 ^c	140	S**, R×D×S*
Gain:Feed	0.40	0.40	0.35	0.35	0.35	0.36	0.34	0.33	0.03	R*, S*, R×S*,
Slaughter parameters										
Slaughter wt, kg	82.0	79.7	78.2	77.6	89.2	88.3	85.9	82.4	5.1	R**, S**
Lean meat, %	60.6	60.1	60.1	60.1	60.6	59.9	59.1	61.4	1.7	D×S*

¹A total of 64 pigs were allocated to 4 experimental groups based on sex and live yeast supplementation in 2 successive replicates. Experimental period started after a 3-day acclimation of at 32°C was thereafter maintained for 28 d. N: non-supplemented diet; Y: live yeast supplemented diet; F- female; EM: entire male

²Data was analyzed using PROC MIXED model with replicate (R), diet (D), sex (D), room (r), and their interactions as fixed effects and litter origin as random effect.

***P* < 0.01, * *P* < 0.05, ^{a,b,c}LSmeans within a row with different superscripts letters differ (*P* < 0.05) according to the experimental group.

³RSD: residual standard deviation.

in replicate 1 ($P < 0.05$). Regardless of the replicate, EM pigs were heavier and had higher average daily gain (ADG) than F pigs ($P < 0.05$) and thus had higher final and slaughter BW ($P < 0.05$). In TN conditions, boars were reported to grow faster and have higher feed efficiency than gilts (Blanchard et al., 1999; Ruiz-Ascacibar et al., 2017). However, the improved efficiency in EM in this study was only observed on lighter (replicate 1) but not on heavier (replicate 2) pigs with significant interaction of replicate and sex on gain:feed of the pigs ($P < 0.05$). This suggests that the negative effect of HS on feed efficiency is heightened in heavier EM but not F pigs probably due to exacerbated effect of HS on their ability to reach their maximal potential for protein deposition or growth (Le Bellego et al., 2002). This might be attributed to higher susceptibility of heavier pigs to HS (Renaudeau et al., 2011) coupled with a higher heat production of EM pigs due to their higher growth potential and protein deposition (Moughan et al., 2006; Labussière et al., 2013). There was a significant interaction between replicate, diet, and sex for average daily feed intake (ADFI) expressed per $BW^{0.60}$ and ADG ($P < 0.05$). Supplemented EM pigs in replicate 2 had higher ADFI per $BW^{0.60}$ ($P < 0.05$) than supplemented EM pigs in replicate 1 and F pigs in replicates 1 and 2, and higher ADG than F pigs in replicate 1 and supplemented F pigs in replicate 2. Meanwhile, supplemented EM pigs only tended to have a higher ADFI per $BW^{0.60}$ ($P = 0.08$) than non-supplemented EM pigs in replicate 2. While this was only a tendency, this suggests that LY supplementation seemed to improve ADFI during HS but only in heavier EM pigs. Indeed, due to the high standard deviation in the ADFI response of the pigs in the study, it would have needed ~400 pigs to detect significance difference of $P = 0.05$ between the diets according to PROC GLMPOWER. Thus, based on our results, the effect of LY supplementation on HS responses of pigs seem to depend on the BW and sex of the animal.

Conclusion

Entire male pigs had higher growth performance than female pigs in HS conditions. Meanwhile, the effect of sex on feed efficiency (higher in EM than F pigs) seem to depend on the BW of the pigs (not observed in heavier pigs). LY supplementation also improved ADFI of HS pigs but only in heavier EM pigs. Thus, the effect of HS and of LY supplementation during HS seem to depend on the age or BW and on sex of the pig.

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General discussion



Figure 24. Bullet point summary and conclusions of the chapters in the thesis

General discussion

The objective of this thesis project was to contribute to the understanding of HS effects in pigs and to evaluate the ability of some feeding strategies to mitigate them. A total of 4 experiments were conducted to:

- to investigate the effects of a chronic prenatal heat challenge,
- to distinguish direct and indirect HS effects,
- to determine whether or not positive effects of LY supplementation were linked to a modified feeding behavior,
- to evaluate possible interactions between LY supplementation and sex in HS pigs, and
- to further understand the dynamic process of how body heat balance (body temperature) changes and is maintained throughout the day within a narrow range.

The main results of the four experiments have already been extensively discussed in the previous chapters and summarized in **Figure 24** and **Table 10**. Thus, this discussion will focus, first, on the possible sources of variation in HS responses among experiments. The second part discusses the dynamic responses of pigs to maintain their homeothermic body temperature in HS conditions and how these responses are influenced by the feeding behavior of the animals. The third part focuses on the possible links among latent HL, water intake, feeding frequency, and insulin sensitivity that makes them important during HS. Finally, the fourth part discusses the possible mechanism of LY during HS in pigs.

Possible sources of variation in the severity of heat stress among experiments

The different heat challenge models employed in our experiments elicited different severity in HS responses. One illustration is the differences in ADFI drop of *ad libitum*-fed pigs as shown in **Figure 25**. In this section, some factors that might contribute to the differences among experiments are discussed.

Animal related factors

As mentioned in the literature review, heavier pigs are more susceptible to HS (Quiniou et al., 2000a; Renaudeau et al., 2011) due to their higher HP and their lower surface area to mass ratio that lowers their ability to dissipate heat. Our results agree with these conclusions wherein the effect of HS on ADFI was less important in Exp. 1 (-7%) in which pigs were lighter

Table 10. Summary of the effects of HS only on *ad libitum*-fed non-supplemented growing pigs in the experiments of the thesis project.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Sex	Females	Females	Entire males	Females & Entire males
Growth				
Pre-challenge mean BW, kg	39.2	74.1	83.2	74.4
Challenge duration, d	58	19	5	27
ADFI, g·d ⁻¹	-332	-547	-846	-328 (vs. adaptation)
ADFI, g DM·d ⁻¹	-295	-489	-753	-368 (vs. adaptation)
ADG, g·d ⁻¹	-127	-279	-737	-
Hormones				
Insulin	-	increase on d 8 of HS	maintain	-
Insulin: Glucose	-	-	maintain	-
T3	decrease (more pronounced on d 3 than 49 of HS)	decrease (more pronounced on d 1 than 20 of HS)	decrease	-
T4	decrease (more pronounced on d 3 than 49 of HS)	decrease (more pronounced on d 1 than 20 of HS)	decrease	-
Metabolites				
Glucose	maintain	maintain	increase basal level	-
Lactate	-	-	maintain	-
Creatinine	increase	increase	increase	-
NEFA	decrease	maintain	decrease	-
Urea	-	-	increase	-
BAP	decrease after 49 d of HS	-	-	-
SOD	decrease on d 3 of HS	-	-	-
Enzyme activity				
Creatinine kinase	increase on d 3 of HS	-	-	-
Lactate dehydrogenase	increase on d 3 of HS	-	-	-

(<40 kg BW) compared to the other experiments (-16 to -29% drop in ADFI) with heavier pigs (>70 kg BW) upon heat exposure. Meanwhile, the effect of sex on the severity of HS was less clear. Female pigs decreased their ADFI by only 16% (in Exp. 2 and 4) while the entire male pigs by 29% or 19% (Exp. 3 and 4, respectively). To our knowledge, sexual dimorphic responses to HS has not yet been established in pigs. Previous reports suggested that barrows had lower feed efficiency than gilts during HS (Renaudeau et al., 2006b) while others reported higher susceptibility of gilts to heat-induced oxidative stress (Rudolph et al., 2021; Wesolowski et al., 2022). Meanwhile in Exp. 4, heavier entire male pigs appeared to be more susceptible to HS. While feed efficiency is reported to be higher in entire males compared to females up to 140 kg BW in TN conditions (Ruiz-Ascacibar et al., 2017), we only observed this sex effect in relatively lighter pigs (initial BW of ~77 kg) but not in heavier pigs (initial BW of ~85 kg) subjected to HS. This lack of sex effect in heavier pigs must be because of a negative effect of HS on entire males but not in females at this BW. As discussed in the literature review, animals with higher growth potential are less able to tolerate HS (Renaudeau et al., 2006b; Rauw et al., 2017; Gourdine et al., 2021). Thus, heightened effect of HS might be related to both the heavier BW (Renaudeau et al., 2011) and to the higher PD and fasting HP of entire male pigs (Moughan et al., 2006; Labussière et al., 2013). This assumption has to be confirmed in further studies.

As another animal factor, early life fetal HS experience of the pig can also influence their future HS responses particularly their performance and their body composition (as reviewed by Johnson and Baumgard, 2019; and Liu et al., 2021). In addition, our results (Exp. 1) demonstrated that a chronic prenatal heat exposure resulted to a decreased ability of the pig to cope with a subsequent heat challenge in their postnatal life. Results from Exp. 1 also shows that even microbiota composition of growing pigs exposed to HS (same growing environment) tended to be influenced by their prenatal environment (**Annex 3**). Although there are increasing *in utero* HS studies, the repercussions of a prenatal HS are still largely unknown and remain to be explored. For example, our study only looked at a chronic (whole gestation) heat challenge but literature seems to suggest that different factors can even influence prenatal HS effects such as the previous HS experiences of the sire and the sows, the duration and pregnancy stage of the heat challenge (Boddicker et al., 2014), the physiological stage of the pig in their postnatal life (Johnson et al., 2015a; Johnson et al., 2015b), and their postnatal thermal environment (Exp. 1) among many others. Meanwhile, in our study (in Exp. 2, 3, and 4), we were not able to detect any prenatal effect of HS.

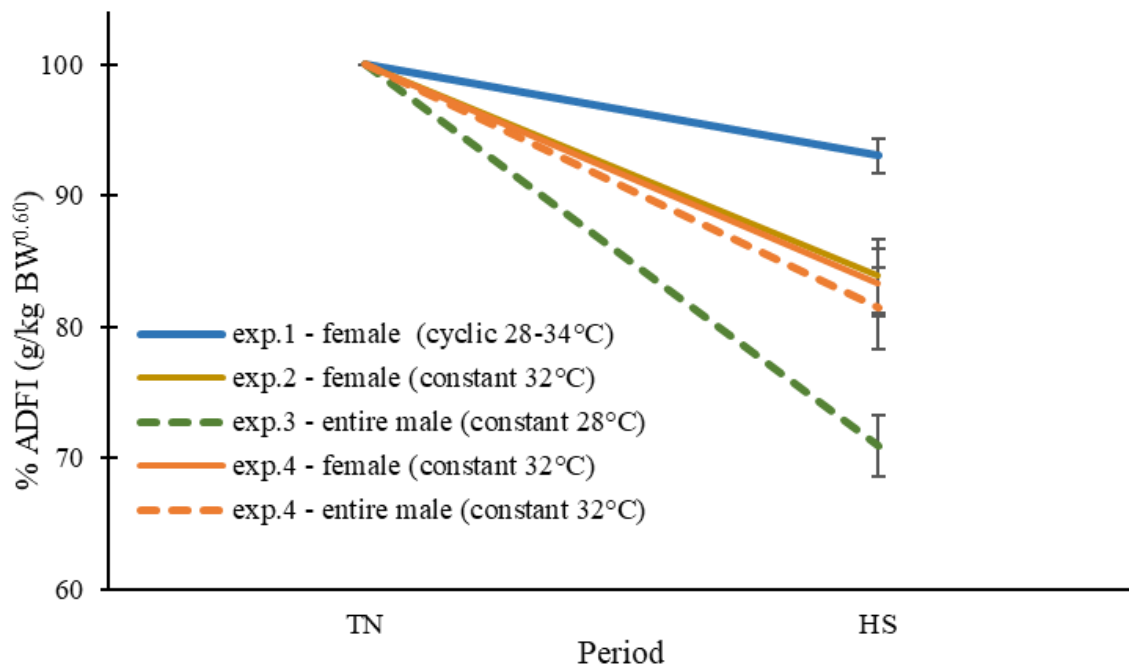


Figure 25. Effects of HS after 1 week of heat exposure on ADFI drop (expressed as % of pre-challenge ADFI) according to the experiments (different colors) and sex of pigs used (solid line for females, broken lines for entire males). After 7 d of HS for Exp. 1 (*PTN—GHS*), Exp. 2 (*Trt 5; HS—AL*), and Exp. 4 (*N—EM* and *N—F*) and after 5 d for Exp. 3 (*N—Unli*).

Housing condition, nature, and severity of the experimental heat challenge

Differences can also arise according to the type of housing conditions. Among the experiments, the highest drop in feed intake observed in Exp. 3 (-29%) indicates that HS level in the respiratory chambers at only 28°C of ambient temperature was greater than in larger rooms with even higher ambient temperatures. With a smaller confinement space and highly isolated equipment, radiant heat exchange which is dependent on distance between two objects (Berman and Horovitz, 2012), i.e. between the pig and its surrounding environment, may have been higher in the respiratory chamber thus eliciting a stronger HS response than in the other experiments. The drop in ADFI in Exp. 3 ($-753 \text{ g} \cdot \text{DM} \cdot \text{d}^{-1}$ in *ad-libitum* fed pigs) was comparable with another study in the respiration chamber ($-730 \text{ g DM} \cdot \text{d}^{-1}$) but using lighter pigs with higher ambient temperature (~50 kg; 32°C) (Renaudeau et al., 2013). Meanwhile, the ADFI drop in our study was higher than in another study using 100-kg finishing pigs ($-410 \text{ g DM} \cdot \text{d}^{-1}$) (Labussière et al., 2021). This difference is probably explained by the more sudden increase of ambient temperature in our study compared to the aforementioned study (increase from 22 to 28°C in 3 h vs. in 2 d, respectively) which gave less chance for the pigs in our study to acclimate to the temperature increase.

Additionally, the nature of the heat challenge may influence the severity of observed HS responses. While the lower ADFI drop in Exp. 1 was probably related to the animal's lower BW, it might also be linked to the fact that a cyclic heat challenge was used compared to the constant ambient temperatures in the other experiments. A cyclic heat challenge was reported to allow pigs more opportunities to increase feed intake during the cooler parts of the day as reported previously (Xin and DeShazer, 1991; Quiniou et al., 2000c; Patience et al., 2005). In fact, this was precisely why a constant heat challenge was chosen in our experiments because feeding behavior was of particular interest in this thesis (Exp. 2 and 3). Nevertheless, housing conditions influenced our results, specifically in Exp. 2 where, even if pigs were individually housed, feeding behavior of *ad libitum*-fed pigs were unintentionally modified as a result of stimulation from hearing or seeing pigs in other pens with different feeding distributions (**Annex 4**). Finally, our results emphasize the importance of housing conditions in evaluating HS effects and that, in line with this, the measured responses for one study may only be specific to the particular experimental conditions for that experiment.

Implications

Many factors influence how pigs respond to a heat challenge and a higher ambient temperature does not always necessarily mean a more severe HS response. The different heat challenge models used in this thesis project demonstrated that these differences might be related to the animals (at least BW, sex) and the housing conditions (room characteristics, stocking density, possible interactions among the pigs, heat challenge used). Additional complex factors that might cause differences in pig response to HS are certainly linked to the pig's prenatal thermal environment since there is still little known about its consequences on the postnatal life of the pig. Nonetheless, these factors should be considered when designing future HS experiments. Future studies might also be needed to elucidate the sexual dimorphic responses to HS in pigs taking into account its possible interaction with BW and age. On the other hand, while prenatal pig studies can be more complicated to design and very expensive, there are still many things to consider in furthering our understanding about the factors at play in a prenatal heat challenge such as the previous HS experiences of sires and sows, the duration of the challenge, the pregnancy stage, and even the possible interaction with the sex of the progeny.

Feeding behavior and its influence on dynamic regulation of body heat balance

One of the key ideas confirmed in this thesis project is related to the dynamic response of pigs in their attempt to maintain a thermal equilibrium (or relatively constant body temperature) during the day. With the pig being in constant heat exchange with its environment and experiencing variations in metabolic HP throughout the day, heat storage (difference of HP and HL) is never constant as reflected by the fluctuations in body temperature. While this is not a new concept, only few studies in pigs focused on the dynamic HS responses within the day (Xin and DeShazer, 1991; Cervantes et al., 2018). Owing to the continuous measurements of body core temperature in Exp. 2 and 3 and HP and HL components from the respiratory chambers in Exp. 3, our results have concretely demonstrated that HP, body temperature, and proportions of sensible and latent HL of growing pigs fluctuates during the day depending on their feeding behavior and ambient temperature. In this subsection, we will describe first how feeding behavior and meal characteristics influence the dynamics of body heat balance in TN conditions and then how this regulation is impacted when pigs are exposed to HS.

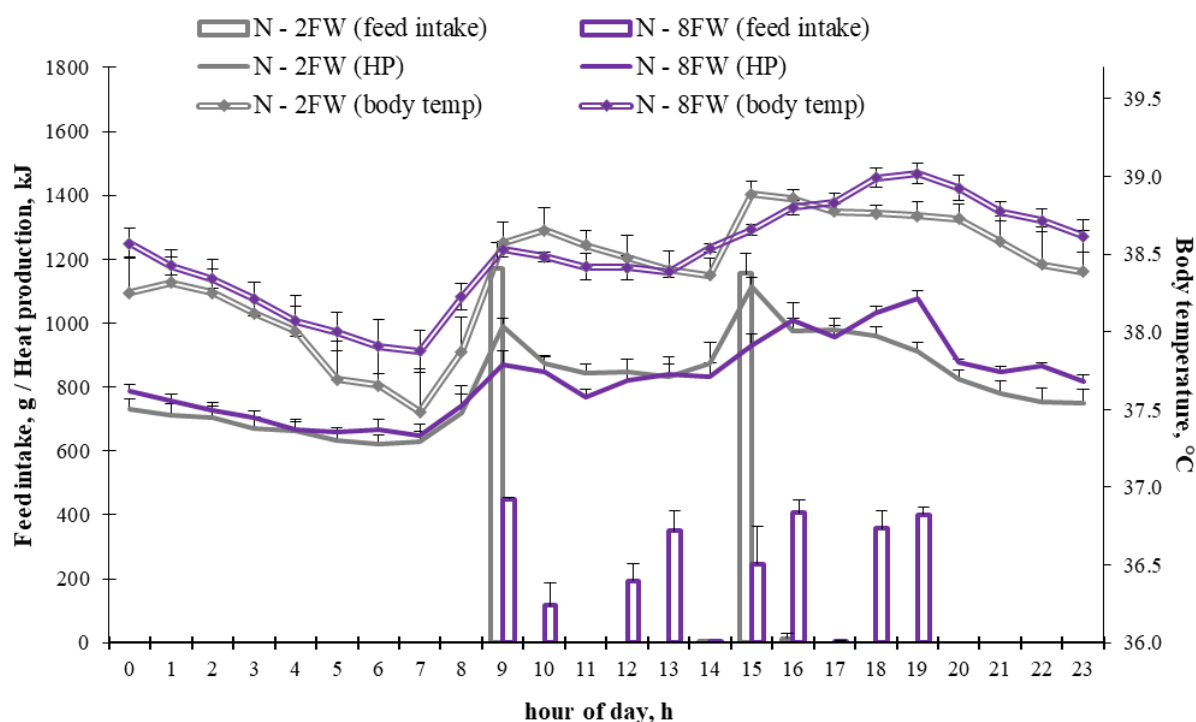


Figure 26. Hourly variations of feed intake, heat production, and body core temperature in growing pigs with restricted feeding windows kept in TN conditions of 22°C (mean±SE). Data from N—2FW (grey) and N—8FW (violet) pigs in Exp. 3.

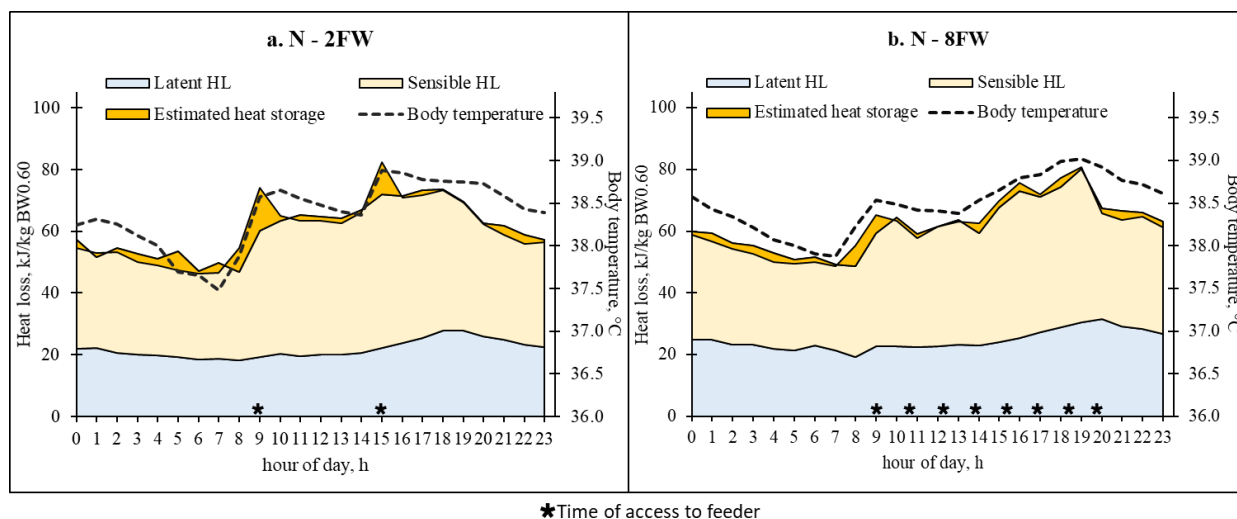


Figure 27. Hourly variations in latent and sensible heat losses, estimated heat storage, and body temperature in TN conditions (22°C) according to the feeding management. Data used from pigs in a) N—2FW and b) N—8FW group in Exp. 3.

Thermoneutral conditions

In agreement with Labussière et al. (2013), a large part of HP variations observed in Exp. 2 and 3 in individually housed pigs was due to heat associated with the feeding activity. As shown in **Figure 26**, this was also reflected in the body temperature fluctuations which increased and decreased according to when the pigs have or do not have a feeding activity. These feeding-related fluctuations are comparable to the intestinal temperature pattern reported in a previous study in TN conditions (Morales et al., 2018). In fact, these increases in body temperature reflect temporary imbalances between HP and HL (Taylor et al., 2014). This transient hyperthermia might be related to the time it takes for heat to be exported (e.g. as the blood travels from the core to the skin and then to the environment) and/ or to the delay in the initiation of HL mechanisms which are activated only after a deviation from the set point temperature is detected (Tan and Knight, 2018).

While pigs are able to lose heat by both the sensible and latent routes, they rely mainly on the changes in the skin blood flow to maintain a constant body temperature in TN conditions. Literature suggests sensible HL represent 70-80% of total HL in pigs in TN environment (Kamada and Notsuki, 1987; Renaudeau et al., 2013) which is comparable to our observations in Exp. 3 when looking at daily averages. However, as demonstrated in our study, HP and body temperature are not constant during the day and thus proportions of sensible and latent HL also fluctuate accordingly. Furthermore, since body temperature remains constant only when all heat produced is equal to heat lost (heat storage), this implies that net heat storage in the body also fluctuates since body temperature is not constant. Thus, for the sake of a more accurate graphical representation, we also tried to roughly estimate heat storage in the body using the changes in the measured body temperature, the mean BW of the pig, and the assumption that the specific heat of lean tissue is at $3.47 \text{ kJ}\cdot\text{kg}^{-1}\cdot^\circ\text{C}^{-1}$ (Vallerand et al., 1992; Giering et al., 1996) as shown in **Figure 27**¹². As seen in the graph, sensible HL increases whenever there is an increase in body temperature caused by the feeding activity, and it can fluctuate from around 28 to $50 \text{ kJ}\cdot\text{kg BW}^{-0.60}\cdot\text{h}^{-1}$ (or 53 to 73% of HP) during the day. Meanwhile, as previously discussed in Exp. 3, transient heat imbalances caused by feeding activity in TN conditions did not significantly increase the amount of heat lost by evaporation and it remained relatively constant at around 18 to $31 \text{ kJ}\cdot\text{kg BW}^{-0.60}\cdot\text{h}^{-1}$ during the day representing around 42-48% of HP

¹²Recall that in estimating sensible and latent HL, latent HL is calculated from the evaporated water in the respiratory chamber (Text box 2) and sensible HL is calculated by difference between total HP and latent HL.

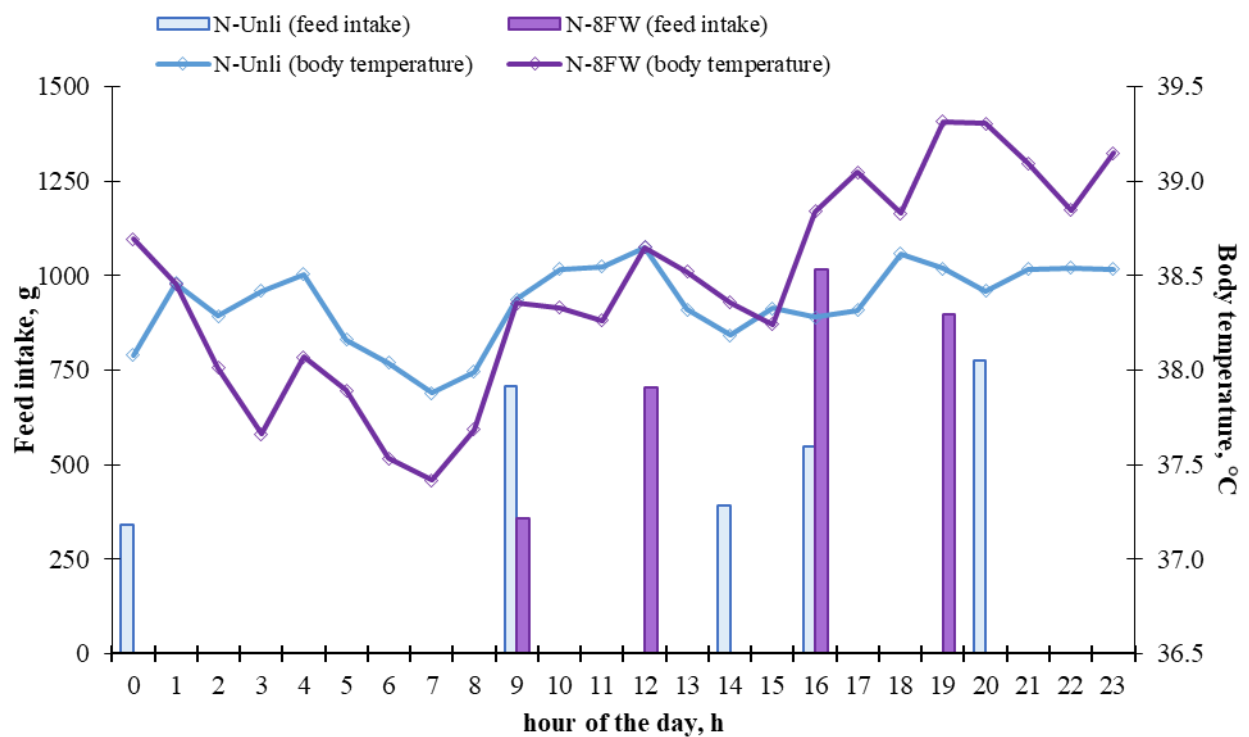


Figure 28. Example of the relationship of feeding management and body temperature fluctuations in growing pigs raised in TN conditions (22°C). Data from pigs N063693 for N—Unli (blue) and N062538 for N—8FW (violet) from Exp. 3

when HP was relatively low (i.e. no feed intake) and only up to 26-34% of HP during times of high HP (i.e. during or after a meal).

While there was a subsequent decrease in body temperature after a meal as result of these HL mechanisms, our results in Exp. 2 and 3 demonstrated that there is an incremental increase in the pre- and postprandial body temperature with each additional meal (also clearly shown in **Figure 26**). This incremental increase in HP per meal agrees with previous studies (Schrama et al., 2003; Bolhuis et al., 2008; van Erp et al., 2020) and was likely from the cumulative heat increment due to nutrient utilization (long-term TEF) that follows each meal. Meal characteristics such as meal size also influenced the degree of HP and body temperature increases. For example, using the data from pigs in Exp. 3 (**Figure 26**), HP and body temperature increased¹³ on average by 361 kJ and 1.08°C, respectively, for the pigs with an average meal size of ~1.20 kg (N—2FW) after the 0900h meal while it was only by 222 kJ and 0.65°C for pigs with an average meal size of ~0.54 kg (N—8FW). Furthermore, HP and body temperature seemed to remain higher for a longer time in the pigs with larger meal sizes which is logical since duration of gastric emptying of larger meals takes longer than smaller meal sizes (Gregory et al., 1990) and thus there is longer influx of nutrients that contributes to the long-term TEF (nutrient utilization).

However, our results have also shown that it is not only the meal size but also the pattern of meal distributions that influenced the pattern of body temperature and heat balance. Looking at the feeding managements applied in this thesis project, specifically in Exp. 2 and 3, the degree of body temperature fluctuations were less apparent when pigs have unrestricted access to the feed or feeder as they were able to eat when and how much they want. This was in contrast to restricted pigs (in time or amount of feed) which were relatively in a “fasted state” during large periods of the day and had to eat the bulk of their meals within a shorter period of the day. An example is shown in **Figure 28** with two pigs from different feeding management groups. Despite similar average body temperatures during the day (~38.4°C), body temperature of the pig restricted to eat for only a total of 2 hours with an 11-hour time frame (N—8FW) can incrementally increase up to 39.3°C during the last meal of the day but can decrease to as low as 37.4°C (-1.9°C) after a 13 hour feeder access restriction. Meanwhile, pigs that had unrestricted feed access (and whose longest meal interval was ~5 hours) maintained their body temperature at a relatively constant range of 37.9-38.6°C (0.8°C difference). Along with the

¹³ Calculated by difference from the value at 0700h and at 0900h.

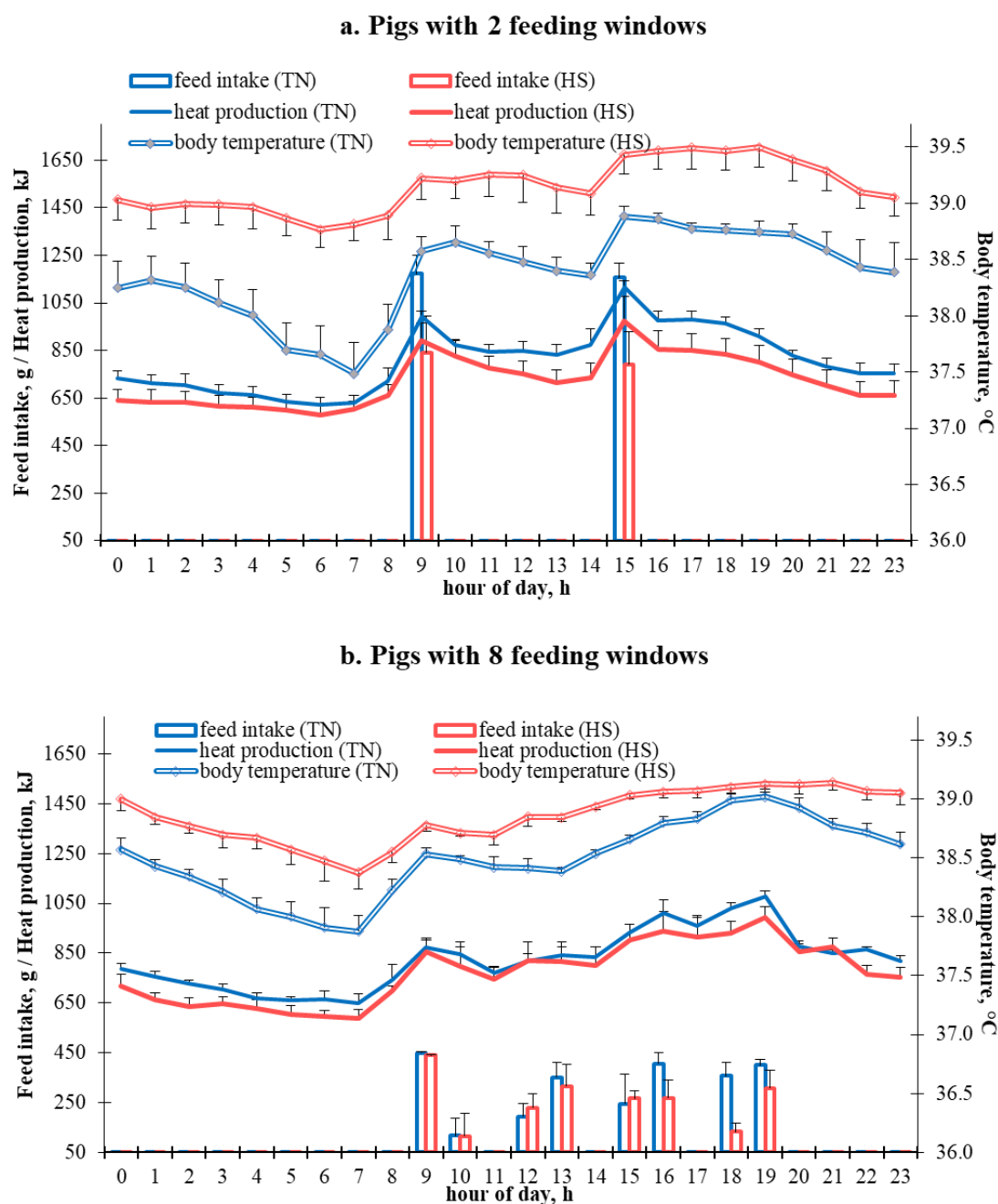


Figure 29. Hourly variations of feed intake, heat production, and body temperature in growing pigs according to the frequency of meal distribution (mean \pm SE). Data from (a) N—2FW and (b) N—8FW pigs raised in TN (blue; 22°C) and HS (red; 28°C) conditions from Exp. 3.

results of Exp. 2 and 3, our results thus demonstrated that meal distribution (alongside duration of meal intervals) can also influence variations in the body heat balance.

The feeding managements employed in the thesis project may admittedly have been oversimplified. With gastric satiation being volumetric and intestinal satiation being nutritive (Powley and Phillips, 2004), one can imagine that it was the gastric volume that limited the pigs with only 2 meals to eat more per meal. On the other hand, it may have been nutrients still present in the intestinal tract which can trigger ileal brake (Ratanpaul et al., 2019) and the relatively short 90-min interval that restricted the pigs with 8 FW to eat at every feeding window. Nevertheless, our results clearly showed the importance of feeding behavior and meal characteristics (especially size and pattern) in looking at the dynamics of body heat balance during the day. Future studies might be needed to elucidate the effects of different meal sizes and interval durations on thermoregulatory and metabolic responses of pigs (e.g., how much time it takes to restore body temperature to its basal value based on the size of a previous meal).

Heat stressed conditions

Upon heat exposure, there was a reduction in metabolic HP (**Figure 29**) which was related to the feed intake reduction as observed across our experiments (Exp. 1, 2, 3, and 4) and in other studies (Collin et al., 2001c; Renaudeau et al., 2013). This reduction in metabolic HP may also be related to a lower metabolic rate (Fausnacht et al., 2021) as indicated by decreased thyroid hormone levels which was consistently observed in HS pigs in Exp. 1, 2, 3 and in other studies (Collin et al., 2002; Campos et al., 2014b; Sanz Fernandez et al., 2015a). Despite this, body temperature of pigs under HS remained higher than those under TN conditions which suggested a higher heat load and that HL pathways are more saturated during HS regardless of the time of day (Exp. 2 and 3).

Similar to the pattern in TN conditions, each additional meal resulted to an incremental increase in the pre- and post-prandial body temperature of the pigs subjected to HS. However, these increases in body temperature were to a lesser degree but were maintained for a longer period compared to that observed in TN conditions (as presented in Exp. 2). This was in contrast to TN conditions wherein there was a clear and continuous body temperature decline after the last meal of the pigs with feed or feed access restriction, results of Exp. 2 and 3 showed that body temperature of the pigs remained high during HS even during periods with no feeding activity. In both experiments, we hypothesized this to be related to a slowed down gastric

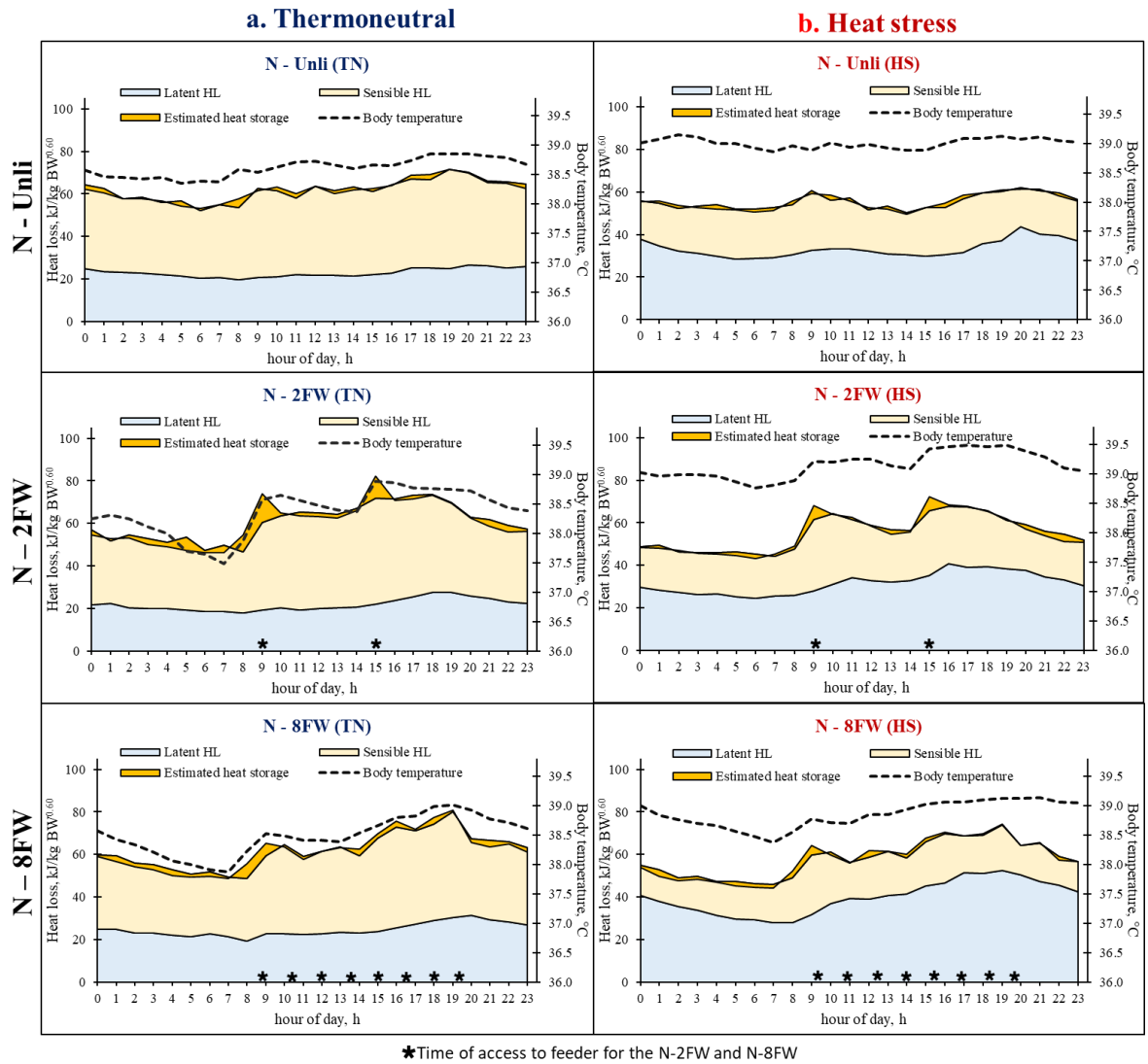


Figure 30. Latent and sensible HL and estimated heat storage according to the ambient temperature (a. TN at 22°C and b. HS at 28°C) and the feeding management (Unli, 2FW, 8FW). Data used from Exp. 3.

emptying and motility as reported in animals under HS (Gonzalez-Rivas et al., 2020) possibly as a result of restricted splanchnic blood flow. The negative effect of HS on gastric emptying and motility remains a theory and has yet to be confirmed in pigs. However, our results support that delayed gastric emptying seems to be related to large meal size and/or high insulin levels (Gregory et al., 1990; Eliasson et al., 1995) since pigs with larger meal sizes and higher levels of insulin (N—2FW) maintained a high body temperature for longer periods than the pigs with smaller meal sizes (N—8FW). Nevertheless, body temperature fluctuations were less apparent during HS than during TN conditions and even more so when pigs were imposed to eat large meals per day.

Aside from the pattern of HP and body temperature, patterns of sensible and latent HL were also modified by heat exposure with increased proportions of latent HL during HS conditions (**Figure 30**). Pigs lost a maximum of only 28-34 kJ·kg BW^{-0.60}·h⁻¹ via sensible route during HS even when the HP is high as compared to ~50 kJ·kg BW^{-0.60}·h⁻¹ in TN conditions. As discussed in the literature review, sensible HL is driven by the temperature gradient between the body and the environment and this gradient is decreased in high ambient temperature. Then, pigs start to dissipate more heat via the evaporative route (Curtis, 1983). According to our results, the ability of pigs to lose heat via the latent pathway seems to be influenced by their feeding behavior. While heat loss via the latent route can be as low as 25-28 kJ·kg BW^{-0.60}·h⁻¹ (50-54% of HP) during periods of low HP, this can increase up to 40 kJ·kg BW^{-0.60}·h⁻¹ (58% HP) in N—2FW pigs and up to 53 kJ·kg BW^{-0.60}·h⁻¹ (71% of HP) in N—8FW pigs when HP is highest (e.g. during or after a meal). Moreover, the pattern of incremental increase in HP during the day was closely followed by an incremental increase in latent HL. In the N—8FW pigs in contrast to N—2FW pigs. Our results have demonstrated that limited ability for sensible HL in hot conditions means an increased importance of the latent route during HS but especially during periods of high HP (such as during or after a meal). Furthermore, the feeding behavior of the pigs seems to influence their ability to dissipate heat via the latent route.

Implications

Body heat balance as reflected in the core body temperature varies throughout the day depending on the variations in metabolic HP and in the ability of the pigs to lose heat. Our results have shown that feeding behavior, meal characteristics, and meal distribution pattern highly influence fluctuations of HP, HL proportions, and body temperature. Our results also further demonstrated the importance of the latent route in maintaining thermal equilibrium

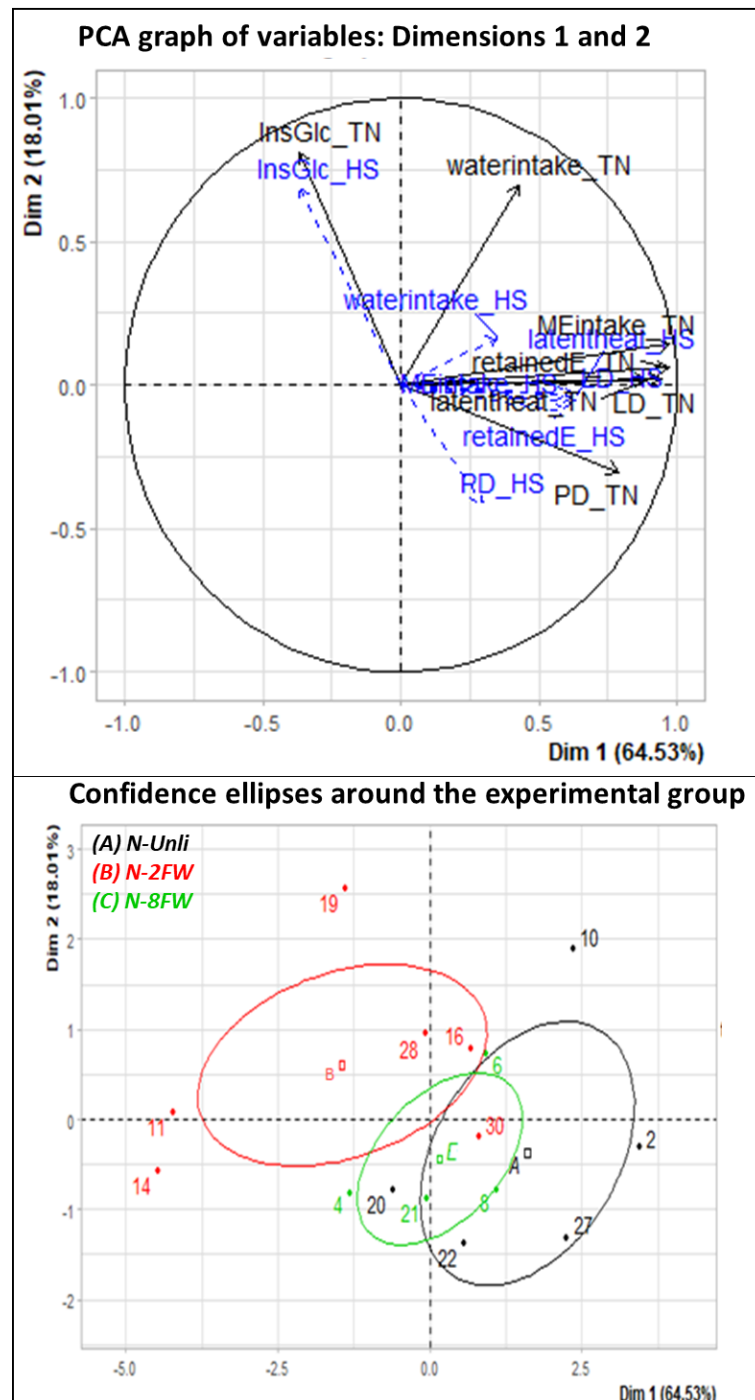


Figure 31. Principal component analysis accounting for 82.5% of the total variance among non-supplemented pigs in Experiment 3 (N-Unli, N-2FW, and N-8FW) according to ambient temperature: TN conditions in black (22°C) and HS conditions in blue (28°C). Parameters included were ME intake, retained energy (retained E), water intake, protein deposition (PD), lipid deposition (LD), and plasma levels of insulin:glucose (InsGlc).

during HS wherein the sensible HL may not be sufficient, especially during periods of high HP such as during and after a meal. It is thus important to consider these dynamic changes in the body heat balance during the day when analyzing how pigs respond to HS.

Importance of latent heat loss, water intake, feeding behavior, and insulin sensitivity during HS

In line with our results on the increased reliance of pigs to the latent route for heat dissipation during HS, this subsection focuses on the possible links between latent HL, water intake, feeding management, and insulin sensitivity and their importance during HS as demonstrated by our study. Principal component analysis (**PCA**) was applied to the data from non-supplemented TN pigs in Exp. 3 to look at the relationship between important parameters. The data collected from HS conditions were then projected on the 2 latent variables of the PCA calculated from TN data. The loading plot shown in **Figure 31** indicates that in TN conditions, latent HL, ME intake and retained energy have large positive loadings on component 1. Meanwhile, low insulin sensitivity (or a high insulin: glucose ratio) and water intake have large positive loadings and PD has moderate negative loading on component 2.

High capacity for heat loss increases ability to maintain feed intake during HS

Projections in the PCA of data collected during TN and HS suggests that regardless of the ambient temperature, there is a positive link between latent HL and ME intake (**Figure 31**). In TN conditions, this link may be due to higher metabolic water produced with a higher amount of substrate oxidation (Van Es, 1969) as it represented ~40% of evaporated water¹⁴. However, this was less likely the case in HS conditions wherein there was lower feed intake and the metabolic water only represented ~20% of evaporated water. It is more likely that the higher latent HL during HS is related to the increased panting (Robertshaw, 2006) as supported by the higher respiration rate observed in Exp. 2 and in other studies (Cottrell et al., 2020; Le et al., 2020).

¹⁴ Metabolic water is the water produced by oxidizing energy-containing substances in the diet. Oxidized carbohydrate and fat were calculated based on the formula in Text box 2 using volumes of O₂ consumed and CO₂ produced while oxidized protein is from the total N lost in the urine, condensates, and air from the experiment in the respiratory chamber. Metabolic water was then calculated using the constants assuming that there is 60, 107, and 42 g water produced per 100 g of carbohydrates, fat, and protein, respectively.

The reduction of feed intake during HS is based on the fact that there is limited capacity for sensible HL in hot conditions and thus there is a need to rely on a reduced HP to still maintain thermal equilibrium of the body. It is thus logical to assume that in order for pigs to rely less on reducing HP and feed intake in hot conditions, one must increase the ability of the pig to dissipate heat via the only other HL route which is the latent pathway. Taking the N—2FW and N—8FW as examples, the improved ability of N—8FW to incrementally dissipate heat via the latent pathway allowed them to dissipate a total of $200 \text{ kJ} \cdot \text{kg BW}^{-0.60}$ more heat during the course of the day than the N—2FW pigs. This higher amount of heat lost from the pig's body can probably mean that the N—8FW pigs can eat at least $200 \text{ kJ} \cdot \text{kg BW}^{-0.60}$ more energy without increasing their body temperature. This can thus partly explain why N—8FW pigs had lower reduction in their ME intake compared to the N—2FW (-500 vs. $-740 \text{ kJ} \cdot \text{kg BW}^{-0.60}$, respectively) upon heat exposure.

High water intake increases capacity to dissipate heat via the latent route

Reasonably, an increased evaporative HL during HS means a higher body water loss (dehydration) which can trigger thirst (Leib et al., 2016) which helps explain the increased water consumption during HS. While increased latent HL during HS disrupts body water balance, body water balance is vital for thermoregulation as demonstrated by a reduced ability for evaporative HL in dehydrated animals (Baker and Doris, 1982; McKinley et al., 2009). Furthermore, dehydration can lead to plasma hyper osmolality¹⁵ that not only increase water retention (Mroz et al., 1995) but has also been shown to delay vasodilation responses with subsequent negative effects on skin blood flow responses (Nakajima et al., 1998; Shibasaki et al., 2009). Thus, the increased water intake of HS pigs may be necessary to replenish water lost and to restore body water homoeostasis thereby allowing the pigs to maintain or increase heat dissipation via the latent route (McKinley et al., 2017). As shown in the PCA (**Figure 31**), in contrast to TN conditions, water intake has a stronger correlation with latent HL (as % HP) and with ME intake in HS conditions. From that, it is assumed that improving water intake increases the ability of pigs to maintain or increase capacity for latent HL and consequently for feed intake.

¹⁵ A high plasma osmolality indicates lack of water to maintain body electrolyte–water balance.

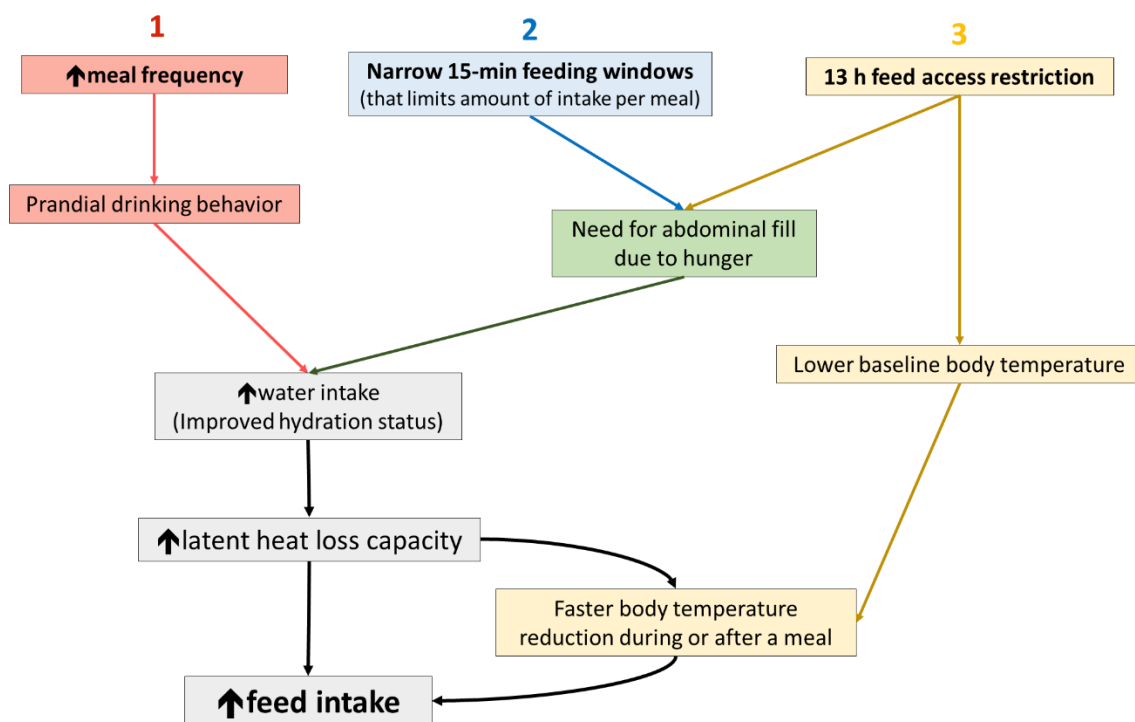


Figure 32. Three mechanisms on how the feeding management imposed for the N—8FW groups in Exp. 3 increased feed intake during HS.

Increasing meal frequency increases drinking frequency and thus water intake

One of the main objectives of this thesis project was to determine whether a modified feeding behavior, specifically an increased meal frequency, can help the pigs to improve feed intake, energy efficiency, and PD during HS. From our results, we have no clear conclusions since the effects of meal frequency on the ability to cope with HS varied according to the experiments (Exp. 2 and 3). However, we theorize that it is the type of feeding management rather than the increased meal frequency per se that helped N—8FW pigs in Exp. 3 to improve performance (or at least ME intake) during HS.

The imposed feeding management in the N—8FW group has three combined elements that may have helped them in improving feed intake during HS compared to the other groups (N—Unli and N—2FW) as summarized in **Figure 32**.

- First, the imposed *increased meal frequency* was theorized to have increased drinking bouts and thus water intake as a result of the prandial drinking behavior of pigs (Yang et al., 1981; Bigelow and Houpt, 1988) which seem to agree with our results when comparing N—8FW to N—2FW pigs (**Figure 33a**).
- Second, the *imposed narrow feeding windows of 15-min each* (that did not allow pigs to eat as much as they want per meal) in addition to the *13 h feed access restriction* during the night might have left the pigs feel hungrier prompting them to drink more water as “abdominal fill” in order to feel satiety (Yang et al., 1981). This does seem to be the case (**Figure 33b**) when comparing water intake (and thus latent HL) of the N—8FW pigs and N—Unli pigs who had unrestricted access to feed.
- Third, the *13 h feed access restriction* itself may have allowed N—8FW to lose excess heat and go back to a lower baseline BT until the next meal in the following day. Indeed, feed withdrawal strategy has already been demonstrated to help pigs (Kpodo et al., 2020a) and poultry species (Francis et al., 1991; Farghly et al., 2018) to cope with HS. It has also been suggested in our results in Exp. 3 and in another study (Cervantes et al., 2018) that a lower pre-prandial body temperature or a lower heat load before a meal is an important factor in the capacity of the animal to lose heat after a meal.

Nevertheless, the improved feed intake in non-supplemented pigs did not translate to an increase in PD (Exp. 3). Looking back in the PCA in **Figure 31**, heat exposure seemed to

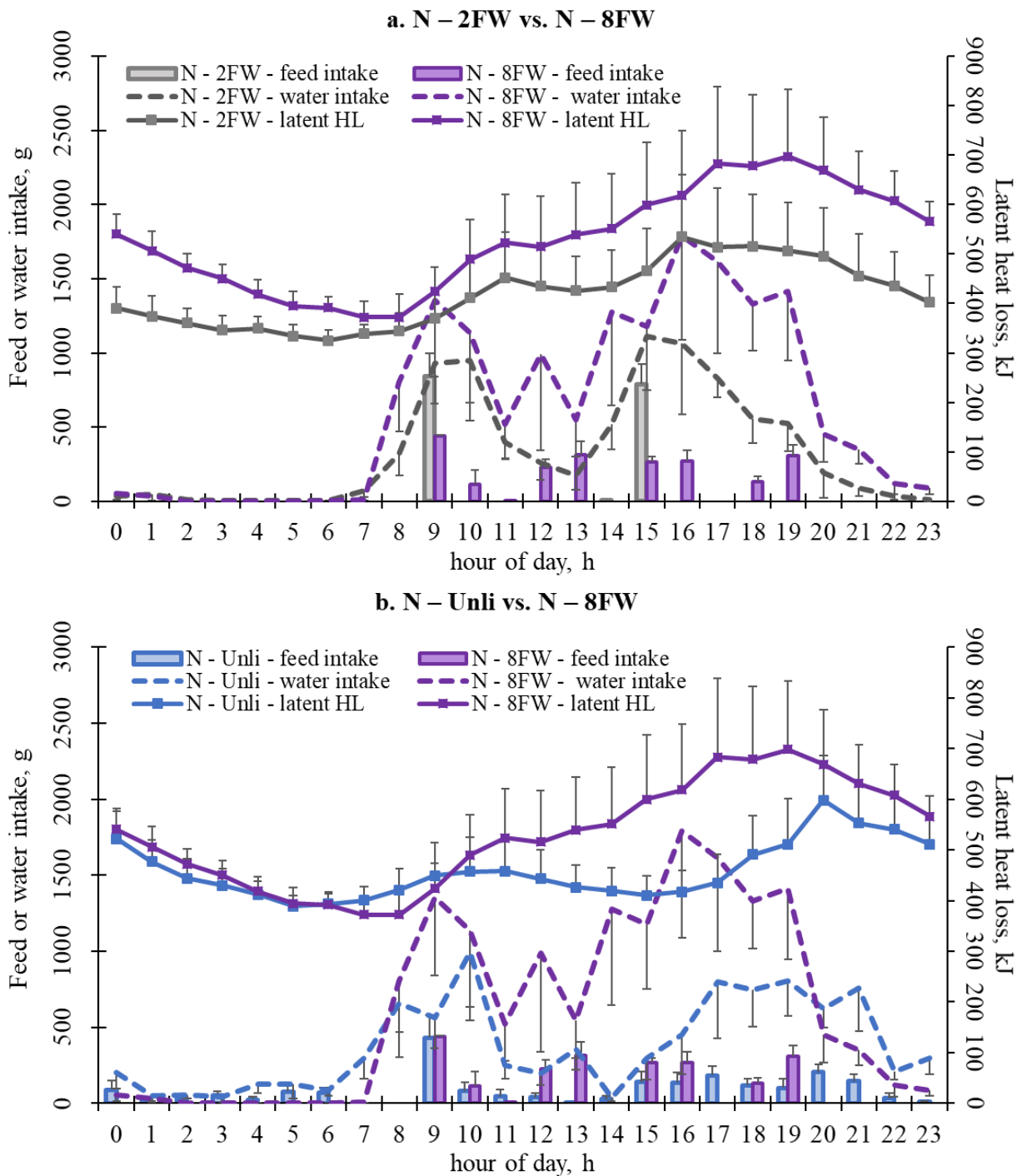


Figure 33. Hourly variations of meal frequency, water intake, and latent heat loss capacity in high ambient temperature (28°C) (mean±SE). Data from Exp. 3 wherein a) non-supplemented 2FW pigs are represented in grey and 8FW pigs in violet b) non-supplemented Unli pigs are represented in blue and 8FW pigs in violet.

modify energy partitioning in pigs. Regardless of the period, LD was strongly correlated to ME intake while this was not the case for PD in which its correlation with ME intake seems to be weaker in HS than in TN conditions. This agrees with reports of a decreased maximum potential for PD of pigs subjected to HS. This effect was attributed to the higher energetic cost (and heat increment) of PD compared to LD (Le Bellego et al., 2002b). This could also help explain the relatively increased fatness (especially visceral adiposity) demonstrated in our results in Chapters 1 and 2 and in many studies (Kouba et al., 2001; Le Bellego et al., 2002b; Wu et al., 2016). Meanwhile, based on the position of the groups (N—Unli, N—2FW, and N—8FW) on the PCA, a higher PD also seem to be related to the shorter length of feed restriction. Since the whole body PD is regulated by the balance between protein synthesis and breakdown, the imposed prolonged feed restriction may have decreased protein synthesis rate thereby contributing to a decreased net protein accretion (Reeds et al., 1980; Arnal et al., 1987).

An increased insulin sensitivity improves PD in HS

Projections in the PCA of data collected under HS suggests a lower insulin: glucose ratio (signifying increased insulin sensitivity) seems to be more correlated with a higher PD during HS than during TN. Furthermore, some studies have attributed increased insulin sensitivity to a decreased fat deposition (Dunshea and Cox, 2008; Salgado et al., 2022) and to leaner pig breeds (Albuquerque et al., 2021; Rodríguez-López et al., 2021). Many studies have already either demonstrated or hypothesized that an improved insulin sensitivity is important in coping with HS (Rhoads et al., 2013; Sanz Fernandez et al., 2015b; Liu et al., 2017; Cottrell et al., 2020). One hypothesis is that insulin sensitivity helps improve capacity to lose heat via the sensible route since it has been reported to improve microvascular circulation and vasodilation (Serné et al., 1999; de Jongh et al., 2004). Another hypothesis may be related to the direct role of insulin on whole body temperature regulation. Insulin is reported to delay heat loss mechanisms by inhibiting warm-sensitive neurons in the hypothalamus (Sanchez-Alavez et al., 2010). From this, lower insulin levels in the blood (to maintain glucose homeostasis) can thus help reduce or maintain a lower body temperature.

Implications

Taking into account what has been discussed in this and in earlier subsections, maintaining body heat equilibrium and body water balance are closely linked. Therefore, improvement of water intake is also highly correlated to the ability of pigs to dissipate heat via the latent route. The ability of pigs to decrease body temperature and to lose heat after a meal seem to be major contributing factors in improving feed intake during HS. Evaluating feeding strategies that stimulate an increase in water intake (e.g., via modification of feeding behavior, liquid feeding) and that rapidly decrease body temperature (e.g., feed withdrawal) might help in maintaining feed intake during HS. However, the exact scheme of the feeding management (e.g., optimal inter-meal intervals, pattern of meal distribution throughout the day, the size of the meal, the duration of feed restriction, and many others) in order to maintain body heat balance and feed intake and performance during HS are also important to take into account when evaluating feeding strategies. With the many roles of insulin on metabolic processes and body temperature regulation, there seems to be potential in evaluating feeding strategies that improve insulin sensitivity to help pigs cope with heat challenges while maintaining their growth performance.

Live yeast mechanism during HS may be due to an improved insulin sensitivity

One of the main objectives of the thesis was to determine whether the positive effects of LY supplementation in HS pigs observed in a previous study, i.e. improved feed intake, energy efficiency, and PD (Labussière et al., 2021), were due to changes in feeding behavior and/or energy metabolism, and if these effects can be replicated by imposing an increased meal frequency. However, as our results demonstrated, increasing meal frequency can only explain improved feed intake and energy efficiency but failed to improve PD during HS. Rather, the altered feeding behavior was not the cause but is theorized to be one of the consequences of an improved insulin sensitivity. We thus theorize that the effect of LY on improved insulin sensitivity 1) allows the pigs to eat more (via shorter inter-meal intervals or increased meal frequency) and thus leads to increase in feed intake and energy efficiency, and 2) improves thermoregulation responses of pigs that mitigates HS effects and allows them to reach a higher maximal potential for PD. **Figure 34** summarizes the hypothesized mechanisms by which the increased insulin sensitivity with LY would improve pig performance during HS as discussed extensively in Exp. 3 and in the previous subsections.

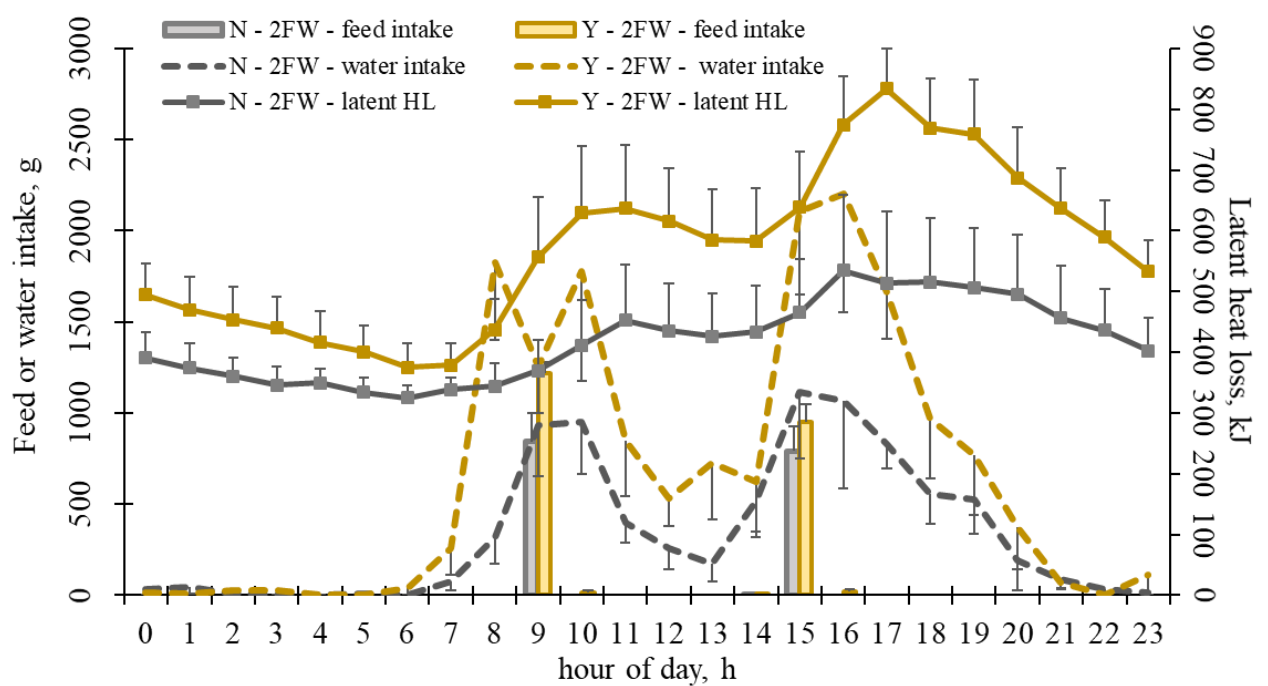


Figure 35. Hourly variations of feed intake, water intake, and latent heat loss capacity of 2FW pigs in high ambient temperature (28°C) (mean±SE). Data from Exp. 3 wherein non-supplemented pigs (N) are represented in grey and supplemented pigs (Y) are represented in yellow.

Mechanisms of LY supplementation in pigs subjected to HS

Improved insulin sensitivity modifies feeding behavior and thus improves feed intake.

High insulin in the blood has been reported to delay gastric emptying (Eliasson et al., 1995) thus we hypothesize that lower levels of circulating insulin needed to regulate blood glucose levels can attenuate HS effect on gastric emptying allowing pigs to eat within shorter inter-meal intervals (as observed in Exp. 3) or to increase meal frequency (Labussière et al., 2021). This theorized effect on gastric emptying may also help to explain why supplemented pigs with only 2 feeding windows (Y—2FW) were able to drink more water (**Figure 35**) and improve both feed intake and PD compared to their non-supplemented counterpart during HS (N—2FW). Furthermore, as mentioned in Exp. 3, duration of inter-meal intervals were similar among the supplemented groups (240 min) during HS which suggests supplemented pigs can maintain their ability to shortly start a new meal after the last one has ended regardless of the imposed feeding behavior. The indirect effect of LY on an increased meal frequency may thus have helped pigs drink more water, increase latent HL especially during or after a meal, return to a lower body temperature, and thus improve feed intake and energy efficiency (**Figure 34**; green box). Field observations reported by Lallemand and previous research work using the same yeast strain supports a better digestive transit in gestating sows and a higher feed intake in sows fed the live yeast during lactation (Chevaux et al., 2015; Domingos et al., 2021) which could imply similar mechanisms. However, an improved feed intake does not always translate to an improvement in PD thus there must be another mechanism aside from the increased meal frequency.

Improved insulin sensitivity improves thermoregulation responses thereby attenuating HS effects on PD.

We hypothesize that since ME intake was less correlated to PD during HS, then the effect of LY on PD must be related to mitigating the direct effect of heat exposure on the maximal potential for PD. It can thus be assumed that this effect might be related to improved thermoregulation effects caused by an improved insulin sensitivity that decreased severity of HS in LY supplemented pigs. Indeed, lower skin temperature were observed in supplemented pigs in a previous study (Labussière et al., 2021). Meanwhile, in our study, improved thermoregulation responses may have also allowed supplemented 8FW pigs in Exp. 3 (Y—

8FW) to maintain similar body temperature with non-supplemented counterparts (N—8FW) in HS conditions without as much reliance on an evaporative HL. Furthermore, Y—8FW were also able to deposit more protein during HS than the N—8FW despite having lower ME intake, suggesting attenuated HS effect on a reduced maximal potential for PD when LY is supplemented.

So how does LY improve insulin sensitivity?

In humans, LY supplementation has been reported to attenuate diabetic-related complications such as hyperglycemia (due to impaired insulin activity or production) by microbiota modulation and improved immune responses (Brandão et al., 2018; Albuquerque et al., 2019; Cunha et al., 2020). Moreover, yeast extract from *S. cerevisiae* has also been reported to potentiate insulin action (Edens et al., 2002). Possible mechanisms in pigs may be related to improved intestinal integrity, bile acid metabolism, and SCFA production as reported in LY supplemented pigs (Bontempo et al., 2006; Oswald et al., 2015; Gresse et al., 2021; Labussière et al., 2021; Bravo de Laguna et al., 2022) since these have been previously correlated to help improve insulin sensitivity (Caricilli and Saad, 2013; Khan et al., 2014). However, the exact mechanism by which LY supplementation improves insulin sensitivity (or at least potentiate insulin action) has not yet been established in pigs so far.

Possible sources of variation in responses among experiments

Similar to the discussed differences in HS severity among experiments, there were also variation in the effect of LY supplementation in pigs subjected to HS that seems to be related to both animal and environmental factors. Exp. 4 has demonstrated that the effect of LY supplementation during HS might be dependent on the age or BW and sex of the animal and its interactions. Improved responses to HS seem to be more consistent in LY-supplemented heavier entire male pigs as shown in Exp. 3, Exp. 4 (Replicate 2), and in a previous study (Labussière et al., 2021). This suggests that the beneficial effects of LY supplementation in high ambient temperature may be more important for more susceptible animals. Indeed, published literature on the improvement of HS responses by LY supplementation is usually in animals susceptible to HS due to high productivity or generally higher HP such as finishing pigs, lactating sows, and dairy cows (Brewster et al., 2017; Lee et al., 2019; Perdomo et al., 2020; Domingos et al., 2021; Labussière et al., 2021).

However, in this thesis project, variation in responses due to the animal factors might be confounded with the feeding management employed and the housing conditions. For example in Exp. 2, the effect of LY supplementation may not have been observed not only because female pigs were used but may be due to the pair-feeding management employed and to the unprecedented effect of the housing conditions and the experimental design on the feeding behavior of the *ad libitum* fed pigs. Indeed, the feeding management applied can be a source of variation. For example, differences in ME intake response were observed even between two LY supplemented groups such as the Y—2FW and Y—8FW (difference of $358 \text{ kJ} \cdot \text{kg BW}^{-0.60} \cdot \text{d}^{-1}$) in Exp. 3 using entire male pigs. When it comes to housing conditions, more consistent results were also observed in experiments in the respiratory chambers which seemed to elicit stronger HS responses than in larger experimental rooms. Similar results were observed in terms of improved feed intake, energy efficiency, and PD (or N retention) in supplemented HS pigs in the previous study in the respiratory chambers (Labussière et al., 2021) and with our results in Exp. 3 using the supplemented HS pigs with the unlimited FW. However, the degree of improvement may have been slightly different such as a higher feed intake improvement by LY in the aforementioned study wherein heavier entire male pigs were used (~100 kg vs. ~80 kg in our study). These variations suggest that the positive effects of LY supplementation during HS seem to be more evident when the stress or challenge is more severe.

Implications

Insulin is key to many metabolic processes especially in energy metabolism that is highly impacted by heat exposure. Although our results have demonstrated that feeding management strategies can help pigs to cope with HS, nutritional interventions that increase insulin sensitivity and increase latent HL capacity of pigs seem to be more efficient not only in helping pigs cope with HS but in helping them maintain their performance during HS. The actual mechanisms of how LY supplementation improve insulin sensitivity, how this increased insulin sensitivity improves pig performance during HS, and the seeming interaction with sex and/or severity of heat challenge can be venues for future research.

Conclusions

Conclusions

The main objectives of this thesis work were to contribute in bridging knowledge gaps in some aspects of heat stress in pigs such as

- 1) to investigate the effects of a chronic prenatal heat challenge on heat stress susceptibility during the growing-finishing period
- 2) to distinguish direct and indirect effects of heat stress on growth performance, hormones, and metabolism
- 3) to determine whether the positive effects of live yeast supplementation were caused by a modified feeding behavior
- 4) to evaluate interactions between live yeast supplementation and sex in HS pigs, and
- 5) to further understand the dynamic process of how body heat balance (body temperature) changes and is maintained throughout the day within a narrow range while ambient temperature is increasing.

The different heat challenge models used in this project demonstrated that many factors could influence pig's ability to cope with heat stress such as animal related factors (body weight, sex, feeding behavior as estimated by the alternation of feeding and fasting periods), the environment (housing conditions, the nature and the severity of the heat challenge), and even the interactions among them. Furthermore, it is not just the immediate surroundings but also the prenatal thermal environment which can affect pig's postnatal performance. This was demonstrated by the decreased ability of the pigs born from heat stressed sows to tolerate a postnatal heat challenge which has consequences on their growth performance and on their body composition. This effect of an altered body composition especially visceral adiposity was also shown to be a direct effect of elevated temperature on energy partitioning between lean and fat deposition.

By using continuous measurements of heat production and losses and of body temperature, our results have demonstrated how feeding behavior (meal characteristics and pattern) and its interaction with ambient temperature influences how the pigs try to maintain homeothermy throughout the day. In line with this, we have also demonstrated the importance of the latent heat loss pathway in maintaining thermal equilibrium in high ambient temperature, in particular during periods coinciding with high heat production (i.e., after a meal).

Data obtained from the thesis project highlights the importance of feeding strategies that stimulate water intake and rapid decrease in body temperature in order to improve feed

intake during heat stress. Live yeast supplementation was found to promote insulin sensitivity, modified feeding behavior, improved thermoregulation responses, and increased latent heat loss capacity of pigs. Thus, nutritional interventions that improve insulin sensitivity seem to be more efficient in helping pigs cope and maintain performance during heat stress.

Perspectives

Perspectives

Improving our knowledge about heat stress and how it deteriorates animal performance must be further understood in order to find ways to mitigate it. While there are increasing studies about prenatal heat stress in pigs, results are still unclear and sometimes inconsistent. Our study only considered a whole gestation heat stress but further studies are needed to determine other factors at play such as the most susceptible stage of pregnancy for the sow and the fetus, duration of a heat challenge (Boddicker et al., 2014), previous heat stress experiences of sires and sows (Gourdine et al., 2006; Rosé et al., 2017), and even the possible interaction with the sex of the progeny. Our results show that prenatal heat stress increase basal body temperature and this was hypothesized to be related to a modified set point due to exposition to increased cytokines in the fetal environment (Glover et al., 2010). However, literature suggests that there may also be an alteration of the energy metabolism in pigs as suggested by reports of a higher fasting heat production of *in utero* heat stressed pigs (Chapel et al., 2017), and higher insulin of pigs born from heat stressed sows and subjected to a postnatal heat challenge (Johnson et al., 2015b). Future research can look more into the possible effects of prenatal heat stress on the energy metabolism and insulin sensitivity of pigs and their ability to cope with a postnatal challenge.

Sexual dimorphic heat stress responses also have not yet been established in pigs. We speculate that, in growing pigs, entire males may be more susceptible due to their higher growth potential and heat production (Labussière et al., 2013; Ruiz-Ascacibar et al., 2017) when compared to females and surgically castrated males. Future studies are needed to determine how sex affects pig responses to heat stress not just in terms of its impact on growth but especially in terms of body composition, and taking into consideration its possible interaction with body weight and age. Furthermore, the effect of sex is usually studied using barrows as the male representative (Renaudeau et al., 2006b; Rudolph et al., 2021) but future studies should also consider both immunocastrated and entire male pigs especially with the increasing ban on surgical castration in the European Union. Research suggests sex effect on feeding behavior of pigs which might help explain their sensitivity to heat stress (Cross et al., 2020). Future studies on the effect of sex and feeding behavior during heat stress, probably along with hormonal differences might be needed.

Since maintaining body heat balance is a dynamic process, management strategies that involves improving heat losses (e.g., fogging, floor cooling, misting) only during periods with

high heat production (after a meal) might also warrant further studies especially on cost and energy savings point of view. In line with the timing, feeding during the night wherein there is relatively low metabolic rate (Ingram and Dauncey, 1985) can also be evaluated. While this has already been shown to improve lactating sow performance (Choi et al., 2019b), it has not yet been studied in growing pigs. In evaluating feeding management strategies, many factors can also be considered such as optimal inter-meal intervals that allows pigs to return to a lower body temperature but still minimize protein losses.

Many studies have postulated about the importance of improving insulin sensitivity in coping with heat stress (Rhoads et al., 2013; Sanz Fernandez et al., 2015b; Liu et al., 2017; Cottrell et al., 2020). Our results on the positive effects of live yeast supplementation exhibited its ability to improve insulin sensitivity but mechanism is still unknown and remains to be elucidated. One can imagine that this is the link or maybe one of the links between the gut microbiota modulation and modified feeding behavior by live yeast during heat stress (Labussière et al., 2021), especially since there have been reports of microbiota being linked with feeding behavior (He et al., 2022). Recent preliminary research suggests that *S. cerevisiae boulardii* CNCM-I 1079 can alter intestinal microbial bile acid metabolism in piglets, leading to an impact on Fibroblast Growth Factor19 expression which could explain postprandial attenuation of both energy expenditure and satiety signals (Bravo de Laguna et al., 2022). At the same time, there are still many gaps about the improvement of pig performance during heat stress that is driven by insulin sensitivity, one of which is gastric emptying. Effects of heat stress on the kinetics of gastric emptying and its highly likely relationship with feeding behavior can also be further studied.

Finally, since our results suggest that live yeast supplementation during heat stress seems to be more beneficial for susceptible animals, it can be interesting to evaluate precision feeding approaches that can provide feed to certain animals that are deemed susceptible to heat stress (e.g., based on BW, sex, growth potential). Since precision feeding systems can also provide feeding behavior data, it would be interesting in the future to set up a system that can detect changes in the feeding behavior patterns of the pigs that signal heat stress (or any perturbation) and couple them with tailored solutions to each individual pig (e.g. provision of live yeast supplemented feed).

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Annex

Annex

Annex 1. Scientific communication no. 1 from Exp. 1

Serviento, A. M., Merlot, E., Prunier, A., Quesnel, H., Louveau, I., Lebret, B., and Renaudeau, D. 2019. *Heat stress in pregnant sows: effects on growth performance and carcass composition of the offspring*. In: Energy and protein metabolism and nutrition Vol. 138. Presented at 6. International Symposium on Energy and Protein Metabolism (ISEP), Belo Horizonte, Brazil, September 9-12, 2019. (Oral presentation)

Abstract

Climatic environment is one of the main limiting factors of production efficiency in the swine industry especially in tropical countries. The aim of this study was to evaluate the effects of heat exposure during most of gestation on the growth performance and carcass composition of the offspring. A total of 24 mixed parity sows pregnant sows were housed at thermoneutrality (PTN, 18-24 °C, n=12) or in HS conditions (PHS, 28-34 °C, n=12) from the 7th to the 106th day of gestation. At 70 d of age, 48 females from the 24 litters were selected, moved to two climatic chambers and exposed to TN (18-24 °C) or HS (28-34 °C) conditions. Prenatal HS did not impair growth performance. Postnatal HS reduced by 15 and 11% the average daily feed intake and BW gain, respectively. Changes in lean and fat tissue accretion were observed only when pigs were exposed to both the prenatal and postnatal HS.

Keywords: Prenatal heat stress, pig, growth, carcass composition

Introduction

Climatic environment is one of the main limiting factors of production efficiency in the swine industry especially when ambient temperature is above the zone of thermal comfort. Although thermal heat stress (HS) is an occasional problem in temperate countries during the 2 to 3 summer months and/or during hot spells, it is a more permanent problem in many tropical countries where pig production has grown at a high rate during the last two decades. Reproductive sows are very susceptible to HS. These effects include anoestrus, delayed puberty, reduced farrowing rates, increase in abortion rate and reduced litter size (Prunier et al., 1996). In addition, HS during pregnancy can induce structural and functional changes in the foetus that may persist through the productive life with subsequent effects on growth

performance and body composition. The aim of this study was to evaluate the effects of heat exposure during almost whole gestation on the growth performance and carcass composition of the offspring.

Material and methods

A total of 24 mixed parity pregnant sows were housed at thermoneutrality (PTN, 18-24 °C, n=12) or in HS conditions (PHS, 28-34 °C, n=12) from the 7th to the 106th day of gestation. From the 107th of gestation to the 28th days of lactation, all sows and their progeny were housed at 24 °C. At 70 d of age, 48 females from the 24 litters were selected based on the climatic environment during pregnancy and transferred to the two climatic rooms (8 pens/room, 3 pigs/pen, 12 pigs/temperature treatment during pregnancy). After a 7-d adaptation period, the first room was maintained at an ambient temperature of 18-24 °C (GTN) during all the growing period. The second room (GHS) was kept at 18-24 °C for 7 d (P1) and thereafter at 28-34 °C for 56 d (P2). During the experimental period, feed and water were offered ad libitum, Pigs were weighted weekly and feed intake was measured daily. All the pigs were slaughtered at 140 d of age for measurements of carcass traits. Data were analysed with a mixed model including the effects of temperature during pregnancy (2 levels), temperature during growth period (2 levels) and interactions.

Results and discussion

Table 1. Effects of climatic conditions (TN vs. HS) during pregnancy (P) and growing periods (G) on pig performance (least square means)^{1,2}

	GTN		GHS		RMSE	Statistics
	PTN	PHS	PTN	PHS		
No. of pigs	12	12	12	12		
Period 1						
Initial BW, kg	35.2ab	37.1a	36.4a	33.4b	3.2	G×P*
ADG, g/d	913	933	983	886	183	
ADFI, g/d	1947	1938	2043	1896	152	
FCR	1.81	1.79	1.73	1.79	0.12	
Period 2						
Initial BW, kg	41.6ab	43.6a	43.2a	39.6b	3.7	G×P*
Final BW, kg	104.8	102.4	98.1	92.9	7.0	G**
ADG, g/d	1124	1051	985	942	99	G**
ADFI, g/d	2615	2694	2257	2250	90	G***
FCR	2.33	2.35	2.28	2.31	0.08	

¹ADFI = average daily feed intake (adjusted for a DM of 88.0%); ADG = average daily gain; BW = body weight; FCR = feed conversion ratio (adjusted for a DM of 88.0%).

²BW and ADG data were analysed with a model including the effects of the temperature treatment during pregnancy (PTN vs PHS) and growing period (GTN vs GHS), the interaction, and litter origin. The pen effect was considered as a random effect. The experimental unit was the animal. ADFI and FCR data were analysed with a model including the effects of the temperature treatment during pregnancy (PTN vs PHS) and growing period (GTN vs GHS), interactions, and litter origin. The experimental unit was the pen within each room.

The interaction between prenatal and postnatal HS effect was significant for initial BW in period 1 and 2 ($P < 0.05$, Table 1). In P1, growth performance was similar for the PTN and the PHS groups. These results are in agreement with those previously reported by Boddicker et al., (2014) and Johnson et al. (2015). In P2, elevated temperature significantly reduced growth performance whatever the prenatal temperature treatment. The FCR did not change in HS conditions (2.31 kg/kg on average; $P = 0.529$). At slaughter, hot carcass weight and length were significantly lower in postnatal HS conditions (95.2 vs 105.1 kg and 94.3 vs 96.7 cm, respectively; $P < 0.05$). However, the carcass composition was similar ($P > 0.05$) in the GTN and GHS groups and in the PTN and the PHS groups. When data were adjusted for a same slaughter BW, carcass lean percentage was numerically lower ($P = 0.109$) and backfat thickness was higher ($P = 0.012$) in the GHS-PHS group. The results of the present study confirm the negative effects of postnatal HS on ADFI and ADG but show that prenatal HS did not impair to impact growth performance. Changes in lean and fat tissue accretion were observed only when pigs were exposed to both the prenatal and postnatal HS.

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Acknowledgements

This project was funded by INRA (PHASE Divison).

Annex 2. Scientific communication no. 2 from Exp. 2

Serviento, A.M., Labussière, E., Castex, M., & Renaudeau, D. 2020. *Effect of heat stress and of feed provision modification on growth and thermoregulation in pigs*. In: Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science. Presented at the EAAP 2020 Virtual meeting, December 1 - 4, 2020. (Oral presentation)

Abstract

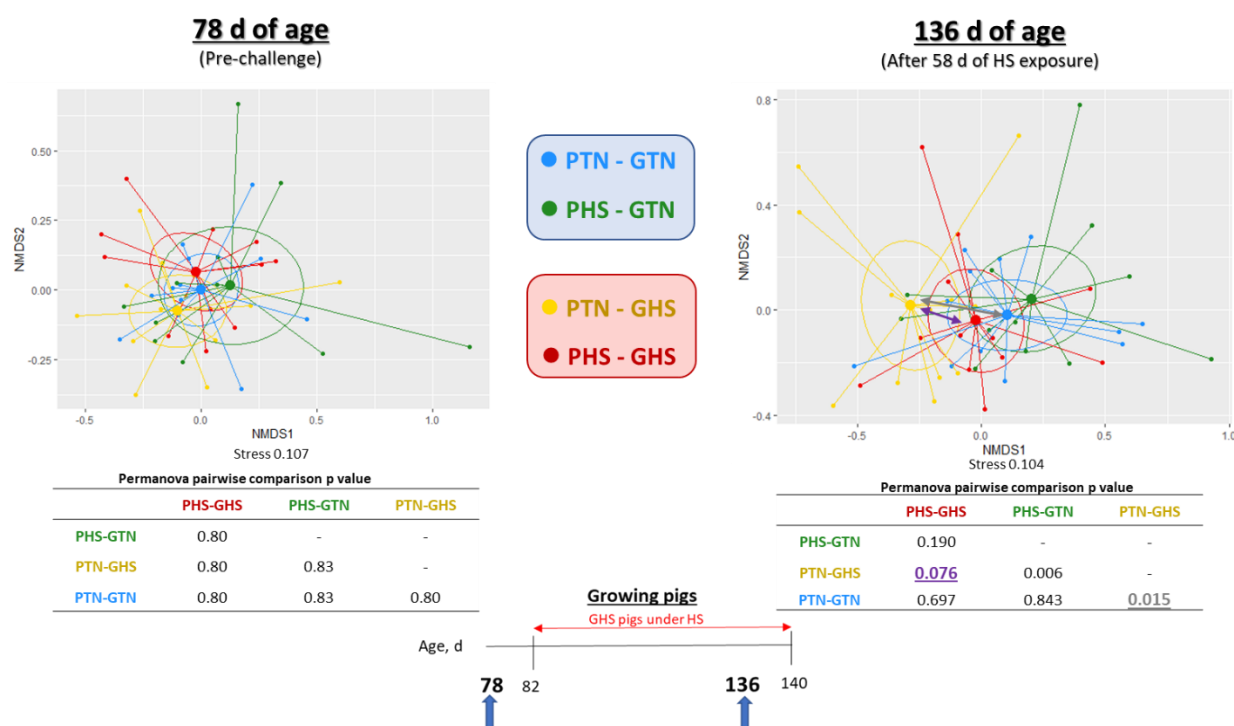
With the increasing global mean temperature and more frequent summer heat waves, there is a need to find techniques to mitigate heat stress (HS) consequences on pig production. The aim of the study was to evaluate the effect of HS and of increasing feed provision frequency on performance and thermoregulation responses in finishing pigs. A total of 48 pigs (66.1 ± 1.7 kg) were allocated to 4 experimental groups in 3 replicates. After being housed at thermoneutrality (TN; 22°C) during 7 days (P1), pigs were subjected to either TN or HS (32°C) conditions during 20 days (P2). In each environment, the diet (14.9% CP, 9.64 MJ NE/kg) was provided either ad libitum (AL; 2 distributions/day) or pair-fed (PF8; 8 distributions/day) with HS pigs fed-AL as the reference group. Thus, there were four experimental groups: TN-AL, HS-AL, TN-PF8, and HS-PF8. In PF8 groups, the calculated daily ration was divided into 8 feed provisions distributed at every 90-min intervals from 09h00 to 19h30. In P2, HS-AL and PF8 pigs had lower ADFI (-19% on average; $P < 0.01$), ADG (-25% on average; $P < 0.01$), and a reduced final BW (-6.1 kg on average; $P < 0.01$) than TN-AL pigs. Rectal temperature was increased by HS ($+0.32^\circ\text{C}$ on average; $P < 0.05$) but did not significantly differ within HS nor within TN groups ($P > 0.05$). The PF8 groups had lower total viscera percentage than TN-AL pigs (-2.1% on average; $P > 0.05$), while HS-AL had intermediate results. Pigs in HS-AL and in PF8 groups had similar slaughter BW ($P < 0.05$) but HS-PF8 had more perirenal fat than TN-PF8 ($+1.6\%$; contrast at $P < 0.05$). Plasma insulin on d 8 of P2 was higher in HS-PF8 than in TN-PF8 pigs ($P < 0.05$). Heat stressed pigs had lower plasma TT3 ($P < 0.05$) even compared to TN-PF8 with the same feeding level. Plasma creatinine was higher in HS pigs within AL ($P < 0.05$) and within PF8 groups ($P < 0.05$). In AL groups, reduced growth performance in HS pigs was mostly due to a reduced feed intake. Increasing feed provision frequency during HS did not seem to improve pig performance. However, the increased fat deposition in internal depot in HS-PF8 could be an adaptation response related to direct effect of HS on pig metabolism.

Annex 3. Scientific communication no. 3 from Exp. 1

Serviento, A.M., Renaudeau D., and Zemb O. 2021. *Effect of heat stress on fecal microbiota composition of pregnant sows and of their female offspring*. In: Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science. Presented at the EAAP 2021 Davos, Switzerland, August 30 – September 3, 2021. (Oral presentation)

Abstract

The objective of this study was to evaluate the impact of chronic heat stress (HS) on microbiota composition of gestating sows and of their female offspring. There were two environmental conditions: thermoneutral (TN; cyclic 18 to 24 °C) and HS (cyclic 28 to 34 °C). Pregnant sows were exposed to either TN (PTN; n=12) or HS (PHS; n=12) conditions from d 9 to 109 of gestation. During the finishing period, two female offspring per sow were allotted to either a growing TN (GTN; n=24) or HS (GHS; n=24) environment. All pigs were fed the same diet all throughout the experiment. From 75 to 140 d of age, GTN pigs (n=24) were in TN conditions, while GHS pigs (n=24) were in TN conditions from 75 to 81 d of age and in GHS conditions from 82 to 140 d of age. Spot faecal samples were collected on d 4 and d 105 of gestation in the sows, and on 78 and 136 d of age in the growing pigs for 16S rRNA analyses of microbiota composition. In both sows and offspring, exposure to HS increased rectal (+0.16 and +0.30 °C, respectively; $P<0.01$) and skin (+2.44 and +1.61 °C respectively; $P<0.01$) temperatures. Firmicutes and Bacteroidetes were the most dominant phyla (~98%) in the samples collected pre-challenge regardless of physiological stage but sows had higher percentage of Firmicutes (+20.4%; $P<0.01$) and lower percentage of Bacteroidetes (-20.2%; $P<0.01$) than the growing pigs. Using a permutational multivariate analysis of variance on the environment effect on microbiota, chronic HS exposure did not significantly affect sow microbiota at d 105 ($P=0.120$). Microbiota from the pigs born from PHS sows was resistant to a further heat stress during the finishing ($P=0.167$), while microbiota of pigs born from the PTN sows were strongly affected ($P=0.027$). Therefore, the prenatal environment of the growing pigs affect how their microbial composition change upon exposure to a thermal stress during their postnatal life. The difference in the effect of heat exposure on gut microbiota of sows and of growing pigs may be related to their microbial composition or to the duration of their thermal stress exposure.



Annex figure 1. Non-metric multidimensional scaling (NMDS) analysis using Bray-Curtis distances of samples according to pregnancy (PTN vs. PHS) and growing (GTN vs. GHS) environment.

Permutational multivariate analysis of variance (PERMANOVA) pairwise test was used to detect significance in the distance between each group. Fecal samples were collected from growing pigs (as described in Chapter 1) before and after a chronic HS challenge on d 78 and 136 d of age, respectively.

Annex 4. Growth and feeder presence of the eight experimental groups in Exp. 2

Annex table 1. Effect of heat stress, live yeast, and feeding management on growth performance of finishing pigs in Experiment 2.¹

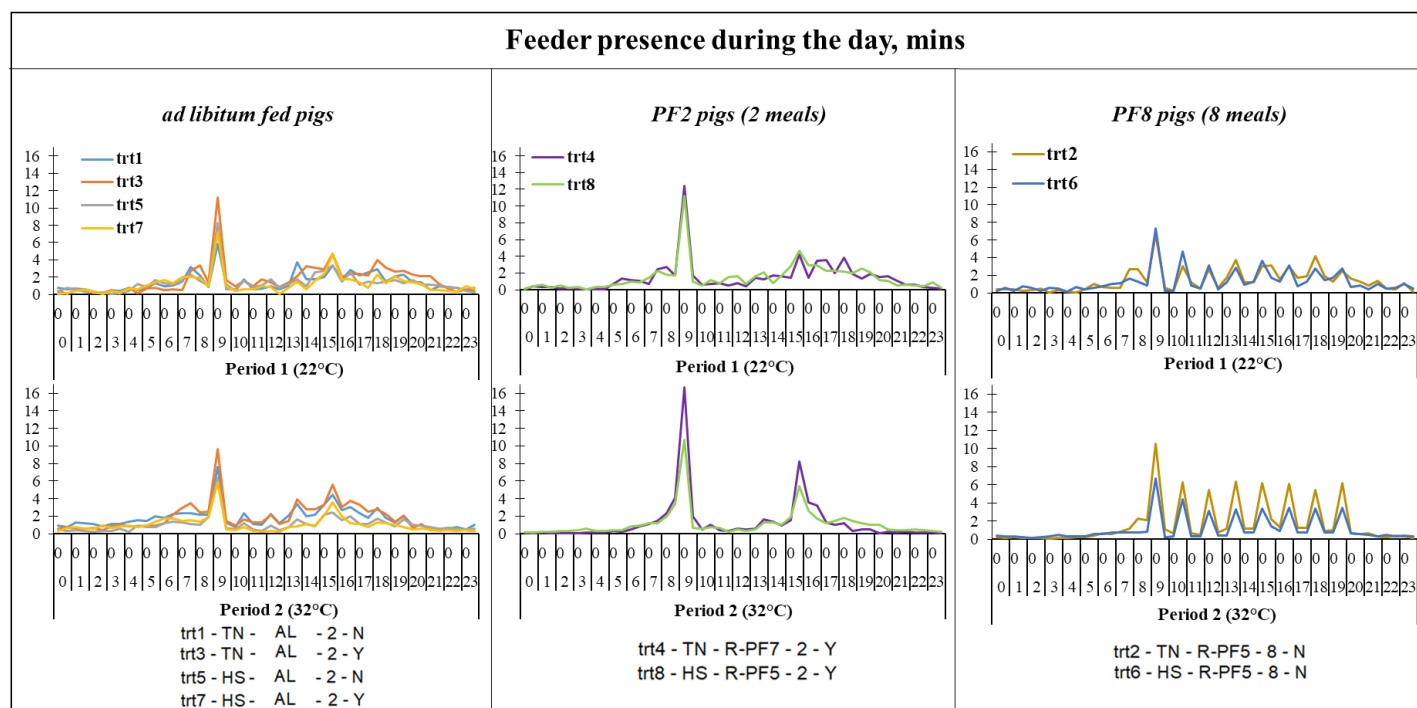
Items	TN				HS				RSD ²	Statistics ³
	N-AL	N-PF8	Y-AL	Y-PF2	N-AL	N-PF8	Y-AL	Y-PF2		
	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8		
Animals, n										
Live BW, kg										
d -7	66.1	66.0	66.3	66.1	65.8	67.0	65.7	66.3		R**, D**, G×D**, R×G×D**
d 0	74.5	73.8	74.2	73.6	73.5	74.6	74.2	73.9	1.4	
d 20	97.6 ^a	91.7 ^{bc}	96.2 ^{ab}	91.7 ^{bc}	91.6 ^{bc}	90.9 ^{bc}	92.5 ^{bc}	89.3 ^c		
ADFI, g·d ⁻¹										G**, P**, R×G ^T , G×P**, R×G×P**
Period 1	2603	2514	2648	2581	2643	2669	2568	2600	132	
Period 2	2927 ^a	2402 ^b	2939 ^a	2421 ^b	2376 ^b	2364 ^b	2417 ^b	2293 ^b		
ADFI per BW ^{0.60} , g·kg BW ^{-0.60} ·d ⁻¹										R**, G**, P**, R×G*, G×P**, R×G×P ^T
Period 1	203	197	206	202	208	207	202	204	9.6	
Period 2	202 ^a	170 ^b	204 ^a	171 ^b	169 ^b	167 ^b	170 ^b	164 ^b		
ADG, g·d ⁻¹										R**, G**, P**, R×G ^T , G×P**
Period 1	1198 ^{ab}	1115 ^{abc}	1124 ^{abc}	1067 ^c	1105 ^{abc}	1092 ^{bc}	1222 ^{aa}	1079 ^{bc}	133	
Period 2	1156 ^a	897 ^{bc}	1101 ^a	905 ^b	905 ^b	817 ^{bc}	915 ^b	773 ^c		
FCR										
Period 1	2.19 ^{ab}	2.28 ^{abc}	2.42 ^{bc}	2.44 ^{bc}	2.45 ^{bc}	2.48 ^{bc}	2.12 ^a	2.49 ^c	0.30	G**, P**, R×G**
Period 2	2.55 ^a	2.70 ^b	2.67 ^{ab}	2.73 ^b	2.63 ^a	2.91 ^b	2.64 ^a	3.01 ^c		

¹All pigs were housed at thermoneutrality (Period 1; d -7 to -1) before being subjected to either thermoneutral (TN) or heat-stressed (HS) conditions (Period 2; d 0 to 19). In each environment, diet was provided either *ad libitum* (AL; 2 feed provisions/day) or pair-fed with two (PF2) or eight (PF8) feed provisions/day). N: non-supplemented diet. Y: live yeast supplemented diet.

²Residual standard deviation.

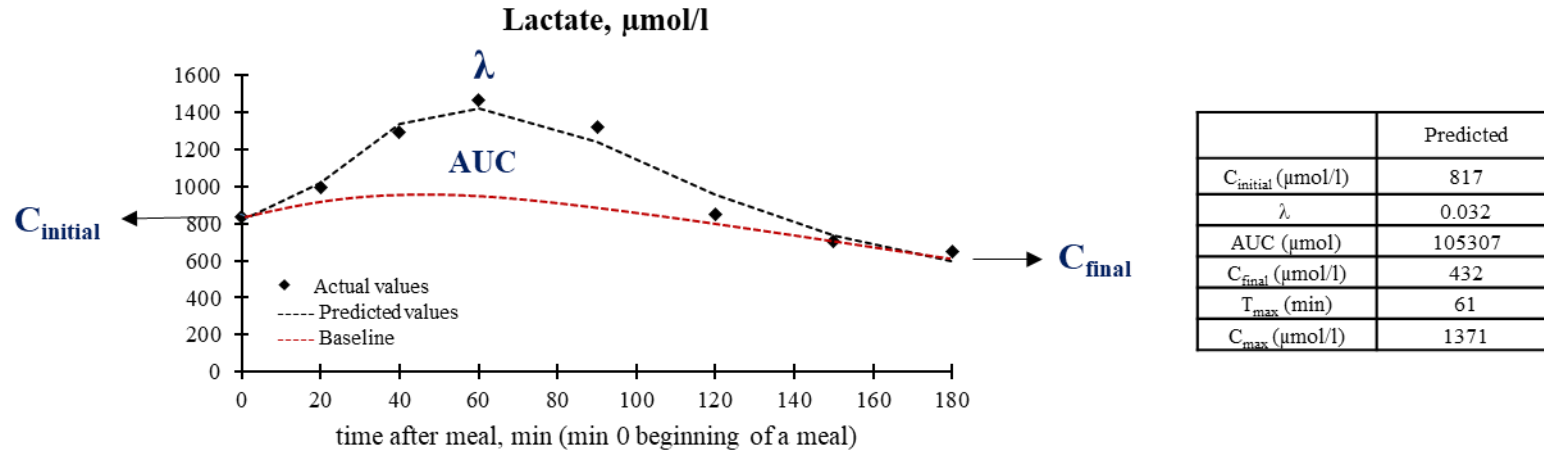
³The pig was considered as the experimental unit. Data was analyzed using PROC MIXED model with replicate (R), experimental group (G), day (D) or period (P), their interactions as fixed effects. * $P < 0.05$, ** $P < 0.01$. ^{a,b,c}LSmeans within a row with different superscripts letters differ ($P < 0.05$) according to the experimental group

Annex 4. Continuation...



Annex figure 2. Presence at the feeder (min) of finishing pigs according to the environmental treatment, feeding management, and live yeast supplementation. From d-7 to d-1 (P1), pigs were housed at thermoneutrality and then from d 0 to 21 (P2), either in thermoneutral (TN; 22°C) or in heat-stressed (HS; 32°C) conditions. In each environment, the diet (N: non-supplemented; Y: live yeast supplemented) was provided either ad libitum (AL; 2 distributions/day) or pair-fed with two (PF2) or eight (PF8) distributions/day. Data included are from d 6 to 19 of the experimental period with d 0 as the transition period of HS groups from TN to HS conditions.

Annex 5. Supplementary information of Chapter 3



Supplementary figure 1. Example of a fitted data into a non-linear model using postprandial plasma lactate concentration of a pig in experimental group N—Unli during Period 1 sampled on the 12th of February 2021. The black dashed line (---) corresponds to the predicted values at minute (time; t) obtained with the equation: $C(t) = C_{final} + (C_{initial} - C_{final}) \exp(-\lambda t) + \frac{1}{2} AUC \lambda^3 t^2 \exp(-\lambda t)$ adapted from (van Milgen et al., 2022) from and the red dashed line (---) represents the baseline considering the dynamics of the change in the steady state concentration from $C_{initial}$ to C_{final} . $C_{initial}$: initial or preprandial concentration, C_{final} : target postprandial concentration, λ : shape factor of the curve, AUC: area under the curve and above the dashed red line; C_{max} : maximum concentration; and T_{max} : time in min at C_{max} .

Annex 5. Continuation...

Supplementary table 1. Parameter estimates describing the postprandial kinetics of plasma insulin plasma metabolites of entire male finishing pigs exposed to high ambient temperature.^{1,2,3}

Items		N- Unli	Y- Unli	N- 2FW	Y- 2FW	N- 8FW	Y- 8FW	RSD ⁴	Statistics
Insulin, μU/mL									
C _{initial}	Period 1 [#]	3.49	7.51	0.74	2.20	2.67	2.35	2.20	FW×P*, D×FW×P*
	Period 2	3.81	0.88	3.03	3.05	1.50	4.35		
λ	Period 1 ^{×#}	0.068	0.108	0.044	0.044	0.054	0.066	0.021	FW ^T , D×P ^T , FW×P*
	Period 2	0.058	0.050	0.058	0.057	0.061	0.053		
AUC	Period 1 ^{+ §}	2051	544	5885	2590	2234	1134	2103	FW*
	Period 2 ^{+§}	1327	4245	5853	2270	1609	1693		
C _{final}	Period 1	10.8	16.1	12.7	10.6	6.9	8.6	6.8	ns
	Period 2	9.4	2.7	8.9	14.2	11.5	4.7		
C _{max}	Period 1 ^{+§}	49.4	31.3	84.6	39.1	33.5	25.3	17.0	FW*
	Period 2 ^{+§}	31.2	60.0	82.4	43.8	38.6	26.6		
T _{max}	Period 1 [#]	30.7	20.5	47.2	48.2	43.2	35.4	14.2	FW*
	Period 2	34.8	40.3	44.7	37.7	33.3	40.1		
Glucose, mg/L									
C _{initial}	Period 1	963	970	883	929	925	940	68	ns
	Period 2 [×]	1039	874	893	952	896	953		
λ	Period 1	0.017	0.025	0.014	0.017	0.017	0.014	0.035	ns
	Period 2	0.017	0.025	0.014	0.017	0.017	0.014		
AUC	Period 1	11901	5448	9774	11110	9278	5003	6554	ns
	Period 2	8214	13318	11955	11870	11945	10053		
C _{final}	Period 1	943	925	1008	961	955	972	75	P ^T
	Period 2	1015	980	1022	1022	982	1005		
C _{max}	Period 1	1227	1092	1208	1120	1146	1098	47	P**
	Period 2	1249	1253	1258	1225	1243	1233		
T _{max}	Period 1	22.6	18.4	26.9	34.7	26.3	20.5	11.6	FW ^T
	Period 2	20.1	26.5	31.1	29.5	27.9	24.9		

Annex 5. Continuation...

Items		N- Unli	Y- Unli	N- 2FW	Y- 2FW	N- 8FW	Y- 8FW	RSD ⁴	Statistics
Insulin :Glucose, μU/g									
C _{initial}	Period 1 [#]	3.90	7.48	0.71	3.21	3.12	2.56	1.97	FW×P*, D×FW×P*
	Period 2	3.98	1.22	3.04	3.62	2.07	4.51		
λ	Period 1 [§]	0.094	0.063	0.041	0.040	0.057	0.046	0.02	FW×P*
	Period 2	0.048	0.048	0.057	0.054	0.058	0.051		
AUC	Period 1 ^{+ §}	1696	697	5755	2256	2293	1277	1791	FW ^T
	Period 2 [†]	1391	3621	4889	1969	1304	1410		
C _{final}	Period 1	10.99	16.08	10.64	10.09	5.83	7.96	6.23	ns
	Period 2	7.75	3.83	7.94	12.85	11.45	4.71		
C _{max}	Period 1 [§]	39.5	29.8	74.6	32.0	30.0	24.3	11.7	FW*, D×P*
	Period 2 [§]	26.6	50.7	66.2	37.0	32.0	22.0		
T _{max}	Period 1 [#]	33.2	26.3	51.0	54.1	47.6	40.5	14.8	FW ^T
	Period 2	41.9	42.1	45.7	39.3	34.8	41.6		
Lactate, μmol/L									
C _{initial}	Period 1 [‡]	788	870	928	936	969	763	97	D×FW×P**
	Period 2	831	752	933	797	822	966		
λ	Period 1	0.042	0.032	0.028	0.031	0.035	0.036	0.007	FW*
	Period 2 [§]	0.042	0.038	0.029	0.032	0.034	0.041		
AUC	Period 1 [†]	68181	115145	161516	83168	116839	95949	40930	ns
	Period 2	67267	121939	128668	107366	108418	82191		
C _{final}	Period 1	762	446	392	592	435	485	240	ns
	Period 2	714	406	626	607	493	639		
C _{max}	Period 1 ^{*†}	1516	1240	1597	1197	1469	1370	163	P*, D× P**
	Period 2	1450	1651	1576	1448	1466	1485		
T _{max}	Period 1	48.7	73.4	72.3	71.3	59.3	57.3	12.6	FW*
	Period 2 [§]	48.9	52.9	73.6	64.8	59.4	50.1		

Annex 5. Continuation...

Items		N- Unli	Y- Unli	N- 2FW	Y- 2FW	N- 8FW	Y- 8FW	RSD ⁴	Statistics
α -amino N, mg/L									
C_{initial}	Period 1 [#]	579	693	547	514	566	525	56	FW*, D×FW×P ^T
	Period 2 [#]	647	592	557	535	585	597		
λ	Period 1	0.041	0.024	0.034	0.039	0.032	0.029	0.011	ns
	Period 2	0.040	0.045	0.032	0.025	0.031	0.035		
AUC	Period 1	18240	31164	25471	15261	36914	31920	17323	P ^T
	Period 2	10270	11602	16195	19108	22907	16553		
C_{final}	Period 1	629	671	647	583	520	528	129	FW ^T
	Period 2	675	731	642	574	482	610		
C_{max}	Period 1 [†]	818	878	847	685	812	780	22	P*
	Period 2	779	873	761	714	671	718		
T_{max}	Period 1	50.5	91.5	63.2	60.7	64.9	72.1	31.5	ns
	Period 2	53.8	45.1	77.3	69.0	64.3	94.5		

¹A total of 27 pigs were allocated to 6 experimental groups in 9 replicates with 2 blocks per replicate. All pigs were housed at 22°C ambient temperature from d -5 to -1 (Period 1 and then at 28°C from d 0 to 4 (Period 2). Serial blood sampling was done on d -2 (P1) and on d 1 (P2). Plasma kinetic profile per variable were then analyzed using non-linear regression using a modified erlang model that was parameterized to estimate C_{initial} , λ , AUC and C_{final} (Supplementary figure 1).

²The predicted values for each parameter per pig and per period from the non-linear regression were analyzed using PROC MIXED model with feeding window (FW), diet (D), period (P), and their interactions as fixed effects, and with pig as a repeated unit per period. **P < 0.01, * P < 0.05, ^TP < 0.10.

³Contrast statements within period (P<0.05): * [N—Unli, N—2FW, N—8FW] vs. [Y—Unli, Y—2FW, Y—8FW]; * (N—Unli vs. Y—Unli); [†] (N—2FW vs. Y—2FW); and [‡] (N—8FW vs. Y—8FW) for diet effect. [#] [N—Unli, Y—Unli] vs. [N—2FW, Y—2FW]; ^{||} [N—Unli, Y—Unli] vs. [N—8FW, Y—8FW]; [§] [N—2FW, Y—2FW] vs. [N—8FW, Y—8FW] for FW effect.

⁴RSD: residual standard deviation.

References:

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Synthèse de la thèse en français

Synthèse de la thèse en français

Résumé

Avec l'augmentation de la température mondiale et des vagues de chaleur plus fréquentes, le stress thermique est un problème pour la production porcine dans de nombreux pays. La diminution des performances de croissance est liée à la fois à une réduction de la consommation alimentaire mais également à des effets directs sur la physiologie des animaux. Ce travail de thèse vise à mieux comprendre : 1) l'effet d'un stress thermique prénatal chronique sur la tolérance des porcs à la chaleur, 2) les effets directs et indirects de l'exposition à la chaleur, 3) les stratégies d'alimentation qui peuvent aider les porcs pendant le stress thermique, et 4) explorer l'idée que le maintien de l'équilibre thermique est un processus dynamique qui varie tout au long de la journée. Ce projet de thèse a confirmé qu'un déséquilibre entre la production et la perte de chaleur (charge thermique) est à l'origine de la réduction des performances de croissance et démontre que la chaleur entraîne une altération du métabolisme énergétique, des réponses physiologiques et une modification de la composition corporelle. Ce dernier effet est exacerbé chez les porcs exposés à un challenge thermique prénatal chronique. L'amélioration de la tolérance à la chaleur chez les porcs dépend de leur capacité à maintenir un équilibre dynamique entre la production et les pertes de chaleur au cours d'une journée, en particulier pendant et après un repas. Nos résultats montrent également l'importance de la consommation d'eau dans l'amélioration des pertes de chaleur par évaporation et plus généralement dans la tolérance à la chaleur. Les effets de stratégies d'alimentation ont également été évalués. Nos travaux montrent que la supplémentation en levures vivantes améliore la tolérance à la chaleur chez le porc via une modification de la sensibilité à l'insuline et de l'efficacité avec laquelle les animaux perdent de la chaleur. Ces réponses se traduisent par une augmentation de l'efficacité énergétique et de la capacité à déposer des protéines corporelles au chaud.

Mots clés : stress thermique, porcs, métabolisme énergétique, thermorégulation, comportement alimentaire, levures vivantes

Introduction

Le changement climatique reste l'un des défis majeurs du 21^{ème} siècle, en particulier avec des vagues de chaleur plus fréquentes et plus sévères, et une température mondiale qui devrait augmenter d'au moins 1,5°C entre 2030 et 2050 (IPCC, 2018). Cela constitue un challenge pour la production animale dans de nombreux pays en raison de ses conséquences sur la santé et le bien-être des animaux et sur la quantité et la qualité des ressources telles que les ressources utilisées pour l'alimentation animale (Rojas-Downing et al., 2017; Lacetera, 2019; Renaudeau and Dourmad, 2021) et particulièrement pour la production porcine en raison de sa demande croissante et de sa croissance dans les pays subtropicaux et tropicaux (OECD-FAO, 2021).

Comme la plupart des animaux d'élevage, les porcs sont considérés comme des animaux homéothermes qui régulent leur température corporelle centrale dans une plage étroite en maintenant un équilibre entre la production et la perte de chaleur. Les porcs sont notamment sensibles au stress thermique (HS), non seulement en raison de leur incapacité à transpirer (Ingram, 1967), mais aussi parce que la sélection d'une croissance maigre plus rapide a entraîné une production de chaleur métabolique plus élevée chez les porcs modernes (Brown-Brandl et al., 2014). Dans des conditions chaudes, le maintien de la température corporelle par des ajustements physiologiques et/ou comportementaux réduit généralement les traits économiquement importants liés à la reproduction et à la production (DeShazer et al., 2009; Renaudeau et al., 2012). Le HS est donc l'un des principaux facteurs limitants de la production porcine, en particulier avec les pertes économiques associées à la réduction de la croissance, de l'efficacité alimentaire, et de la qualité de la carcasse chez les porcs en croissance et à la réduction des performances de reproduction chez les truies, entre autres (Mayorga et al., 2019; Liu et al., 2021). Alors que l'effet le plus largement rapporté du HS chez les porcs en croissance est la réduction de la consommation alimentaire qui se traduit par une réduction de la croissance (Renaudeau et al., 2011), des études de la dernière décennie ont démontré qu'il existe d'autres conséquences à prendre en compte, telles que effets directs de l'exposition à la chaleur et même de l'environnement thermique prénatal des porcs (Ross et al., 2017; Johnson and Baumgard, 2019; Liu et al., 2021). En effet, afin de développer des stratégies pour atténuer ses effets de stress, les mécanismes par lesquels les porcs maintiennent l'équilibre thermique et par lesquels le HS détériore les performances des animaux doivent être mieux compris.

Par conséquent, l'objectif général de ce projet de thèse est de contribuer aux connaissances sur le HS chez les porcs. Ainsi, la thèse vise à déterminer comment un stress thermique prénatal peut influencer la réponse d'un porc à un stress thermique postnatal et à distinguer les effets directs de l'exposition à la chaleur (i.e. ceux qui ne sont pas liés à la fois à la consommation et au comportement alimentaire). De plus, le projet vise également à comprendre comment les porcs maintiennent l'équilibre thermique au cours de la journée, en particulier celui lié au comportement alimentaire. Enfin, les stratégies d'alimentation (i.e. la modification de la fréquence d'alimentation et la supplémentation en levures vivantes) sont évaluées afin d'atténuer les effets du HS chez les porcs.

Sommaire de la revue de la littérature

Des études dans la littérature ont démontré que quelles que soient les conditions environnementales, les porcs essaient de maintenir une température corporelle constante propice aux processus biologiques. Le non-respect de cette consigne peut entraîner des conséquences négatives dues à la chaleur et, dans des cas extrêmes, la mort. Au cours d'un challenge thermique, les animaux maintiennent un équilibre thermique via des ajustements biochimiques, physiologiques et/ou comportementaux appelés collectivement réponses de thermorégulation. L'exposition à une température ambiante élevée limite leur capacité pour dissiper la chaleur, perturbant ainsi l'équilibre thermique. Lorsque leur charge thermique dépasse leur capacité de dissipation thermique et qu'ils ne peuvent plus maintenir leur température corporelle, ils entrent dans un état du stress thermique (HS). La diminution de la croissance lors d'un HS est principalement liée à la réduction de la consommation alimentaire puisqu'il s'agit de la principale réponse d'adaptation du porc pour limiter la production de chaleur.

Des études récentes ont montré que ce n'est pas seulement l'environnement immédiat mais aussi l'environnement thermique prénatal qui affecte les performances des porcs en termes de température corporelle centrale, de composition corporelle, et de leurs réponses à un HS postnatal. Cependant, les résultats sont incohérents, peut-être en raison des différences de durée et de stade du challenge prénatal d'une étude à l'autre. Un nombre croissant d'études a également examiné les effets directs de l'exposition à la chaleur (non liés à la consommation alimentaire), comme l'augmentation de l'adiposité et la diminution de la flexibilité métabolique. Ces travaux ont utilisés des stratégies d'alimentation appelées « pair-feeding ». Dans ce modèle, un groupe dans un environnement thermoneutre (TN) est rationné sur le niveau de consommation de ses congénères exposés à la chaleur. Cependant, il est difficile d'évaluer quels effets sont dus à l'exposition à la chaleur et lesquels sont confondus avec la stratégie d'alimentation (nombre de repas, heures de distribution, etc.).

Le niveau d'alimentation et le comportement contribuent largement aux pics instantanés de la production de chaleur (HP) dus à l'activité et à l'effet thermique du repas. Ces variations de HP et de température corporelle impliquent que le maintien de l'équilibre thermique est un processus dynamique tout au long de la journée. Pourtant, la plupart des stratégies nutritionnelles visant à atténuer les effets du HS se concentrent principalement sur la réduction

de la charge thermique globale (par exemple, l'amélioration de l'utilisation de l'énergie ou la réduction de l'augmentation de la HP). Il n'y a que quelques études sur les porcs qui étudient les stratégies de l'alimentation (par exemple, la fréquence de l'alimentation). Cependant, il a été récemment démontré que la supplémentation avec des levures vivantes (LY) modifiait le comportement alimentaire des porcs et des vaches laitières. Ces modifications du comportement alimentaire pourraient expliquer pourquoi les animaux résistent mieux à la chaleur comparativement à ceux ne recevant pas de levure.

Il existe certainement de nombreux autres éléments concernant le HS qui doivent être compris. L'objectif principal de ce projet de thèse est de contribuer aux connaissances sur certains aspects émergents de l'HS chez les porcs en quatre parties :

1. Étudier les effets d'un HS prénatal chronique sur la croissance, la composition corporelle et les réponses physiologiques des porcs dans des conditions postnatales de TN ou de HS,
2. Évaluer et confirmer les effets indirects (causés par une consommation alimentaire réduite) et directs de l'HS (indépendamment de la consommation et du mode d'alimentation) sur les performances des porcs,
3. Déterminer si les effets positifs de la supplémentation en LY chez les porcs HS sont dus à des modifications du comportement alimentaire ou du métabolisme énergétique, et si ces effets peuvent être reproduits en imposant une fréquence de repas accrue, et
4. Pour mieux comprendre le processus dynamique de maintien de l'équilibre thermique corporel en examinant les variations des réponses de thermorégulations et physiologiques au cours de la journée des porcs soumis à une température ambiante élevée.

Stratégie expérimentale générale

Quatre expériences ont été planifiées pour répondre aux principales questions de la thèse. Les effets d'un HS prénatal chronique ont été évalués dans l'Expérience 1. Les mécanismes et facteurs possibles affectant les effets positifs de la supplémentation en LY pendant l'HS ont été évalués dans les Expériences 2, 3, et 4. L'Expérience 2 a été conçue pour tester si les effets positifs de LY étaient liés à une modification du comportement alimentaire (i.e., un nombre plus élevé de repas). Cette expérience a également été conçue pour distinguer les effets directs et indirects d'une température élevée en utilisant une approche d'alimentation « pair-fed » basée à la fois sur la restriction alimentaire et le changement dans la distribution de la fréquence des repas. L'Expérience 3 a été menée dans les chambres respiratoires afin de déterminer si l'effet LY sur le métabolisme énergétique des porcs était dû ou non à l'altération du comportement alimentaire et/ou à la modification du métabolisme énergétique (Labussière et al., 2021). Contrairement à l'Expérience 2, la restriction alimentaire dans l'Expérience 3 n'était limitée qu'au temps et à la durée de d'accès à la mangeoire, mais pas à la quantité d'aliment disponible. Enfin, l'Expérience 4 a été conçue pour avoir un lien entre l'Expérience 2 (toutes femelles) et l'Expérience 3 (tous des mâles entiers) et avec l'idée que la tolérance à la chaleur et la réponse à l'adaptation à la supplémentation en LY peuvent différer entre les sexes.

Résultats

Cette section se compose de quatre chapitres correspondant à chacune des quatre expériences :

Chapitre 1. Le stress thermique prénatal chronique modifie la croissance, la composition de la carcasse et la réponse physiologique des porcs en croissance soumis au stress thermique postnatal

Chapitre 2. Effet du stress thermique et de la gestion de l'alimentation sur les performances de croissance et les réponses physiologiques des porcs en finition

Chapitre 3. Effet de la supplémentation en levures vivantes et de la fréquence d'alimentation chez les porcs stressés par la chaleur

Chapitre 4. Effet du sexe et de la supplémentation en levures vivantes chez les porcs stressés par la chaleur

Chapitre 1. Le stress thermique prénatal chronique modifie la croissance, la composition de la carcasse et la réponse physiologique des porcs en croissance soumis au stress thermique postnatal

Les effets du stress (HS) thermique postnatal sur la physiologie et les performances des porcs sont largement étudiés, mais les études traitant du stress thermique prénatal sont peu nombreuses. L'objectif de cette étude était d'évaluer les effets d'un stress thermique appliqué pendant la période fœtale sur les réponses des porcs en croissance élevés en environnement postnatal thermoneutre (TN) ou en environnement HS. Pour l'environnement prénatal (PE), les truies en gestation à parité mixte ont été exposées soit à des conditions TN (PTN ; cycliques de 18 à 24°C ; n = 12) soit à des conditions HS (PHS; cycliques de 28 à 34°C; n = 12) entre les jours 9 et 109 de gestation. Deux progénitures femelles par truie ont été sélectionnées à 10 semaines d'âge et réparties dans l'un des deux environnements de croissance postnatale (GE) : GTN (cyclique de 18 à 24°C ; n = 24) et GHS (cyclique de 28 à 34°C ; n = 24). De 75 à 140 jours, les porcs GTN sont restés dans les conditions GTN et les porcs GHS étaient dans les conditions GTN de 75 à 81 jours et dans les conditions GHS de 82 à 140 jours. Indépendamment de PE, le HS post-natal a augmenté les températures rectale et cutanée (+0,30 et +1,61°C en moyenne, respectivement ; $P < 0,01$) et a diminué la consommation moyenne journalière (-332 g/j ; $P < 0,01$), ce qui a entraîné une baisse du gain quotidien moyen et du poids vif final (respectivement -127 g/j et -7,9 kg ; $P < 0,01$). Les porcs GHS présentaient une épaisseur de lard dorsal plus importante ($P < 0,01$), un pourcentage de longe de carcasse plus faible ($P < 0,01$), une augmentation de niveau de créatinine plasmatique ($P < 0,01$) et une diminution du glucose plasmatique, des acides gras non estérifiés, des taux de T3 et de T4 ($P < 0,05$). Un HS prénatal a augmenté la consommation alimentaire main uniquement lors des deux dernières semaines de l'essai (+10 g·kg PV^{-0,60}·j⁻¹ pour les porcs PHS $P = 0,02$) mais n'a pas augmenté la vitesse de croissance ($P > 0,10$). Le HS prénatal a diminué le niveau plasmatique de superoxyde dismutase au jour 3 du GHS (tendance à $P = 0,08$) et de T4 au jour 49 ($P < 0,01$). Le HS prénatal a augmenté les températures rectale et cutanée et diminué le gradient de température entre les températures rectale et cutanée chez les porcs GTN (+0,10, +0,33 et -0,22°C, respectivement ; $P < 0,05$) mais pas chez les porcs GHS ($P > 0,10$). Des interactions PE × GE ont également été trouvées avec un poids corporel inférieur ($P = 0,06$) et un gras dorsal et une adiposité périrénale plus élevés ($P < 0,05$ pour les porcs GHS – PHS

comparativement aux autres groupes. Dans l'ensemble, l'augmentation de la température corporelle, l'altération des fonctions thyroïdiennes et des réponses physiologiques au stress suggèrent une diminution de la tolérance à la chaleur et de la capacité de dissipation des porcs soumis à un HS prénatal chronique pendant toute la gestation. Un HS postnatal a diminué les performances de croissance, augmenté l'adiposité de la carcasse et diminué les traits métaboliques et les fonctions thyroïdiennes, en particulier chez les porcs précédemment soumis à un HS prénatal.

Chapitre 2. Effet du stress thermique et de la conduite alimentaire sur les performances de croissance et les réponses physiologiques des porcs en finition

Cette étude visait à déterminer si les réponses des porcs au stress thermique (HS) étaient directement dues à l'exposition à la chaleur (indépendamment du niveau et de la stratégie d'alimentation) ou étaient indirectement dues à la réduction de la consommation alimentaire mais également de déterminer si l'augmentation de la fréquence d'alimentation (fractionnement des repas) pouvait améliorer la réponse du porc au chaud. Un total de 48 porcs ($66,1 \pm 1,7$ kg) a été réparti en quatre groupes en trois répétitions. Après 7 jours dans des conditions thermoneutres (TN) (22°C ; période 1 [P1 ; jours -7 à -1]), les porcs ont été placés dans des conditions TN ou HS (32°C) pendant 20 jours (période 2 [P2 ; jour 0 à 19]). Le régime alimentaire a été fourni soit ad libitum (AL ; 2 distributions/j) soit en utilisant la stratégie du pair-feeding avec la distribution de 8 repas (PF8 ; 8 distributions/j) en utilisant des porcs HS–AL comme groupe de référence. Ainsi, les quatre groupes expérimentaux étaient TN–AL, HS–AL, TN–PF8 et HS–PF8. La ration quotidienne des porcs PF8 a été distribuée toutes les 90 minutes de 0900 à 1930 heures. Les données ont été analysées à l'aide de la procédure PROC MIXED avec répétition ($n = 3$), groupe expérimental ($n = 4$) et leurs interactions en tant qu'effets fixes, et la fonction REPEATED a été utilisée pour les données de mesures répétées. Les porcs avaient une consommation alimentaire journalière (CMJ) similaire pendant P1 ($P > 0,05$). Dans P2, les porcs HS–AL et PF8 avaient une CMJ (-19 %), un gain quotidien moyen (-25 %) et un poids corporel final (-6,1 kg) inférieurs à ceux des porcs TN–AL ($P < 0,01$). Les porcs TN–AL avaient une épaisseur de gras dorsal plus importante que les porcs TN–PF8 ($P < 0,05$), et les porcs HS avaient des résultats intermédiaires. Les porcs HS avaient un pourcentage de graisse périrénale plus élevé sur la base de l'analyse de contraste entre les porcs PF8 ($P < 0,05$). Les réponses de thermorégulation des porcs augmentaient avec l'exposition au HS mais ne différaient pas entre les groupes HS ou TN ($P > 0,05$). Pour les porcs TN, la variation de la température musculaire (T_{muscle}) dépendait de l'alimentation et de l'activité physique, comme pour les porcs HS, T_{muscle} augmentait progressivement tout au long de la journée. La T_{muscle} des porcs PF8 a augmenté avec chaque repas supplémentaire, mais a atteint un plateau plus tôt pour HS–PF8 que pour les porcs TN–PF8 ; une augmentation de T_{muscle} par repas était également plus faible dans HS–PF8 que dans TN–PF8 ($P < 0,05$). L'exposition au HS a diminué la T3 et la T4 plasmatiques ($P < 0,05$) et augmenté la créatinine plasmatique ($P < 0,05$). Entre les

groupes PF8, les porcs HS ont également eu une augmentation transitoire de l'insuline plasmatique au jour 8 ($P < 0,05$). L'effet du HS sur la CMJ a diminué la croissance des porcs, mais il existe des effets induits par la chaleur, tels que des réponses physiologiques altérées, qui pourraient expliquer les effets directs du HS observés dans d'autres publications, notamment en termes d'augmentation de l'adiposité. L'augmentation de la fréquence d'alimentation dans la présente étude n'a pas amélioré la réponse HS des porcs.

Chapitre 3. Effet de la supplémentation en levures vivantes et de la fréquence d'alimentation chez les porcs exposés la chaleur

Chez les porcs en croissance, une croissance réduite pendant le stress thermique (HS) est principalement liée à une diminution de la consommation d'aliments. L'étude visait à déterminer si les effets positifs rapportés de la supplémentation en levures vivantes (LY) chez les porcs HS étaient dus à une modification du comportement alimentaire et/ou du métabolisme énergétique, et si ces effets peuvent être reproduits en imposant une augmentation de la fréquence de repas. L'effet de la supplémentation en LY [0 (NS) vs. 100 (LY) g/tonne d'aliment], et de la fenêtre d'alimentation (FW) [illimité ou Unli, 2FW de 1 h chacun et 8FW de 15 min chacun] ont été mesurés chez des porcs mâles entiers en finition (n=36). La température ambiante était de 22°C pendant la période thermoneutre (TN; 5 j) et de 28°C pendant la période HS (5 j). L'exposition à la chaleur a diminué la consommation en matière sèche (DMI) et la quantité d'énergie retenue (RE) (-627g et -460 kJ·kg PC^{-0,60}·j⁻¹, P<0,01). Au cours du HS, la supplémentation en LY chez les porcs Unli a diminué les intervalles entre les repas (P = 0,02), atténuant l'effet du HS sur le DMI qui avait tendance à améliorer la RE (P = 0,09). Le lot NS-8FW avait une DMI et une RE plus élevées que NS-2FW (P <0,05) mais le dépôt de protéines était similaire. Les porcs supplémentés avaient un dépôt de protéines plus élevé pendant HS indépendamment de FW (+18 g·d⁻¹ ; P = 0,03). En comparant les groupes 2FW, l'amélioration de la tolérance à la chaleur des porcs supplémentés en LY était due à une meilleure sensibilité à l'insuline (P<0,05) et à une capacité accrue à perdre de la chaleur par voie latente après un repas (P<0,05) leur permettant d'augmenter leur DMI (via un nombre accru de repas) et donc leur efficacité énergétique. L'augmentation de la fréquence des repas a amélioré le DMI chez les porcs HS mais n'a pas reproduit les effets positifs de LY sur le dépôt de protéines.

Chapitre 4. Effet du sexe et de la supplémentation en levures vivantes chez les porcs stressés par la chaleur

La réduction des performances de croissance des porcs pendant le stress thermique (HS) est principalement induite par une diminution de la consommation alimentaire (CMJ). Il a été rapporté que la supplémentation en levures vivantes (LY) améliorait les réponses HS en augmentant la consommation alimentaire et l'efficacité alimentaire chez les porcs mâles entiers (EM) (Labussière et al., 2021), mais cela n'a pas été confirmé chez les femelles (F) (Chapitre 2). En effet, un effet du dimorphisme sexuel à le stress thermique a déjà été rapporté avec des castrats ayant une efficacité alimentaire inférieure à celle des femelles pendant l'HS (Renaudeau et al., 2006) mais aucune étude n'existe à ce jour concernant la différence entre EM et F. Ainsi, l'objectif de l'étude était de déterminer l'effet du sexe et de la supplémentation en LY chez des porcs exposés à un stress thermique. Un total de 64 porcs de finition logés individuellement (32 EM et 32 F ; $80,8 \pm 0,9$ kg de poids corporel) a été utilisé pour évaluer les effets de la supplémentation en LY [0 (N) vs 100 g/tonne d'aliment (Y ; 1×10^6 CFU/g d'aliment de *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079)] et chez les porcs selon leur sexe (EM ou F) et soumis à HS au cours de 2 répétitions successives. Ainsi, il y avait quatre groupes expérimentaux : N-EM, Y-EM, N-F et Y-F. Les porcs ont été répartis dans deux salles expérimentales en fonction des traitements. La température ambiante des deux pièces a été maintenue à 22°C pendant 7 jours puis à 32°C pendant 3 jours pendant la période d'adaptation avant la période expérimentale de 27 jours à 32°C. La croissance et les performances d'abattage ont été mesurées. Le porc a été considéré comme l'unité expérimentale et les données ont été analysées à l'aide du modèle PROC MIXED avec répétition (R), sexe (S), supplémentation en LY (D ; régime), leurs interactions comme effets fixes et avec l'origine de la portée comme effet aléatoire. La répétition a eu un effet significatif sur le poids vif et à l'abattage, les porcs de la répétition 2 étant plus lourds que les porcs de la répétition 1 ($P < 0,05$). Indépendamment des effets de la répétition les porcs EM étaient plus lourds et avaient un gain quotidien moyen (GMQ) plus élevé que les porcs F ($P < 0,05$). Dans les conditions TN, les porcs mâle entiers croissent plus vite et ont une meilleure efficacité alimentaire que les femelles (Blanchard et al., 1999 ; Ruiz-Ascacibar et al., 2017). Cependant, l'amélioration de l'efficacité alimentaire des porcs EM dans cette étude n'a été observée que sur des porcs plus légers (répétition 1) mais pas sur des porcs plus lourds (répétition 2) avec une interaction

significative de la répétition et du sexe sur le ratio gain de poids: alimentation des porcs ($P < 0,05$). Cela suggère que l'effet négatif de l'HS sur l'efficacité alimentaire est accru chez les porcs EM plus lourds, mais pas chez les porcs F, probablement en raison de l'effet exacerbé de l'HS sur leur capacité à atteindre leur potentiel maximal de dépôt de protéines ou de croissance (Le Bellego et al., 2002). Cela pourrait être attribué à une sensibilité plus élevée des porcs plus lourds à un HS (Renaudeau et al., 2011) associée à une production de chaleur plus élevée des porcs EM en raison de leur potentiel de croissance et de leur dépôt de protéines plus élevés (Moughan et al., 2006 ; Labussière et al., 2013). Il y avait une interaction significative entre la répétition, le régime alimentaire et le sexe pour la consommation alimentaire (CMJ) exprimé par $BW^{0,60}$ et ADG ($P < 0,05$). Lors de la répétition 2, les porcs EM supplémentés avaient un CMJ plus élevé par $BW^{0,60}$ ($P < 0,05$) que les porcs EM supplémentés de la répétition 1 et les porcs F des répétitions 1 et 2. Leur GMQ était également plus élevé que les porcs F dans la répétition 1 et les porcs F supplémentés dans la répétition 2. Les porcs EM supplémentés avaient seulement tendance à avoir un CMJ plus élevé par $BW^{0,60}$ ($P = 0,08$) que les porcs EM non supplémentés dans la répétition 2. Bien qu'il ne s'agisse que d'une tendance, cela suggère que la supplémentation en LY semble améliorer la CMJ pendant le HS, mais uniquement chez les porcs EM et en particulier quand ils sont lourds. En effet, en raison de l'écart type élevé de la réponse CMJ des porcs de l'étude, il aurait fallu environ 400 porcs pour détecter une différence significative de $P = 0,05$ entre les régimes selon PROC GLMPOWER. Ainsi, sur la base de nos résultats, l'effet de la supplémentation en LY sur les réponses HS des porcs semble dépendre du poids corporel et du sexe de l'animal.

Conclusions

Les principaux objectifs de ce travail de thèse étaient de

- 1) étudier les effets d'une exposition à la chaleur en période prénatale chronique sur la sensibilité au stress thermique des animaux pendant la période de croissance-finition
- 2) distinguer les effets directs et indirects du stress thermique sur les performances de croissance, les réponses physiologiques et le métabolisme
- 3) mieux comprendre les mécanismes expliquant les effets positifs de la supplémentation en levures vivantes sur les porcs exposés au chaud
- 4) évaluer les interactions entre la supplémentation en levures vivantes et le sexe chez les porcs exposés à la chaleur, et
- 5) caractériser la dynamique des réponses de thermorégulation au cours de la journée et évaluer les conséquences sur la tolérance à la chaleur chez le porc

Les différents modèles de stress thermique utilisés dans ce projet ont démontré que de nombreux facteurs peuvent influencer sur la capacité du porc à faire face au stress thermique, tels que les facteurs liés à l'animal (poids corporel, sexe, comportement alimentaire tel qu'estimé par l'alternance des périodes d'alimentation et de jeûne), l'environnement (conditions de logement, la nature et la sévérité du challenge thermique), et les interactions entre tous ces facteurs. De plus, notre travail montre que l'environnement thermique prénatal peut avoir des conséquences à long terme sur les performances et les réponses physiologiques des porcs. En utilisant des mesures continues de la production et des pertes de chaleur et de la température corporelle, nos résultats ont démontré comment le comportement alimentaire et son interaction avec la température ambiante influencent la façon dont les porcs tentent de maintenir l'homéothermie tout au long de la journée. Dans cette optique, nous avons également démontré l'importance des pertes de chaleur latente dans le maintien de l'équilibre thermique au chaud, en particulier pour compenser les pics de production de chaleur liés à l'effet thermique des repas. Les données obtenues à partir du projet de thèse soulignent l'importance des stratégies d'alimentation destinées à stimuler la consommation d'eau afin d'améliorer la prise alimentaire pendant le stress thermique. Il a été constaté que la supplémentation en levures vivantes favorisait la sensibilité à l'insuline, ce qui pourrait induire une modification du comportement alimentaire et des conséquences positives sur les réponses de thermorégulation chez les porcs.

End.

Titre : Réponses dynamiques des porcs en croissance au stress thermique modulées par la vie prénatale et les pratiques d'alimentation

Mots clés : stress thermique, porcs, métabolisme énergétique, thermorégulation, comportement alimentaire, levures vivantes

Avec l'augmentation de la température mondiale et des vagues de chaleur plus fréquentes, le stress thermique réduit la production porcine dans de nombreux pays. La diminution de la croissance est principalement liée à une réduction de la consommation alimentaire mais d'autres aspects du stress thermique chez les porcs sont apparus au cours des dernières années. La thèse vise à contribuer aux connaissances actuelles avec les objectifs suivants : 1) étudier l'effet d'un stress thermique prénatal chronique sur la tolérance des porcs à la chaleur, 2) confirmer les effets directs et indirects de l'exposition à la chaleur, 3) évaluer les stratégies d'alimentation qui peuvent aider les porcs pendant le stress thermique, et 4) explorer l'idée que le maintien de l'équilibre thermique est un processus dynamique qui varie tout au long de la journée. Ce projet de thèse a montré qu'un déséquilibre entre la production et la perte de chaleur (charge thermique) et la capacité de perte de chaleur entraîne une réduction des performances, non seulement une croissance réduite, mais également une altération du

métabolisme énergétique, des réponses physiologiques et une composition corporelle exacerbée par une exposition au stress thermique pendant la période fœtale. De plus, l'amélioration de la tolérance à la chaleur des porcs est liée dans leur capacité à maintenir un équilibre dynamique entre la production et la perte de chaleur tout au long de la journée, en particulier pendant et après un repas en raison de la forte contribution du comportement alimentaire à la production de chaleur. Nos résultats ont également confirmé la tolérance à la chaleur est en partie déterminée par la capacité des animaux à perdre de la chaleur par la voie évaporative. Les stratégies nutritionnelles liées à un comportement alimentaire modifié ont été évaluées. La supplémentation en levures vivantes a amélioré la sensibilité à l'insuline et l'efficacité de la perte de chaleur qui a aidé les porcs à faire face au stress thermique en modifiant le comportement alimentaire avec des effets positifs sur l'efficacité énergétique lors d'un défi thermique.

Title : Dynamic responses of growing pigs to heat stress modulated by prenatal life and feeding practices

Keywords : heat stress, pigs, energy metabolism, thermoregulation feeding behaviour, live yeast

Abstract : Heat stress remains to be one of the major limiting factors in swine production in many countries and this concern will rise in the future especially with increasing global temperature and more frequent heat waves due to warming climate. While decreased growth is mainly related to a reduction in feed intake, other aspects of heat stress in growing pigs have emerged in the recent decades. The thesis thus aims to contribute to the current knowledge with the following objectives: 1) to investigate effect of a chronic prenatal heat challenge on pig's heat tolerance, 2) to confirm direct and indirect effects of heat exposure on pig performance, 3) to evaluate feeding practices that can help pigs cope to a heat challenge, and 4) to explore the idea of maintaining thermal balance as a dynamic process that varies throughout the day. This thesis project has demonstrated that an imbalance between heat

production and loss (heat load) results in reduced growth and in altered energy metabolism, physiological responses, and body composition. This later effect is exacerbated in pigs exposed to a chronic prenatal heat challenge. Improved heat tolerance in pigs was found to depend on their ability to maintain dynamic equilibrium between heat production and losses within a day, especially during and after a meal. Our results also confirmed the importance of water intake in improving evaporative heat losses in the ability of pigs to cope with heat stress. Nutritional management practices linked to modified feeding behavior were evaluated. Live yeast supplementation improved insulin sensitivity, increased heat loss efficiency under heat stress, and modified feeding behavior with positive effects on energy efficiency and protein deposition during a heat challenge.