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

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Abstract

In growing pigs, reduced growth during heat stress (HS) is mainly related to decreased feed intake. 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 (NS) *v.* 100 (LY) g/ton of feed), and of feeding window (FW) (unlimited or Unli, 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 the thermoneutral (TN) period (5 d) and at 28°C during the HS period (5 d). Heat exposure decreased DM intake (DMI) and retained energy (RE) (–627 and −460 kJ·kg $BW^{-0.60} \cdot d^{-1}$, respectively; $P < 0{\cdot}01$). During HS, LY supplementation in Unli pigs decreased inter-meal intervals ($P = 0{\cdot}02$) attenuating HS effect on DMI which tended to improve RE ($P = 0{\cdot}09$). NS – 8FW had higher DMI and RE than NS – 2FW ($P < 0{\cdot}05$) but protein deposition (PD) were similar. Supplemented pigs had higher PD during HS regardless of FW ($+18\ g \cdot d^{-1}$; $P = 0{\cdot}03$). Comparing the 2FW groups, improved heat tolerance of LY-supplemented pigs were due to improved insulin sensitivity ($P < 0{\cdot}05$) and latent heat loss capacity after a meal ($P < 0{\cdot}05$) allowing them to increase their DMI (via an increased number of meals) and thus their energy efficiency. Imposing an increased meal frequency improved DMI in HS pigs but did not replicate positive effects of LY on PD.

Keywords: **Heat stress: Energy metabolism: Live yeast: Meal frequency: Pig: Thermoregulation**

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 heat production (HP) in modern pigs[2] making them more susceptible to HS, especially entire male pigs due to higher basal HP[3] and higher protein deposition (PD) 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 utilisation and to reduce heat increment) have thus been studied to mitigate HS effects in livestock animals[6,7]. However, most of these solutions aim at reducing the average daily HP, even though maintaining the balance between HP and loss is a dynamic process that varies throughout the day.

Indeed, HP and body temperature are not constant during the day but are closely related to variations in feeding behaviour 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] possibly mediated by a modified feeding behaviour changes (i.e. higher number of meals) 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[10,11] 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 behaviour and/or of energy

Abbreviations: AHP, activity heat production; BW, body weight; DMI, DM intake; FW, feeding window; HP, heat production; HS, heat stress; LY, live yeast; ME, metaboliszable energy; PD, protein deposition; RE, retained energy; TEF, thermic effect of feed; TN, thermoneutral; Unli, unlimited.

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metabolism and to determine if increasing the meal frequency can replicate the observed positive effects of LY supplementation in HS pigs.

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 (Authorisation: APAFiS #18 973–2019020622003043).

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 two levels of Levucell SB TITAN supplementation: 0 (diet NS) *v.* 100 g/ton of feed (diet LY; 1×10^6 CFU *Saccharomyces cerevisiae var. boulardii* CNCM I-1079/g of feed), and three feeding management practices called feeding windows (FW): unlimited access (Unli; 09.00 h to 15.45 h and 16.00 h to 07.00 h), 2FW of 1 h each (from 09.00 h to 10.00 h and from 15.00 h to 16.00 h) and 8FW of 15 min each (at every 90-min interval from 09.00 h to 19.30 h). 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 PD among the groups. Titanium dioxide (TiO_2) was added in the diets (3 g/kg feed) as indigestible marker. Feed was automatically distributed twice for groups with unlimited and 2FW (at 08.55 h and at 14.55 h) and eight times for groups with 8FW (5 min before each FW). There were thus six experimental groups (NS – Unli, LY – Unli, NS – 2FW, LY – 2FW, NS – 8FW and LY – 8FW) in which feeding behaviour, energy metabolism and thermoregulation responses were measured.

In order to have six pigs per experimental group, thirty-six individually housed and fed entire male finishing pigs (Pietrain × (Large White × Landrace); 62·2 ± 1·0 kg initial live body weight (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 nine replicates with four pigs coming from the same litter per replicate. There were two 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 m^3 open circuit respiration chamber as described by Vermorel *et al.*[12] for 10 d of 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 (TN period; day −5 to day −1) and was increased for the last 5 d at HS conditions of 28·0°C (actual 27·4 ± 0·5°C) (HS period; day 0 to 4) with the transition on day 0 (fixed at 25°C at 08.00 h and an increase of 1°C/h from 08.00 h to 11.00 h). The cage (3·3 m^2) 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 as described by Melchior *et al.*[13] to allow for successive blood samplings and a temperature probe (Anipill, Body Cap) was implanted 2 to 3 cm deep into the brachiocephalic muscle of the neck of the pig as described by Renaudeau[14]. 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 acclimated to the feed and feeding distribution schedule with unlimited feed access for the first 10 d, and then the FW were imposed in the last 7 d of adaptation. All throughout the experimental period, relative humidity was kept constant at 65 % (actual 67·1 ± 2·7 %), light was turned on from 07.30 h to 19.30 h, feeder was blocked from 07.00 h to 09.00 h for animal care and samplings, and water was provided *ad libitum*.

Table 1. Composition of the experimental diets*

Ingredients, % as-fed	Non-supplemented (NS)	Supplemented (LY)
Wheat	26·732	26·728
Barley	26·732	26·728
Maize	26·732	26·728
Soyabean meal	4·659	4·658
Rapeseed meal	4·621	4·624
Soyabean 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†	0·500	0·500
Stearin	0·005	–
LSB	–	0·010
Indigestible marker (TiO_2)	0·300	0·300
Analysed chemical composition, % as fed‡		
Actual DM	89·0	88·9
DM	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		
NE (MJ/kg)	9·60	9·60
SID lysine : NE (g/MJ NE)	0·65	0·65

LY, live yeast-supplemented diet; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; SID, standardised ileal digestible; NE, net energy.
* Diet fed in pellet form.
† Provided per kilogram of complete diet: vitamin A, 1 000 000 µg; vitamin D_3, 200 000 µg; vitamin E, 4000 mg; vitamin B_1, 400 mg; vitamin B_2, 800 mg; calcium pantothenate, 2170 mg; niacin, 3000 mg; vitamin B_{12}, 4 mg; vitamin B_6, 200 mg; vitamin K_3, 400 mg; folic acid, 200 mg; biotin, 40 mg; choline chloride, 100 000 mg; iron (sulphate), 11 200 mg; iron (carbonate), 4800 mg; copper (sulphate), 2000 mg; zinc (oxide), 20 000 mg; manganese (oxide), 8000 mg; iodine (iodate), 40 mg; cobalt (carbonate), 20 mg; and selenium (selenite), 30 mg.
‡ As-fed basis. Aside from actual DM content, values are calculated for the same DM content (89·0 %).

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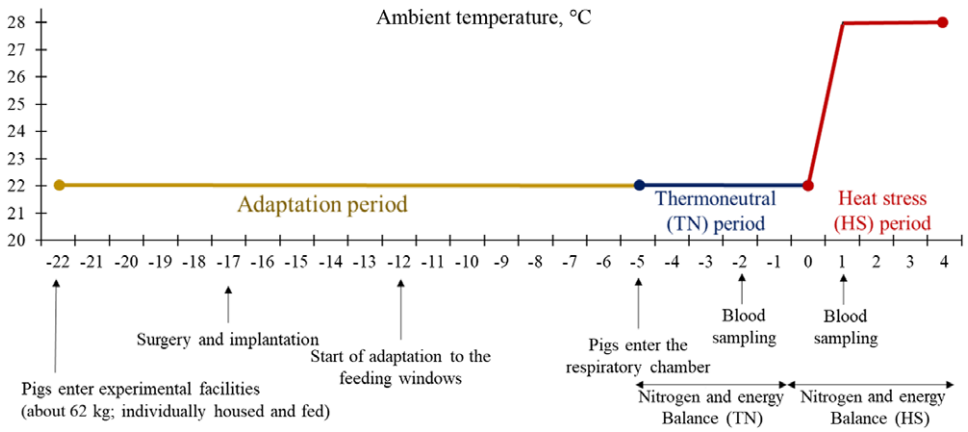

Fig. 1. Description of experimental design and the timing of the measurements.

Measurements and samplings

The pigs were manually weighed twice at 08.30 h: 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 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 and quantity) were continuously recorded through weight sensors placed below the feeding trough and the water storage tank (outside the chamber), respectively. Total faecal collection was done at the end of the 10-d measurement in the chamber, and spot faecal sampling was done twice during each period between 08.00 h and 09.00 h (day −3 and day −1 during the TN period, and day 1 and day 4 during the HS period) in order to have separate digestibility coefficients per period. The spot faecal samples were pooled per period. Urine was collected in a bucket containing 250 ml of 10 % H_2SO_4 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 based on the methods described by Noblet et al[15].

Gas concentrations of O_2, CO_2 and CH_4 of outgoing air were continuously measured with a paramagnetic differential analyzer (Oxymat 6, Siemens AG) for O_2 and with two infrared analyzers (Ultramat 6, Siemens AG) for the CO_2 and CH_4 concentration. Gas extraction rate of the air of the chamber was measured with a mass gas meter (Teledyne Brown Engineering) and corrected for humidity content. The cage in the respiration chamber was mounted on force sensors (9104A, Kistler) producing an electrical signal proportional to the physical activity of the pig[16]. Gas concentrations, the signals of the force sensors, the weight of the trough and water tank, gas flow rate, relative humidity, ambient temperature, and atmospheric pressure in the respiration chamber were measured sixty times per second, averaged over 10 s intervals and recorded for further calculations as described by van Milgen *et al.*[17]. The recovery of known amounts of CO_2 and N_2 was measured before and after the experiment; it averaged 99·7 and 99·9 % for CO_2, and 100·2 and 99·5 % for N_2 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 day −2 (TN) and on day 1 (HS) 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 08.20 h and 08.40 h (referred to as preprandial), 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 (3000 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 DM content. Representative samples of each experimental diet per feed fabrication were analysed for ash, starch, diethyl ether extract and crude fibre contents according to Association of Official Analytical Chemists[18]. Feed samples and total faecal samples (one per pig) were analysed for DM, ash, nitrogen (Dumas method) and gross energy, and TiO_2 content[19]. Periodic faecal samples were also analysed for DM and TiO_2 content. Urine was analysed for nitrogen content using fresh samples and for gross energy content using urine (about 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).

For plasma samples, commercially available kits were used to measure plasma levels of glucose (HK), lactate (lactic acid, ABX Pentra), NEFA (FUJIFILM Wako Chemicals Europe GmbH), creatinine (Jaffe, Thermo Fisher Scientific Oy), TAG (Thermo Fisher Scientific Oy) and urea (Thermo Fisher Scientific Oy). 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 nitrogen (Protéines-kit; bioMérieux), insulin (ST AIA-PACK IRI, Tosoh Corporation), total triiodothyronine or T3 (ST AIA-PACK T3, Tosoh Corporation), and total thyroxin or T4 (ST AIA-PACK T4, Tosoh Corporation) were also determined. Intra-assay CV were 5·20 %, 2·3 %, 3·8 % and 3·99 %, respectively.

Calculations

DM intake per period was measured as the difference between total DM feed offered per period (total feed offered × % DM content) and the sum of the DM feed refused per period. Feeding behaviour parameters were calculated from the continuous data collected from the feeding trough. The meal criterion is the maximal duration between two 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 ($P > 0{\cdot}05$). 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 the calculation of daily feeding behaviour parameters such as number of meals, feeding duration, inter-meal interval and rate of feed intake as described by Renaudeau *et al.*[20]. 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 09.00 h (first FW of the day) and the last meal taken before 09.00 h of the following day.

Apparent faecal digestibility of DM, nitrogen and energy was measured per pig and was split into two periods based on TiO_2 content in the periodic faecal samples. Nitrogen retention was calculated per period as the difference between nitrogen intake and nitrogen lost in the feces, urine and as ammonia. The resulting nitrogen retention was multiplied by 6·25 to determine PD per period. Digestible energy and metabolisable energy (ME) intake were computed according to standard methods and considering CH_4 production. Total HP was calculated from respiratory gas exchanges, CH_4 and urinary nitrogen (including evaporated nitrogen) according to the formula of Brouwer[21]. Energy retention (RE) was the difference between ME intake and HP. Fat or lipid deposition (LD) was then determined based on the energy balance, assuming that the RE was only deposited as protein (REp = PD × 23·6 kJ · g^{-1}) or as fat (LD = (RE − REp)/39·5 kJ · g^{-1}). Components of HP were partitioned into activity heat production (AHP), TEF and minimal heat production (MHP) calculated using simultaneous measurements of O_2 and CO_2 concentrations in the respiration chamber, of force sensor signals, and of physical characteristics of the gas in the chamber based on the modelling approach by van Milgen *et al.*[17] but with the modifications described by Quemeneur *et al.*[22]. The first days of the TN (considered as adaptation) and of the HS periods (ambient temperature varied during the transition on day 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 behaviour calculations. Evaporated water and latent evaporative heat losses were calculated using the relative humidity and temperature measurements in the chambers based on the principle described by Renaudeau *et al.*[23]. 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 and 28°C for TN and HS periods, respectively. 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 ($BW^{0{\cdot}60}$), and the heat dissipation parameters were afterwards expressed as %HP.

Body temperature data were 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 preprandial 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 five pigs per treatment) was determined using GLMPOWER[24] procedure (based on previous measurements of ME intake of pigs submitted to a HS challenge[23] 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 α level. The experimental design allowed measuring each pig at two periods, under TN and then afterwards under HS conditions. For the statistical analyses, $P < 0{\cdot}05$ was considered as significant, and $P < 0{\cdot}10$ was considered as a trend.

During the experiment, one pig died (NS – 8FW) and two pigs got sick (1 NS – 8FW and 1 LY – 2FW) with reasons unrelated to the treatments, and two pigs (1 NS – Unli and 1 LY – 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 behaviour, faecal digestibility coefficients, components of nitrogen and energy balance, thermoregulation responses, and body temperature were

summarised per period and were analysed using the REPEATED statement of the PROC MIXED procedure[24] with diet (D), 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 ((NS – Unli, NS – 2FW, NS – 8FW) *v.* (LY – Unli, LY – 2FW, LY – 8FW)) and within each FW ((NS – Unli *v.* LY – Unli), (NS – 2FW *v.* LY – 2FW) and (NS – 8FW *v.* NS – 8FW)), and to compare the FW ((NS – Unli, LY – Unli) *v.* (NS – 2FW, LY – 2FW)), ((NS – Unli, LY – Unli) *v.* (NS – 8FW, LY – 8FW)) and ((NS – 2FW, LY – 2FW) *v.* (NS – 8FW, LY – 8FW)).

Body temperature and postprandial plasma parameters were analysed using the REPEATED statement of the PROC MIXED procedure[24] 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 with the maximum body temperature (BT_{max}) or evaporated water per group and per period. 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, day of sampling and their interactions.

Postprandial plasma kinetics of insulin, glucose, lactate and α-amino nitrogen followed a bell-shaped curve and a modified Erlang model developed by van Milgen *et al.*[25] was adapted (online Supplementary Fig. 1) in order to describe their kinetics. This takes into account four parameters: initial or preprandial concentration in the plasma ($C_{initial}$), the postprandial concentration after 180 min (C_{final}), the shape factor of the curve (λ) and the AUC above C_{inital} and C_{final}. Maximum concentration (C_{max}) and time at C_{max} (T_{max} in min) were calculated from the estimated parameters as described in the aforementioned paper. Data per pig per period were fitted into the model using PROC NLIN[24], and the resulting estimates were subjected to REPEATED measure of the PROC MIXED procedure[24] with D, FW, P (based on the day of sampling) and their interactions as fixed effects.

Results

Growth performance and feeding behaviour

Table 2 shows the summary of the growth performance and feeding behaviour traits of the finishing pigs according to their experimental group. The effect of period on DM intake (DMI) and live BW was significant with lower DMI (–527 g DM · d^{-1}; $P < 0·01$) and higher mean BW (+4·0 kg BW; $P < 0·01$) in HS than in TN conditions. Supplementation of LY increased DMI during HS period (+205 g DM · d^{-1}; $P = 0·03$) especially for the 2FW group (+551 g DM · d^{-1}; contrast $P < 0·01$). For water intake, the interaction between diet, FW and period was significant wherein it increased during HS period (+3848 g · d^{-1}; $P < 0·01$) albeit being statistically significant only for the LY – 2FW group (+8919 g · d^{-1}; $P = 0·01$). For feeding behaviour traits, heat exposure decreased feeding duration (–16·4 min · d^{-1}; $P < 0·01$) and increased rate of feed intake (+4·3 g · 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 meals · d^{-1} on average; $P < 0·01$) but not in 2FW and 8FW groups and also affected meal size for pigs in 2FW (–237 g·$meal^{-1}$; $P < 0·01$) 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 the TN period ($P = 0·07$). During the HS period, supplemented pigs had generally faster rate of feed intake ($P = 0·04$) than non-supplemented pigs 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 ($P = 0·02$) during the HS period.

N retention and energy utilisation

As shown in Table 3, period significantly affected components of nitrogen retention and energy utilisation ($P < 0·01$) with higher faecal digestibility coefficient (+0·52 points) and AHP (+40 kJ kg $BW^{-0·60}$ · d^{-1}), and lower ME intake, HP and RE (–627, –166, –460 kJ kg $BW^{-0·60}$ · d^{-1}, respectively) during HS than during TN period. Regardless of period, FW significantly affected AHP ($P = 0·04$) with 8FW pigs having higher AHP than Unli pigs (+54 kJ kg $BW^{-0·60}$ · d^{-1} on average), while 2FW pigs had intermediate values. In TN conditions, NS – Unli and LY – Unli had higher ME intake ($P = 0·04$ and trend at $P = 0·06$, respectively) and RE ($P < 0·04$) than NS – 2FW. Unli groups also had higher MHP + TEF than both 2FW and 8FW groups (contrast at $P < 0·04$) during this period. Meanwhile, upon being subjected to HS, supplemented pigs had generally higher ME intake ($P = 0·03$) with the difference most pronounced between the 2FW groups (+570 kJ kg $BW^{-0·60}$ · d^{-1}; $P < 0·01$) but only numerical in the Unli pigs (+245 kJ/kg $BW^{-0·60}$ · d^{-1}; $P = 0·16$). Supplemented pigs also had higher RE ($P = 0·01$) within the Unli (+204 kJ kg $BW^{-0·60}$ · d^{-1}; trend at $P = 0·09$) and the 2FW group (+461 kJ kg $BW^{-0·60}$ · 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 g · d^{-1}; $P < 0·01$) and was only numerical in the Unli groups (+54 g · d^{-1}; $P = 0·16$). Nevertheless, supplemented pigs had higher PD regardless of FW (+18 g · d^{-1}; $P = 0·03$) and most notably for pigs between the Unli groups (+28 g · d^{-1}; trend at $P = 0·06$).

Themoregulation 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 during the HS period, these increased ($P < 0·01$) to 56 % of HP and to 39·0°C on average, respectively. Under HS, proportion of heat dissipated via the sensible route and used to heat the ingested

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Table 2. Effect of live yeast supplementation and feeding window on feed and water intake, growth and feeding behaviour of male finishing pigs exposed to high ambient temperature†,‡,§

	Unli		2FW		8FW			
Items	NS	LY	NS	LY	NS	LY	RSD†††	Statistics
Animals, n	5	5	6	5	4	6		
Growth parameters								
Mean BW (kg)								
TN	81·4	80·6	81·7	82·6	80·9	81·1	1·2	P**
HS	85·2	85·0	84·2	87·0	85·4	85·2		
DMI (g DM per · d)								
TN§§	2567	2566	2229	2365	2345	2394	246	D***, P**
HSII,††	1814	2060	1584	2135	1947	1765		
Water intake (g/d)								P**, D × FW***, D × FW × P**
TN	7045	6013	5200	5242	5126	5149	3498	
HS††,‡‡	8637	7723	7075	14 161	13 251	6015		
ADG (g/d)								
TN	1221	1146	1082	1101	1150	1152	364	P**
HS††	484	768	253	776	858	551		
Meal parameters								
Meal (n/d)								
TN§§,IIII,¶¶	5·6	7·1	2·0	2·4	5·1	4·2	0·8	FW**, P*, FW × P*
HS§§,¶¶	4·5	5·6	2·1	2·6	4·8	4·0		
Meal size (g/meal)								FW**, P**, FW × P***
TN§§,¶¶	694	409	1198	1124	543	659	152	
HS§§,¶¶	622	471	839	1008	495	560		
Total feeding duration (min/d)								FW*, P**, D × FW × P***
TN¶¶	61·8	59·2	52·1	53·4	72·3	63·8	5·0	
HS‡‡,¶¶	42·2	42·6	37·3	42·5	59·4	42·0		
Inter-meal intervals within a day (min/d)								FW**, P**, FW × P*
TN	244	160	314	277	151	181	52	
HS¶	341	220	310	266	184	233		
Rate of feed intake (g/min)								D***, FW**, P**
TNIII,¶¶	45·7	51·0	51·1	51·2	35·7	41·2	3·2	
HSII,¶¶	48·6	54·7	54·1	57·8	38·8	48·1		
Meal size at day of blood sampling (g)‡‡‡								ds**
day −2 (TN)§§,¶¶	693	396	1208	1190	544	554	161	
day 1 (HS) ††,§§,¶¶	616	329	948	1283	558	533		

Unli, unlimited; FW, feeding window; NS, non-supplemented diet; LY, live yeast-supplemented diet; RSD, residual standard deviation; BW, body weight; TN, thermoneutral; P, period; HS, heat-stressed; D, diet; DMI, DM intake; ADG, average daily gain; ds, day of sampling.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·10$.
† A total of thirty-one pigs were allocated to six experimental groups in nine replicates with two blocks per replicate. All pigs were housed under thermoneutral conditions (TN period; 22°C) from day −5 to −1 and then under heat-stressed conditions (HS period; 28°C) from day 0 to 4.
‡ Data were analysed using PROC MIXED model with FW, D, P and their interactions as fixed effects, with pig as a repeated unit per period.
§ Contrast statements within period ($P < 0·05$).
II (NS – Unli, NS – 2FW, NS – 8FW) *v.* (LY – Unli, LY – 2FW, LY – 8FW).
¶ (LY – Unli *v.* LY – Unli).
†† (NS – 2FW *v.* LY – 2FW).
‡‡ (NS – 8FW *v.* LY – 8FW)) for diet effect.
§§ (NS – Unli, LY – Unli) *v.* (NS – 2FW, LY – 2FW).
IIII (NS – Unli, LY – Unli) *v.* (NS – 8FW, LY – 8FW).
¶¶ (NS – 2FW, LY – 2FW) *v.* (NS – 8FW, LY – 8FW) for FW effect.
††† RSD.
‡‡‡ A total of twenty-eight pigs out of the thirty-three pigs in the experiment. Data were analysed using PROC MIXED model with FW, D, ds and their interactions as fixed effects, with pig as a repeated unit per period. ** $P < 0·01$.

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 ($P < 0·01$). The diet, FW and period interaction for evaporative water losses was significant ($P < 0·05$), wherein evaporative water was significantly higher when pigs were in HS than when they were in TN conditions for all groups ($P < 0·05$) except the LY – 8FW ($P > 0·10$). Based on the contrast analyses during the HS period, 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. 2(a)) during the TN period with peaks at approximately 09.00 h and 14.00 h, and this variation was more pronounced in the 2FW (Fig. 2(b)) and 8FW (Fig. 2(c)) groups. In the 2FW groups, body temperature peaked to similar level (BT_{max}) at 09.00 h and at 15.00 h ($P > 0·10$) during the TN period,

Table 3. Effect of feed access and live yeast supplementation on energy and nitrogen metabolism of male finishing pigs exposed to high ambient temperature†,‡,§

	Unli		2FW		8FW			
Items	NS	LY	NS	LY	NS	LY	RSD†††	Statistics
Faecal DM digestibility (%)								
TN	85·3	84·5	85·0	85·7	85·0	85·2	0·64	P**
HS	85·5	85·1	86·2	85·8	85·8	85·7		
Energy balance (kJ·kg $BW^{-0.60}$ · per d)								
ME intake								
TN§§	2765	2727	2398	2568	2524	2588	243	D***, P**
HSII,††	1890	2136	1658	2228	2027	1870		
Heat production (kJ kg $BW^{-0.60}$ ·per d)								
Total HP								
TN	1484	1503	1401	1461	1426	1446	68	P**
HS††	1257	1328	1225	1357	1295	1255		
MHP + TEF								
TN§§,IIII	1234	1230	1128	1179	1117	1141	56	FW***, P**
HS††	964	1030	914	1018	932	930		
AHP								
TNIIII	247	268	278	285	309	310	34	FW*, P**
HSIIII	289	294	317	343	363	331		
RE								D*, FW***, P**, D × FW*,
TN§§	1265	1239	988	1122	1088	1126	182	D × FW × P***
HSII,††	617	821	423	884	722	599		
RE/ME intake (%)								D**,P**, D × FW* D × P***, D × FW × P***
TN	38·0	38·5	33·3	36·8	36·0	35·5	6·0	
HSII,††	27·1	32·6	15·8	33·2	29·6	25·7		
Nutrient deposition (g/d)								
Protein (PD)								
TN	160	158	135	146	150	150	24	D*, FW*,
HSII	83	109	70	91	82	90		P**
Fat (LD)								D*, P**, D × FW*, D × FW × P***
TN§§	361	347	271	308	293	313	61	
HSII,††	180	234	120	268	215	165		

Unli, unlimited; FW, feeding window; NS, non-supplemented diet; LY, live yeast-supplemented diet; RSD, residual standard deviation; TN, thermoneutral; P, period; HS, heat-stressed; BW, body weight; ME, metabolisable energy; D, diet; HP, heat production; MHP, minimal HP; TEF, thermic effect of feeding; AHP, activity heat production; RE, retained energy; PD, protein deposition; LD, lipid deposition.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·10$.

† A total of thirty-six pigs were allocated to six experimental groups in nine replicates with two blocks per replicate. All pigs were housed under thermoneutral conditions (TN period; 22° C) from day −5 to −1 and then under heat-stressed conditions (HS period; 28°C) from day 0 to 4.

‡ Data were analysed using PROC MIXED model with FW, D, P and their interactions as fixed effects, with pig as a repeated unit per period.

§ Contrast statements within period ($P < 0·05$):

II (NS – Unli, NS – 2FW, NS – 8FW) *v.* (LY – Unli, LY – 2FW, LY – 8FW).

†† (NS – 2FW *v.* LY – 2FW).

§§ (NS – Unli, LY – Unli) *v.* (NS – 2FW, LY – 2FW).

IIII (NS – Unli, LY – Unli) *v.* (NS – 8FW, LY – 8FW).

††† RSD.

whereas in 8FW groups, BT_{max} measured in the evening was significantly higher than the body temperature peak measured 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 under TN conditions at about 131 g/h.

Compared with the TN period, the diurnal variation of body temperature in Unli pigs was less apparent upon being subjected to HS, wherein body temperature was at BT_{max} 80 % of the day ($P > 0·10$). During this HS 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 non-supplemented 2FW pigs was higher in HS than in TN conditions throughout the day, while that of supplemented 2FW pigs were similar between periods except during non-feeding times when it was higher in HS than TN conditions (at hours 0 to 9, 14 and 23 ($P < 0·05$)). For 8FW groups during HS, body temperature was at BT_{max} for 11 h (14.00 h to 00.00 h) in non-supplemented pigs, while it lasted only for 8 h (17.00 h to 00.00 h) for the supplemented pigs. In contrast to the TN period, pattern of evaporative water loss closely followed time of feeding and body temperature pattern when pigs were subjected to HS, except for the LY – 8FW group in which it remained relatively constant compared with the other groups. Nevertheless, evaporative water loss increased for all groups during HS ($P < 0·05$). In the 2FW groups, evaporative water losses was higher in the supplemented pigs starting from 09.00 h to 00.00 h ($P < 0·05$), while

Table 4. Effect of feed access and live yeast supplementation on thermoregulation responses of male finishing pigs exposed to high ambient temperature†,‡,§

	Unli		2FW		8FW			
Items	N	Y	N	Y	N	Y	RSD†††	Statistics
Evaporated latent water (g/ d)								
TN	2659	2676	2249	2592	2628	2417	592	P**, D × FW*, D × FW × P*
HS††,‡‡	4158	4061	3729	5215	4864	3609		
Latent heat loss (% HP)								
TN	31·0	30·8	28·7	31·1	32·5	29·6	5·1	P**, D × FW*, D × FW × P**
HS††,‡‡	55·6	51·8	51·9	65·1	63·3	49·0		
Sensible heat loss (% HP)								
TN	66·3	66·8	69·0	66·7	65·2	68·2	5·7	P**, D × FW*, D × FW × P**
HS††,‡‡	42·0	46·0	45·9	30·9	32·7	49·2		
Heat loss due to water intake (% HP)								
TN	2·32	1·95	1·89	1·77	1·78	1·80	1·01	P*, D × FW***, D × FW × P*
HS††,‡‡	2·25	1·94	1·94	3·75	3·71	1·50		
Heat loss due to feed intake (% HP)								
TN§§,‖‖	0·45	0·47	0·43	0·44	0·44	0·44	0·02	D*, FW***, P**
HS††,‡‡	0·24	0·27	0·22	0·27	0·25	0·24		
Body temperature (°C)								
TN	38·58	38·70	38·42	38·57	38·47	38·39	0·18	P**, D × FW × P*
HS‖	38·89	39·11	39·18	38·90	38·84	38·89		

Unli, unlimited; FW, feeding window; N, non-supplemented diet; Y, live yeast-supplemented diet; RSD, residual standard deviation; TN, thermoneutral; P, period; D, diet; HS, heat-stressed; HP, heat production; LY, live yeast-supplemented diet.
* $P < 0·05$,
** $P < 0·01$,
*** $P < 0·10$.
† A total of thirty-six pigs were allocated to six experimental groups in nine replicates with two blocks per replicate. All pigs were housed under thermoneutral conditions (TN period; 22° C) from day −5 to −1 and then under heat-stressed conditions (HS period; 28°C) from day 0 to 4.
‡ Data were analysed using PROC MIXED model with FW, D, P and their interactions as fixed effects, with pig as a repeated unit per period.
§ Contrast statements within period ($P < 0·05$):
‖ (NS – Unli, NS – 2FW, NS – 8FW) *v.* (LY – Unli, LY – 2FW, LY – 8FW).
†† (NS – 2FW *v.* LY – 2FW).
‡‡ (NS – 8FW *v.* LY – 8FW)) for diet effect.
§§ (NS – Unli, LY – Unli) *v.* (NS – 2FW, LY – 2FW).
‖‖ (NS – Unli, LY – Unli) *v.* (NS – 8FW, LY – 8FW).
††† RSD.

it was the opposite in the 8FW groups (higher in the non-supplemented pigs from 11.00 h to 21.00 h ($P < 0·05$)). The maximum evaporated water was recorded for non-supplemented 8FW pigs for 9 h starting at 15.00 h (1 h after they attained BT_{max}), while it was recorded only for 5 h for supplemented 8FW pigs starting at 20.00 h (3 h after they attained BT_{max}).

Plasma metabolite and hormone concentrations

The average meal size at time 0 of blood sampling on day −2 (TN period) and day 1 (HS period), 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 day 1 (HS) for 2FW pigs (+335 g for Y pigs; $P = 0·04$), while the difference was only numerical for Unli pigs for TN and HS periods (–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 (including that of insulin: glucose) 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 TN ($P \leq 0·04$), and than 8FW during HS ($P = 0·04$), 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 TN conditions and within the 2FW groups during both TN and HS conditions ($P \leq 0·04$). As shown in Supplementary table 1, AUC and C_{max} of plasma insulin: glucose were also significantly lower in supplemented pigs within the 2FW groups during TN ($P < 0·05$) and during HS ($P = 0·05$) periods. Supplemented pigs also showed lower C_{max} for plasma lactate ($P = 0·02$) and glucose ($P = 0·06$) than non-supplemented pigs in TN conditions, 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 TN conditions.

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 and NEFA decreasing after a meal. There was an interaction between

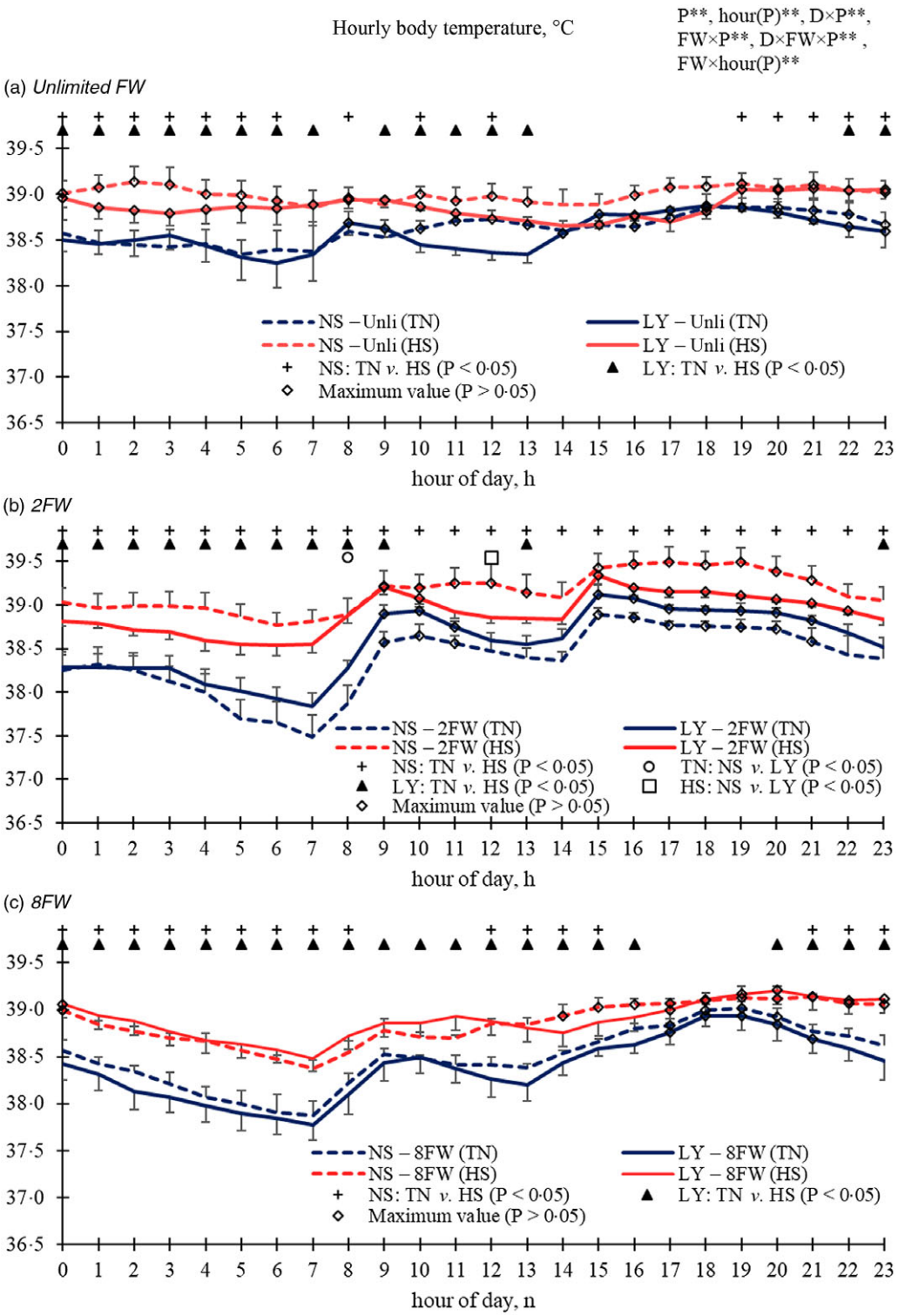

Fig. 2. Effect of diet (0 (NS) *v.* 100 (LY) g/ton live yeast supplementation) within each feeding window ((a) Unli *v.* (b) 2FW *v.* (c) 8FW) on the hourly body temperature (°C) of heat-stressed entire male finishing pigs (means ± SE). The broken line (—) represents pigs with NS diet and the solid line (—) pigs fed with LY diet during the thermoneutral or TN period (22°C; blue line) and the heat stress or HS period (28°C; red line). D: diet, FW: feeding window and P: period. $^{**}P < 0{\cdot}01$.

diet and FW, wherein plasma levels of creatinine and NEFA were lower for supplemented pigs in 2FW group ($P < 0{\cdot}05$) 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 ($P < 0{\cdot}01$). During the TN period, plasma T3 level was higher in 2FW than in Unli pigs ($P = 0{\cdot}02$) in non-supplemented but not in LY-supplemented pigs ($P = 0{\cdot}42$). Meanwhile, LY – 2FW had

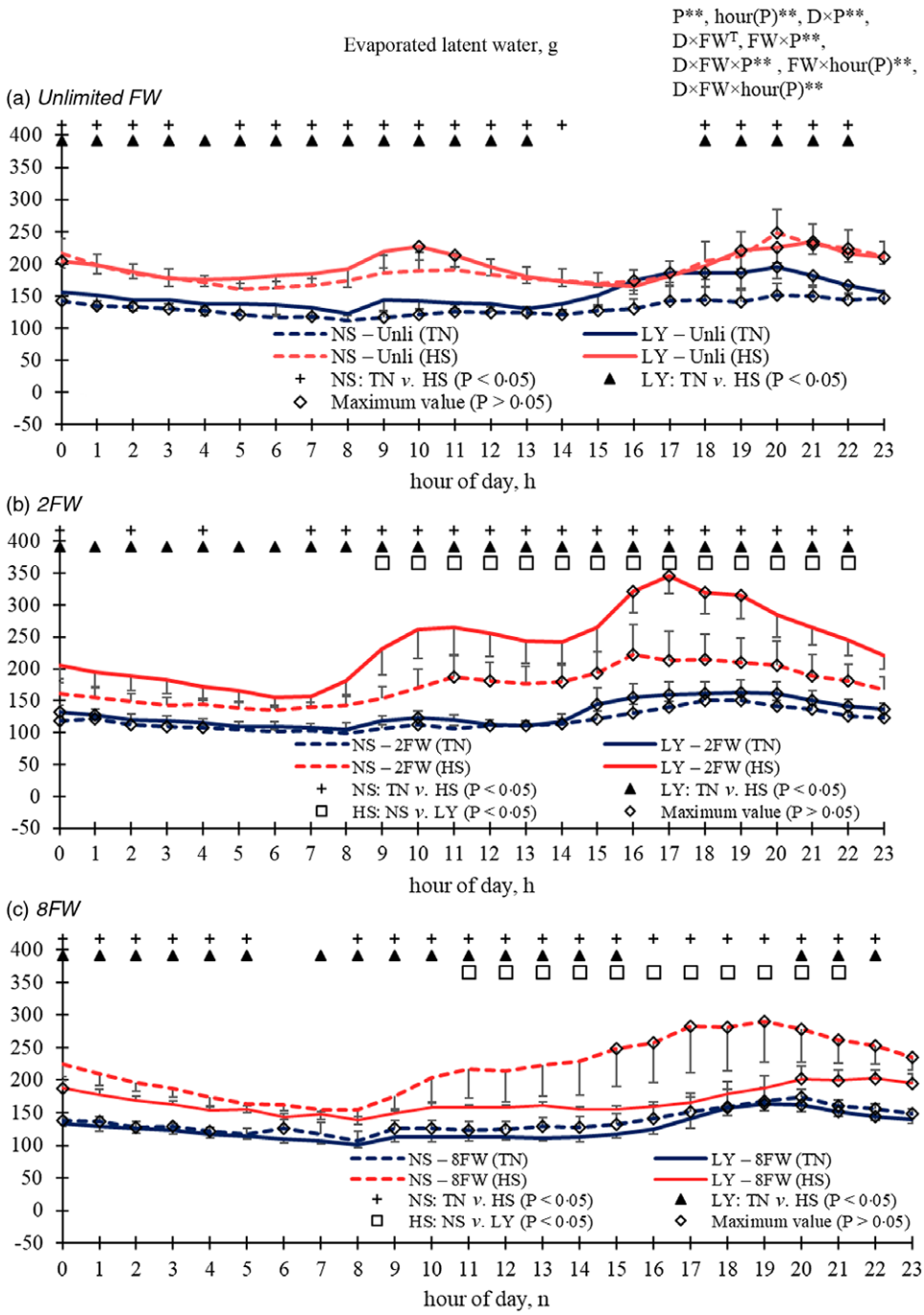

Fig. 3. Effect of diet (0 (NS) *v.* 100 (LY) g/ton live yeast supplementation) within each feeding window ((a) Unli *v.* (b) 2FW *v.* (c) 8FW) on the hourly evaporated latent water (g) of heat-stressed entire male finishing pigs (means ± SE). The broken line (—) represents pigs with NS diet and the solid line (—) pigs fed with LY diet during the thermoneutral or TN period (22°C; blue line) and the heat stress or HS period (28°C; red line). D: diet, FW: feeding window and P: period. $^{T}P < 0{\cdot}10$, $^{**}P < 0{\cdot}01$.

higher plasma T4 during HS than NS – 2FW and NS – 8FW groups ($P < 0{\cdot}05$).

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 behaviour 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[26]. In high ambient temperature, heat loss via

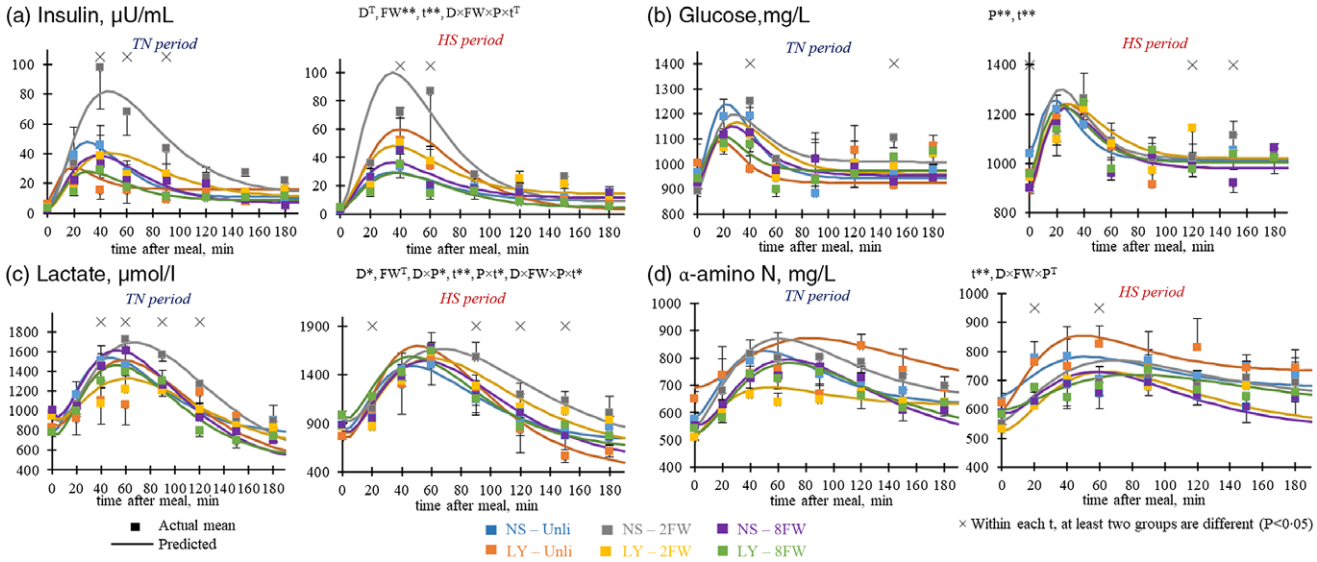

Fig. 4. Effect of diet (0 (NS) *v.* 100 (LY) g/ton live yeast supplementation) and feeding window (Unli *v.* 2FW *v.* 8FW) on the postprandial plasma concentrations of (a) insulin, (b) glucose, (c) lactate and (d) α-amino nitrogen of heat-stressed entire male finishing pigs (means ± SE). Pigs were housed under thermoneutral conditions (TN period; 22°C) from day −5 to −1 and then under heat-stressed conditions (HS period; 28°C) from day 0 to 4. Blood sampling was done on day −2 (TN) and on day 1 (HS). The line (—) corresponds to the predicted values at minute (time; t) obtained with the non-linear model (Supplementary Fig. 1). $^{T}P < 0{\cdot}10$, $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

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 NS – Unli pigs kept in HS conditions which is in agreement with the 60 % already reported by Renaudeau *et al.*[23] for pigs kept at 33°C and 75 % relative humidity. Body water balance is vital for thermoregulation[27]; thus, evaporative losses must be replenished explaining the increased water consumption in high ambient temperature. The water intake which increased by 274 % in HS compared to the TN period (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 NS – Unli pigs by −753 $g \cdot d^{-1}$ (or −30 %) to reduce metabolic HP related to nutrient utilisation (TEF)[28]. This value is higher than the −400 g DM $\cdot d^{-1}$ reported in the literature[29] 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 behaviour such as less time spent in the feeder, faster rate of feed intake, and decreased number of meals[20,30] and resulted to lower HP, RE, and thus lower nutrient deposition. The reduced HP during HS also accounts for the decreased thyroid hormones of HS pigs in the present and in previous studies[31,32]. Heat exposure did not affect plasma insulin levels in this study in contrast to previous reports[8,33], but it slightly increased glucose levels suggesting reduced insulin sensitivity in agreement with previous studies[34]. It can be hypothesised that dietary glucose is a less preferred energy source in hot conditions[35] which could partly explain the need for higher muscle breakdown during HS indicated by higher postprandial creatinine and urea levels during the HS period[36].

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

Between the two Unli groups, LY supplementation attenuated HS effects on DMI reduction (−20 % in LY – Unli *v.* −30 % in NS – Unli) that tended to improve energy efficiency (+5·5 points) and PD (+26 $g \cdot d^{-1}$) in agreement with Labussière *et al.*[9] Although the effect of LY on meal frequency was not significant unlike the aforementioned study, shorter inter-meal intervals were observed in supplemented pigs in the present study. 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 behaviour.

The following subsections focus 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 HP 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.

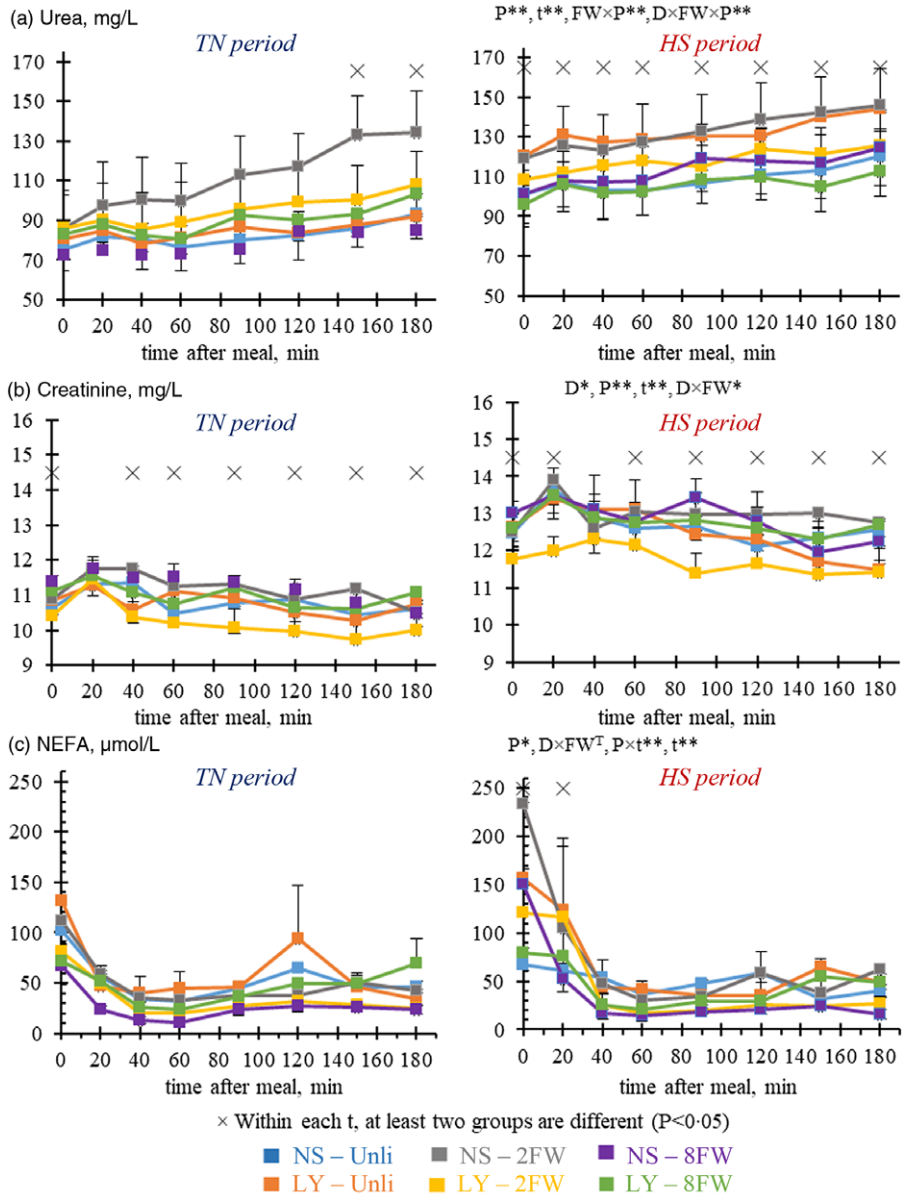

Fig. 5. Effect of diet (0 (NS) *v.* 100 (LY) g/ton live yeast supplementation) and feeding window (Unli *v.* 2FW *v.* 8FW) on the postprandial plasma concentrations of (a) urea, (b) creatinine and (c) NEFA of heat-stressed entire male finishing pigs (means ± SE). Pigs were housed under thermoneutral conditions (TN period; 22°C) from day −5 to −1 and then under heat-stressed conditions (HS period; 28°C) from day 0 to 4. Blood sampling was done on day −2 (TN) and on day 1 (HS). $^{T}P < 0{\cdot}10$, $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

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 about two meals a day. However, the 8FW strategy did not achieve the expected increase in the number of meals (NS – 8FW and NS – Unli had similar number of meals per d). As undigested nutrients in the ileum can trigger ileal brake which signals satiety and inhibits gastric emptying and feed intake[37], the imposed 90 min meal interval may not have been enough time for satiety signals to end prompting the NS – 8FW to skip a meal or two. Nonetheless, NS – 8FW still had higher meal frequency than the NS – 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 NS – 8FW than in NS – 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

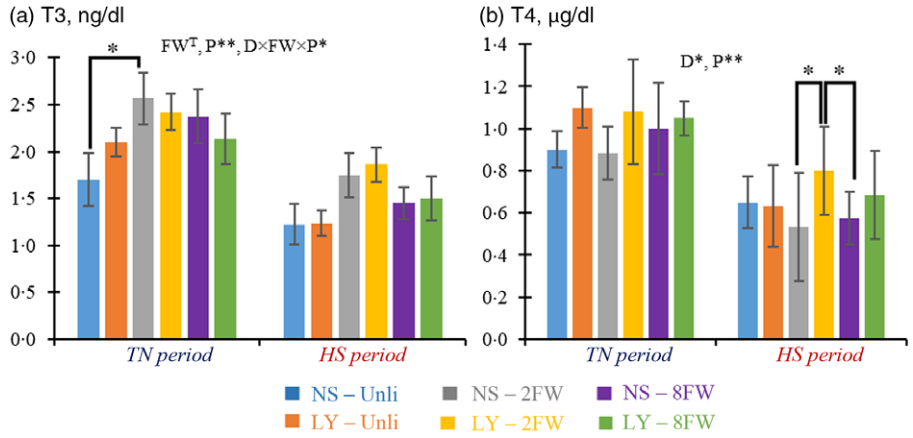

Fig. 6. Effect of diet (0 (NS) *v.* 100 (LY) g/ton live yeast supplementation) and feeding window (Unli *v.* 2FW *v.* 8FW) on the plasma concentrations of (a) T3 and (b) T4 of heat-stressed entire male finishing pigs (means ± SE). Pigs were housed under thermoneutral conditions (TN period; 22°C) from day −5 to −1 and then under heat-stressed conditions (HS period; 28°C) from day 0 to 4. Blood sampling was done on day −2 (TN) and on day 1 (HS). $^TP < 0{\cdot}10$, $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

sufficient to maintain constant core body temperature immediately after a meal in TN conditions.

The observations that body temperature (1) remained at BT_{max} over a longer period after a meal in NS – 2FW pigs and (2) reached BT_{max} earlier in the HS than in the TN period (14.00 h *v.* 17.00 h) in NS – 8FW pigs suggest that the ability to maintain thermal balance after a meal seemed to decrease upon heat exposure. Based on the higher preprandial body temperature of pigs during HS than during TN, 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[38]. 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 for heat dissipation when subjected to HS. It is unclear why BT_{max} was maintained longer in during HS even during non-feeding times. One hypothesis could be related to the reported decrease of gastric emptying rate in hot conditions[39] 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 NS – 2FW pigs compared with the NS – 8FW pigs in terms of reduction of DMI and energy efficiency (–30 % *v.* –17 % and –17·5 *v.* –6·5 points, respectively). Large meal size and high insulin levels have been reported to delay not just gastric emptying but also carbohydrate absorption[40,41]. Moreover, as insulin promotes glycolysis, it may have caused the prolonged and elevated lactate levels observed in NS – 2FW pigs from excess pyruvate production.

The NS – 8FW pigs in the present study seemed to have had better heat tolerance when compared with 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[42]. In addition, the higher water intake and evaporative heat losses observed in the NS – 8FW pigs when compared with the NS – 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', that is, their drinking bouts are stimulated by feed ingestion[43]. As a result, the higher water intake may have contributed to the increased latent heat losses of NS – 8FW pigs, since an animal's hydration status is closely linked to its evaporative heat loss capacity in order to maintain body water balance[44]. These mechanisms might have helped the NS – 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 NS – Unli and NS – 2FW pigs.

Despite the relatively higher RE measured in NS – 8FW compared with the other nitrogen groups during HS, their PD did not differ from NS – Unli pigs and did not reach the highest PD observed for the LY – 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[45] 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 with the Unli pigs in TN or HS conditions. However, it does not explain the +35 g/ d higher fat deposition of the NS – 8FW compared with NS – Unli pigs. PD entails high thermal load; thus, capacity of pigs to reach their maximum potential for PD is usually limited in hot conditions[46]. Since the NS – 8FW pigs already reached BT_{max} early at hour 14 during HS conditions, it can be assumed that additional ME intake from here onwards was deposited as fat, since the animal needed to maintain homoeostasis and deposition of fat has a lower thermal load than that of protein.

This present study therefore demonstrates that the thermal balance is a dynamic process highly influenced by the feeding level and behaviour 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 preprandial body temperature is a contributing factor, together with the ability of the animal to lose heat after a meal, to improve DMI and PD 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 with an increase in meal frequency in the present study. Comparing the NS – 8FW pigs to LY-supplemented pigs, the increased DMI during HS in the former group was not to the same extent as the latter (LY – Unli), neither did it result to an increase in PD. Furthermore, based on RE and PD measured in the HS period, supplemented pigs with low meal frequency (LY – 2FW) tolerated heat better than NS – 8FW pigs. However, it remains unclear why LY – 8FW pigs did not perform as well as the LY – Unli. As already mentioned, the feeding management employed in the present study may have been an oversimplified sense of 'frequent feeding' in supplemented pigs. Durations of inter-meal intervals were similar among the supplemented groups during the HS period (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, supplemented 8FW pigs still deposited more protein than non-supplemented 8FW pigs despite having lower ME intake. The LY – 8FW pigs attained BT_{max} at 1700 h whether in TN or HS conditions, which means their reduced PD in the HS period was not due to the effect of HS on PD potential, but rather due to lower feed intake. This inclination to prioritise and use higher proportion of ME intake for protein instead of lipid deposition has been observed in restricted-fed pigs in TN conditions[47]. One can thus hypothesise that LY – 8FW pigs were experiencing a lower level of HS than NS – 8FW pigs as supported by their relatively constant evaporative water loss in the HS period, suggesting that the sensible route was still the main route of LY – 8FW pigs for maintaining 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 behaviour with more meals taken during the cooler parts of the day in studies with cyclic or natural HS challenges[48,49]. 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 behaviour 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 LY – 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 based on the lower plasma insulin and insulin:glucose AUC than the non-supplemented 2FW pigs. A yeast extract from *S. cerevisiae* has already been reported to potentiate insulin action[50], and *S. boulardii* has been demonstrated to attenuate diabetic-related complications such as hyperglycaemia (due to impaired insulin activity or production) by microbiota modulation and improved immune responses[51–53]. As previously discussed, high insulin levels are associated with elevated lactate levels possibly from the excess pyruvate production as insulin promotes glycolysis. 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[54] 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 preprandial body temperature is important in alleviating effects of HS on feed intake. The lower preprandial body temperature and higher evaporative heat loss and water intake of supplemented 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 non-supplemented 2FW pigs. The hypothesised 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 BT_{max} of LY – 2FW and their slightly shorter inter-meal intervals (–44 min · d^{-1}) compared with NS – 2FW pigs. This shorter inter-meal interval in LY – 2FW pigs might have indirectly promoted water intake by prompting them to drink more often because of the prandial drinking behaviour of pigs[43]. Thus, the improved evaporative heat loss in the supplemented 2FW pigs may be 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[55] 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[56,57], but there are currently no studies on LY effect on respiratory health during a heat challenge. Nevertheless, with a more efficient ability to dissipate heat via the latent pathway after a meal, one can assume that LY – 2FW pigs had to rely less on a reduction in metabolic HP (alongside their T4 levels) to maintain homoeothermic status during hot

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conditions. Thus, the supplemented 2FW pigs were able to maintain higher DMI and higher potential for PD during HS which is also reflected in their lower plasma creatinine as there was less need to mobilise 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 with 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 HP and loss throughout the day. LY 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 HS. The lower thermal load in supplemented pigs helped them reach a higher maximum potential for PD, 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 behaviour is not the cause but a mere consequence of the improved energy metabolism and thermoregulation responses of LY supplementation in pigs.

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There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522002513

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