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Saccharomyces cerevisiae boulardii CNCM I-1079 supplementation in finishing male pigs helps to cope with heat stress through feeding behaviour and gut microbiota modulation

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Abstract

Pigs subjected to heat stress (HS) decrease their feed intake and growth. The objectives of the experiment were to determine the effects of live yeast (LY) supplementation (*Saccharomyces cerevisiae var boulardii* CNCM I-1079) on feeding behaviour, energy metabolism and faecal microbiota composition of finishing boars (*n* 10) housed in a respiration chamber at thermoneutrality (7 d at 22°C) or during HS (seven plus six days at 28°C). Dietary LY supplementation increased DM intake ($P = 0{\cdot}01$) whatever the ambient temperature, whereas HS decreased feed intake whatever the dietary supplementation ($P = 0{\cdot}01$). Dietary LY supplementation increased the number of meals ($P = 0{\cdot}02$). Energy retention was higher with dietary LY supplementation ($P < 0{\cdot}01$) but decreased during HS ($P < 0{\cdot}01$). The skin temperature of the supplemented pigs was lower at thermoneutrality and increased during HS to a lesser extent than that of non-supplemented pigs ($P < 0{\cdot}01$). Faecal microbiota composition was determined using 16S rRNA gene sequencing. *Treponema, Christensenellaceae R-7*, *Ruminococcaceae UCG-002, Rikenellaceae RC9, Clostridium sensu stricto 1* and *Romboutsia* genera and some bacteria belonging to *Alloprevotella*, *Oxalobacter* and *Anaeroplasma* genera were more abundant under HS. LY supplementation attenuated HS effects on *Romboutsia* abundance, while decreasing the abundance of some bacteria from *Ruminoccocus*, *Coprococcus*, *Peptococcus* and *Oxalobacter* genera and increasing the abundance of beneficial bacteria from *Lactococcus* and *Subdoligranulum* genera. Our results suggest that higher level of the keystone species *Ruminococcus bromii* at thermoneutrality may be one of the causes for higher energy retention observed under subsequent HS.

Key words: **Feed intake: Heat stress: Probiotics: Nutrition: Boars: 16S rDNA**

In the near future, global warming will lead to more frequent and intensive events of summer heatwave, even in temperate countries. For pig production, heatwaves induce an increase in ambient temperature in fattening buildings which can increase above the upper critical temperature for pigs, which is the upper limit of their thermoneutral zone. It has been shown that the upper critical temperature decreases from 26 to 21°C as pigs get older because finishing pigs are more sensible to heat stress (HS) than growing pigs[(1)]. Under HS, pigs face difficulties in maintaining their body temperature[(2)] because of a decreased ability in exporting heat caused by the decreased difference between body temperature and ambient temperature and associated sensible heat loss[(3)], whereas pigs are deprived of sweat glands[(4)]. Because of their high heat production (HP) for maintenance purposes[(5)], entire male pigs may be more sensible to HS than other genders. The principal consequence of HS in pigs is the decreased feed intake[(1)], to limit the oxidation of nutrients and associated HP. In order to mitigate the consequences of HS on feed intake that lead to altered growth performance, nutritional strategies have been implemented to limit HP while maintaining the same level of energy retention. For example, decreased dietary protein content, or increased dietary net energy density, by dietary addition of fat is techniques to avoid the utilisation of extra amino acid or volatile fatty acid for ATP synthesis, which is usually done with a lower energetic efficiency than with other nutrients[(6)]. Nevertheless, all these techniques

Abbreviations: AHP, heat production due to physical activity; BW, body weight; D, diet; FHP, fasting heat production; HP, heat production; HS, heat stress; LY, live yeast; ME, metabolisable energy; OTU, Operational Taxonomic Units; P, period; RE, energy retention; TEF_s, short-term thermic effect of feeding.

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only limit the negative consequences of HS, but they do not succeed in restoring the growth performance to the level achieved at thermoneutrality. These techniques have been thought to decrease the global load on energy metabolism, as estimated from the daily HP, although it can be considered that HP is not constant during the day. Indeed, HP varies principally according to variations in physical activity and feeding behaviour[7]. After a feed intake, bout, digestion, absorption and metabolic utilisation of nutrients lead to the so-called thermic effect of feeding, which is a transient increase in instantaneous HP. According to the thermostatic theory[8], such an increase induces an increase in body temperature that may exert a strong but transient negative control on feed intake.

Dietary live yeasts (LY) are widely used as probiotics in livestock, with numerous studies demonstrating a beneficial effect on health and performance[9]. We thus suppose that dietary supplementation with LY could help pigs facing HS. Indeed, a dietary supplementation with a *Saccharomyces cerevisiae boulardii* strain has been shown to induce an increased feed intake in lactating sows, that is, during a period when the ambient temperature is above their thermoneutral zone[10]. In dairy cows, LY supplementation has been shown to induce an increased meal frequency and a decreased meal size[11], that may limit the increase in HP shortly after a meal (by decreasing the amount of nutrients and energy consumed at each meal). Moreover, in dairy cows submitted to HS, LY dietary supplementation induces changes in feeding behaviour associated with improved lactation performance[12]. Another study furthermore evidenced a decrease in rectal temperature of 0·2°C[13] in LY-supplemented cows that may increase the temperature difference between the animal and its environment, thus helping them to face HS. However, the mechanisms underlying the beneficial effect of different strains of *S. cerevisiae* supplementation under HS conditions are not fully understood.

In close interaction with its hosts, the gastrointestinal microbiota is a major player modulating several physiological functions in humans and animals. With the development of high-throughput sequencing techniques, it has been extensively studied and shown to be involved in the modulation of immunity, nutrient digestion and host metabolism[14,15]. HS effects on the gut microbiota composition have been evidenced in rats, broilers, growing pigs and gestating sows[16–20]. We thus hypothesise that changes in microbiota composition through dietary probiotic intervention could modulate the deleterious effects of HS, specifically by modulating feed intake and energy retention.

The objectives of the study were to determine the effects of dietary supplementation with the LY *S. cerevisiae var. boulardii* (CNCM I-1079) on feeding behaviour and thermal heat acclimatisation in male finishing pigs as well as investigate the potential interactions between the gut microbiota composition and energy balance and performances.

Materials and methods

The experiment was conducted according to the current French law on animal experimentation and ethics and under the direction of E. Labussière (authorisation number by the French Ministry of Research: 02280.03).

Experimental design, animals, housing and feeding

The experiment was designed to study the effect of dietary LY supplementation on acclimation to HS of finishing pigs through characterisation of energy utilisation. Five replicates of two male pigs (Pietrain × (Landrace × Large White)) from five different litters were used for measuring their feeding behaviour, HP, energy and nitrogen balances. For each replicate, the experiment was conducted during three consecutive periods of 7, 7 and 6 d when the pigs were fed, followed by a supplementary day when the pigs received no feed to assess their BMR.

From 80 kg body weight (BW) onwards, the two littermate pigs were housed in individual metabolism crates with an ambient temperature at 22°C and fed either a standard finishing diet based on wheat, maize, barley and soyabean meal (online Supplementary Table S1) or the same diet supplemented with 100 g/ton of feed of Levucell SB TITAN (corresponding to 1×10^6 CFU/g of feed of *S. cerevisiae var. boulardii* CNCM I-1079). After this 2-week adaptation period, the animal in its cage was placed in a 12-m^3 open-circuit respiration chamber[21]. During the first 7 d in the respiration chamber (P1 – thermoneutrality), the ambient temperature was maintained at 22°C and it was then increased up to 28°C for the last 14 d in the respiration chamber that was spread over a short- (P2 – from days 8 to 14) and long- (P3 – from days 15 to 20) term acclimatisation to high ambient temperature (Fig. 1). For the first replicate, the ambient temperature varied from 22 to 28°C on the eighth day in the respiration chamber according to a 2°C/h increase from 11.00 hours onwards. Because pigs had difficulties to cope with the challenge, the ambient temperature for the other replicates varied from 22 to 25°C on the eighth day, and then from 25 to 28°C in the ninth day in the respiration chamber according to a 1·5°C/h increase from 11.00 hours onwards. The relative humidity was kept constant at 70 %, and a 12-h lighting time span (from 07.30 to 19.30 hours) was used during the 21 d in the respiration chamber.

The cage where the pig was housed was mounted on force sensors (9104A, Kistler) producing an electrical signal proportional to the physical activity of the pig[22]. Apart from 06.00 to 09.00 hours and from 15.45 to 16.00 hours, the pigs had free access to the diet that was distributed twice daily. On the days of ambient temperature increase, the pigs were only offered 500 g of diet from 09.00 to 16.00 hours. The pigs had free access to water that was provided in a drinking bowl.

Measurements and samplings

The animals were weighed five times at 08.30 hours: at the entrance in the respiration chamber on the first day, on the 8th, 15th and 21st d and at the end of the measurements in the respiration chamber (i.e. on the morning of the day after the 21st d in the chamber). The daily feed allowance was weighed, and the diets were sampled during each period. Samples of each diet were pooled over the whole experiment. Feed refusals and spillages (if any) were collected at the end of each period of measurements and were oven-dried to calculate the refused DM. Additionally, the feed intake was continuously recorded (time, duration and amount ingested at each meal) using a weight sensor placed under the trough.

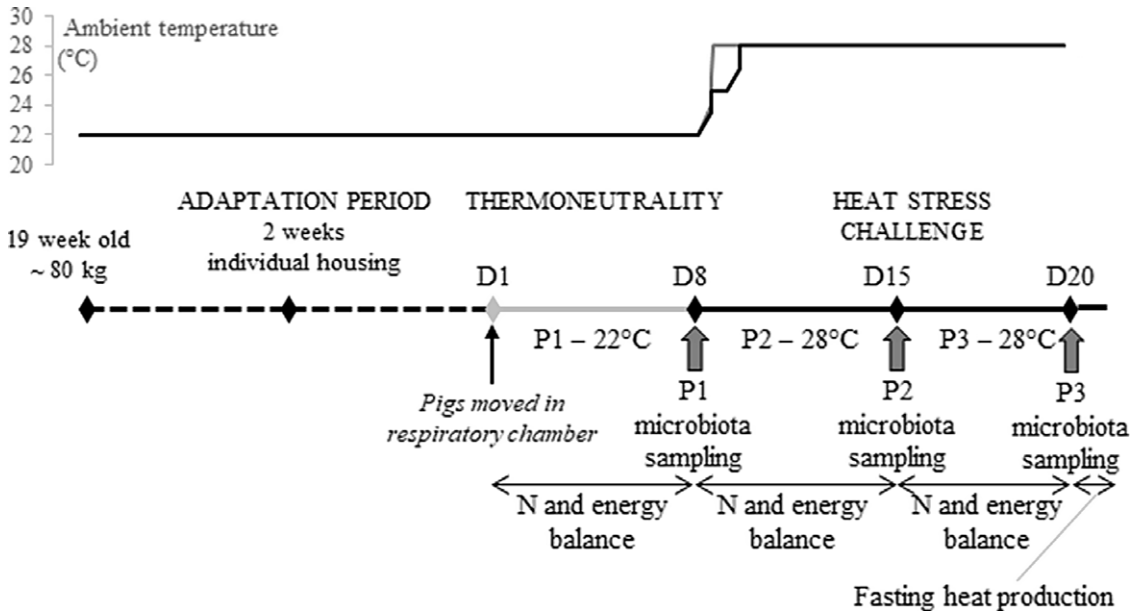

Fig. 1. Description of experimental design to determine the effects of heat stress on feeding behaviour, energy metabolism and faecal microbiota in pigs. ——, Replicate 1; ——, Replicates 2–5.

Similarly, water consumption was measured using a weight sensor placed under the tank where the water was stored outside the respiration chamber. In order to measure skin temperature at every 1-min interval, a contact temperature probe was fixed onto the neck of the pig using a medical adhesive tape. The faeces were collected and pooled over each period; they were weighed at the end of each period and sampled for DM determination and for laboratory analyses after freeze-drying and grinding through a 1 mm grid. The urine was collected daily in a bucket that contained 120 ml of sulphuric acid (1·8 mol/l) to prevent from microbial fermentations resulting in ammonia losses. Urine was weighed daily, and a representative sample was cumulated over each period and stored at −20°C for laboratory analyses. Ammonia losses (evaporated N) recovered in condensed water, and outgoing air of the chamber were collected and cumulated over the three stages as described by Noblet *et al.*[23]. Grab samples of faeces were also collected in the rectum of the pigs at the end of each period. They were immediately frozen in liquid N_2 and then stored at −80°C pending microbiota analyses.

The O_2 concentration of the outgoing air was measured with a paramagnetic differential analyser (Oxymat 6, Siemens AG), whereas the CO_2 and CH_4 concentration was measured with an infrared analyser (Ultramat 6, Siemens AG). Gas extraction rate of the air of the chamber was measured with a mass gas meter (Teledyne Brown Engineering). Gas concentrations, the signals of the force sensors, the weight of the trough, gas flow rate, ambient temperature and relative humidity in the respiration chamber were measured sixty times per second, averaged over 10-s intervals and recorded for further calculations[24]. Two similar chambers were used during the experiment. The recovery of known amounts of CO_2 and N_2 was measured before and after the experiment; it averaged 100·1 and 100·4 % for CO_2 and 100·7 and 101·1 % for N_2 for respiration chambers 1 and 2, respectively.

Laboratory analyses

Periodic samples of each diet (three per pig) were analysed for DM determination. The pooled samples of each diet and the individual faecal samples at each period were analysed for DM, ash, N (Dumas method) and gross energy contents[25]. The pooled samples of each diet were analysed for ether extract, starch and crude fibre contents. Samples of urine were analysed for N content on fresh material. Gross energy content in the urine was obtained after freeze-drying approximately 30 ml of urine in polyethylene bags.[25] The ammonia content of condensed water and extracting air was determined on fresh material using an enzymatic method (Enzytec fluid, Scil Diagnostics GmbH).

Calculations

The daily DM intake was calculated as the difference between average daily offered DM and average refused DM over each period of measurements. The apparent faecal digestibility coefficients for DM, ash, N and energy were calculated from nutrient intake and faecal excretion. Nitrogen retention was calculated as the difference between ingested N and N lost in faeces, urine and evaporated in the chamber. Digestible energy and metabolisable energy (ME) intakes were calculated according to the standard methods including CH_4 production. Total HP was calculated from respiratory gas exchanges and urinary N (including evaporated N) according to the formula of Brouwer[26]. The first day of each period in the respiration chamber was considered as an adaptation day, and it was not included in the calculations. Energy retention (RE) was calculated as the difference between ME intake and HP. Energy retained as protein was calculated from N balance, assuming an energy content of protein (Nitrogen retention × 6·25) of 23·6 kJ/g, and energy retained as fat was calculated as the difference between total energy retained and energy retained as protein. Fat deposition was then calculated assuming an energy content of 39·7 kJ/g fat. Data from

the feeding trough were used to describe feeding behaviour through the number of meals per day (considering 20 min as a meal criterion), the feeding rate and the duration of feeding[27]. Simultaneous measurements of O_2 and CO_2 concentrations in the respiration chamber, signals of force sensors, meals information (time of intake and ingested quantity) and physical characteristics of the gas in the chamber were used to calculate the components of HP according to the modelling approach of van Milgen *et al.*[24]. Parameters of the differential equations for gas concentrations in the respiration chamber involved in the model were estimated in R software[5]. The HP due to physical activity (AHP), feed intake (short-term thermic effect of feeding, TEF_s) and resting metabolism were calculated from respective volumes of O_2 consumption and CO_2 production according to the formula of Brouwer[26] excluding urinary N losses and CH_4 production. Data from the last day in the respiration chamber when the pigs received no feed were used to determine fasting HP (FHP) according to the modelling approach of van Milgen *et al.*[24]. All energy traits were expressed relative to metabolic BW calculated as $BW^{0.60}$[28].

Microbial DNA extraction and 16S rRNA gene sequencing

Microbial DNA was extracted from 40 to 60 mg of faeces using a ZR-96 Soil Microbe DNA Kit™ (Zymo Research) according to the manufacturer's instruction. A 15-min bead beating step at 30 Hz was applied using a Retsch MM400 Mixer Mill. The V3 and V4 hypervariable regions of the 16S rRNA gene were amplified using the primers F343 (CTTTCCCTACACGACGCTCTTCCGATCT ACGGRAGGCAGCAG) and R784 (GGAGTTCAGACGTGTGCTC TTCCGATCTTACCAGGGTATCTAATCCT). High-throughput sequencing was performed on a MiSeq sequencer using the Reagent Kit version 3, according to the manufacturer's instruction (Illumina Inc.) in the Genomic and Transcriptomic Platform (INRA) and as previously described[29].

Bioinformatics analyses

Generated paired-end 250 bp sequences were assembled using Flash software[30] (10 bp minimum overlap, 10 % maximum mismatch). Assembled sequences were processed using FROGS pipeline[31]. Briefly, sequences were clustered in Operational Taxonomic Units (OTU) using SWARM algorithm[32]. Chimeric sequences were then detected by samples using UCHIME algorithm[33] and removed from all samples. After quality filtering and chimaera removal, 38 507 ± 4815 reads were kept per samples. Taxonomic annotation of the OTU was performed using the SILVA SSU Ref NR 128 database[34] and BLAST+[35] and RDP[36] algorithms. BLAST hits with identity and coverage alignments higher than 99 % were kept for annotation. Otherwise, species were annotated as unknown, and RDP classifier results were used for higher rank. Bootstrap thresholds were set to 0·9 and 0·8, respectively, for annotation at the genus rank and higher ranks.

Sequences were deposited in Sequence Read Archive: accession number PRJNA649405.

Statistical analyses

The number of animals (n 10) was determined using GLMPOWER procedure[37] based on previous measurements of ME intake of pigs submitted to a HS challenge[3] 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 three periods, at thermoneutrality and under short- and long-term acclimation to HS.

Analysis of growth performance and nutrient and energy balance. Growth performance, apparent digestibility coefficients, components of N and energy balance were tested for the effects of diet (D), period (P) and their interaction (D × P). The effect of replicate was also included in the model, but it is not described in detail as it did not affect the interpretation of the results. The FHP was only measured once per pig at the end of period P3; the data were tested for the effect of diet in a GLM procedure.[37] Components of feeding behaviour, which were measured daily, were analysed for the effects of replicate, P, D, their interaction and the effect of the day as repeated effect. Skin temperature (only four replicates were available) was averaged per hour and analysed for the effects of replicate, P, D, their interaction, and the interaction between the day and hour as repeated effect. A pairwise comparison of skin temperature between diets was also tested day by day in a GLM procedure[37]. Unless stated otherwise, the statistical models were tested in a MIXED procedure with the pig as repeated unit assuming a 'compound symmetry' covariance structure[37].

Analysis of faecal microbiota composition. A rarefaction step was applied – that is, the same number of sequences was kept in all samples to avoid bias due to differences in sequencing depth (random sampling with 27 397 reads kept per samples), followed by a filtering step to remove all singletons (i.e. OTU represented by only one read). The generated OTU count table was normalised by total sum scaling – that is, relative abundance was calculated and given as percentage of the total counts per sample. The table of relative abundances containing 5555 OTU was used to calculate richness and Shannon indices and perform differential analyses after taxonomic binning.

Multivariate analyses were performed using MixOmics R package[38] in R software. The OTU count table was first filtered to keep only OTU that represent at least 0·001 % of the total sequences (1698 OTU), then normalised by total sum scaling and subjected to centred log-ratio transformation (with a 0·001 offset) as advised for compositional data[39]. Sparse Partial Least Square Discriminant Analysis, a supervised classification method, was applied to identify the OTU that contribute the most to discriminate the samples according to the sampling date and the diet[40]. Tuning steps were performed to determine the number of components and the number of OTU for each component to keep. An iterative cross-validation step allowed to estimate the stability of the selected OTU (200 repeats, 10 folds).

A mixed linear model accounting for the fixed effect of the date of sampling, the diet and their interaction, as well as the random effect of the animal, was applied to analyse microbiota compositional data after double square root transformation. Normality of the residuals and homoscedasticity was tested using Shapiro and Bartlett test, respectively. ANOVA with Satterthwaite correction of df and Tukey's multiple comparisons of means were performed using lmerTest and emmeans R packages.

Table 1. Effect of dietary live yeast supplementation (*Saccharomyces cerevisiae var. boulardii*; CNCM I-1079, 1 × 106 CFU/g of feed) on feed and water intake, feeding behaviour, growth and skin temperature in finishing male pigs housed at thermoneutrality or high ambient temperature (*n* 10; LS-means and standard deviations)

	Period (ambient temperature)			Significance*			
	P1 (22°C)	P2 (28°C)	P3 (28°C)	RSD	Diet	Period	D × P
Mean BW (kg)				0·8	0·40	<0·01	<0·01
Non-supplemented diet	100·5^{a}	108·1^{bc}	114·9de				
Supplemented diet	102·1^{ab}	111·0^{cd}	119·5^{e}				
ADG (kg/d)				0·28	0·03	0·56	0·40
Non-supplemented diet	1·26	0·98	1·19				
Supplemented diet	1·23	1·29	1·33				
Water intake (kg/d)				4·1	0·23	0·04	0·48
Non-supplemented diet	6·7	9·3	9·4				
Supplemented diet	10·5	17·3	16·9				
DM intake (g/d)				205	0·01	0·01	0·18
Non-supplemented diet	2542	2132	2120				
Supplemented diet	2732	2558	2662				
Number of meals per day†				2·2	0·02	0·06	0·26
Non-supplemented diet	5·7	6·2	4·7				
Supplemented diet	6·4	7·3	6·7				
Total duration of eating (min/d)†				18	<0·01	<0·01	<0·01
Non-supplemented diet	70^{b}	55^{a}	46^{a}				
Supplemented diet	73^{b}	67^{b}	72^{b}				
Feeding rate (g/min)†				9	0·02	<0·01	<0·01
Non-supplemented diet	40^{a}	47^{b}	56^{c}				
Supplemented diet	41^{a}	44^{ab}	44^{ab}				
Skin temperature‡ (°C)				0·5	<0·01	<0·01	<0·01
Non-supplemented diet	36·9^{a}	38·0^{d}	38·0^{d}				
Supplemented diet	36·8^{a}	37·8^{c}	37·7^{b}				

ADG, average daily gain; BW, body weight; RSD, residual standard deviation.
^{abcde}LS-means for one parameter with different superscripts differ ($P < 0·05$).
* Unless stated otherwise, data were analysed for the effects of diet, period and their interaction (D × P) with the pig as repeated unit between periods.
† Data were obtained on a daily basis and were analysed for the effects of diet, period and their interaction (D × P) with the pig as repeated unit between days.
‡ Data (only four replicates were available) were averaged on an hourly basis and were analysed for the effects of diet, period and their interaction (D × P) with the pig as repeated unit between hours and days.

A Kruskal–Wallis Rank Sum Test followed by Pairwise Test for Multiple Comparisons of Mean Rank Sums (Nemenyi test, PMCMR R package) was used to analyse microbiota data that did not fulfil ANOVA prerequisites.

Regularised canonical correlation, an exploratory approach, was used to highlight the correlation between the OTU data sets (P1, P2 and P3) and performances and energy balance data[41]. The threshold values of the canonical correlations were set using an iterative procedure.

Results

Growth performance and feeding behaviour

Between periods P1 and P3, the mean BW significantly increased from 100·5 to 114·9 kg and from 102·1 to 119·5 kg for the pigs fed the standard diet and the LY-supplemented diet, respectively ($P < 0·01$; Table 1), in agreement with a higher average daily gain when the diet was supplemented with LY (1·28 *v.* 1·14 kg/d, respectively; $P = 0·03$). The water intake was significantly higher at 28°C rather than at 22°C. The DM intake was significantly lower during periods P2 and P3 than during period P1, whereas it was increased by LY supplementation (+380 g/d on average during the 3 periods; $P = 0·01$). The number of meals per day was significantly higher when the diet was supplemented with LY (6·8 *v.* 5·5 meals per day), and it tended to increase during period P2. The duration of eating in pigs fed the standard diet significantly decreased when ambient temperature increased up to 28°C, whereas duration of eating of pigs fed the supplemented diet remained unaffected by ambient temperature ($P < 0·01$). Similarly, the feeding rate of the non-supplemented diet pigs increased between periods P1 and P3, whereas the feeding rate of the supplemented diet pigs averaged 43 g/min during the three periods. The skin temperature was monitored on a minute basis and then averaged per hour. It did not significantly differ during the first period between the two diets (36·9°C). The pigs fed the non-supplemented diet exhibited an increased skin temperature during periods P2 and P3 (38·0°C), whereas the pigs fed the supplemented diet exhibited a moderately increased skin temperature during P2 (37·8°C) followed by a decrease during period P3 (37·7°C; $P < 0·01$). These variations are mainly due to the significantly lower skin temperature from day 16 onwards when pigs were fed the diet supplemented with LY (Fig. 2).

Digestive and metabolic utilisations of nutrients and energy

The digestibility coefficients of nutrients and energy did not significantly differ between diets and periods and averaged 85·3, 87·0, 85·9 and 85·3 % for DM, organic matter, N and energy, respectively (online Supplementary Table S2). The increased methane production between periods P1 and P3 tended to be higher when the diet was supplemented with LY. The associated energy loss and the amount of energy lost in urine resulted in a tendency for a lower ME/digestible energy for the supplemented

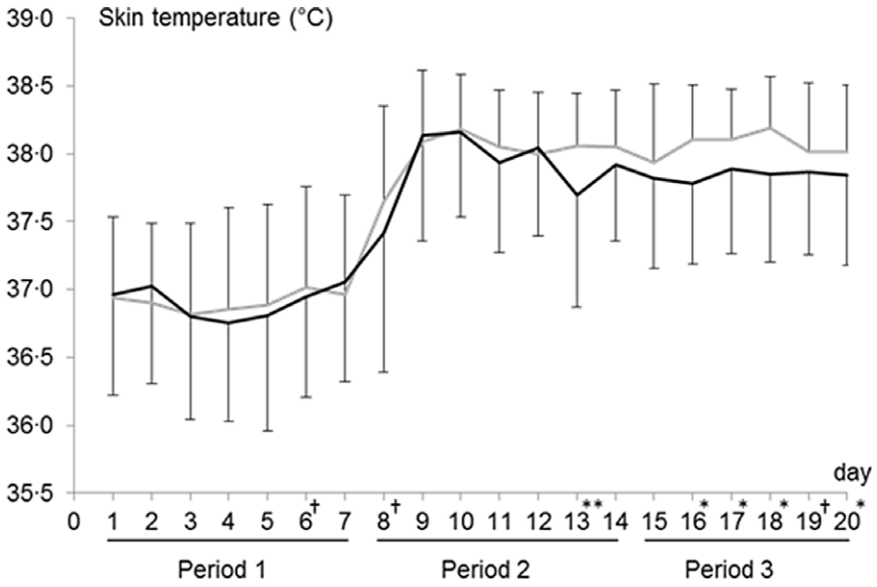

Fig. 2. Effect of dietary live yeast supplementation (*Saccharomyces cerevisiae var. boulardii*; CNCM I-1079, 1×10^6 CFU/g of feed) on skin temperature in finishing male pigs housed at thermoneutrality (22°C, Period 1) or high ambient temperature (28°C, Period 2 and Period 3) (*n* 10; values are means and standard deviation). Skin temperatures were analysed by day to test the effect of live yeast dietary supplementation: †$P < 0{\cdot}10$; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$. ——, Non-supplemented diet; ——, Supplemented diet.

diet and a higher ME/digestible energy for the non-supplemented diet during period P2 than during other periods. In line with the variations in feed intake, the digestible N intake significantly differed between diets and periods but N retention was not significantly affected by period (34 g/d on average that is 53 % of digestible N intake). Logically, the LY dietary supplementation tended to increase N retention ($P = 0{\cdot}10$).

The components of energy balance are described in Table 2. The ME intake was significantly higher when the diet was supplemented with LY but decreased significantly between period P1 and periods P2 and P3 whatever the dietary treatment. Total HP significantly decreased between periods P1 and P3 because of the associated decrease in HP related to resting metabolism and thermic effect of feeding (TEF_s), whereas HP associated with physical activity (AHP) increased. The LY dietary supplementation increased significantly the TEF_s, especially during period P3 (+22, +22 and +75 kJ/kg $BW^{0{\cdot}60}$ per d during periods P1, P2 and P3, respectively; $P = 0{\cdot}09$). It also tended to increase the AHP. The FHP was only estimated at the end of period P3; it was similar for the two dietary treatments and averaged 796 kJ/kg $BW^{0{\cdot}60}$ per d. Total RE was significantly higher during period P1 than during periods P2 and P3 and when the diet was supplemented with LY. The variation in RE between periods was associated with variations in both energy retained as protein and energy retained as fat, whereas the variation in RE between diets was only associated with variations in energy retained as fat. When expressed as a percentage of ME intake, RE decreased significantly when ambient temperature increased (from 42 to 39 % between period P1 and periods P2 and P3), whereas the dietary LY supplementation increased RE from 38 to 42 % of ME intake ($P = 0{\cdot}03$). The proportion of ME dissipated as AHP was not significantly affected by dietary supplementation, but it increased when ambient temperature increased (from 7·7 to 11·4 % between period P1 and periods P2 and P3). The proportion of ME lost as TEF_s tended to be lower when ambient temperature increased. In line with the variations in ME intake and RE, the respiratory quotient was the highest during the first period and was higher when the diet was supplemented with LY during all periods.

The protein deposition did not significantly vary between periods but tended to be higher when the diet was supplemented with LY (227 *v.* 193 g/d, on average between periods; $P = 0{\cdot}10$). The fat deposition was significantly reduced when ambient temperature increased but was higher with the supplemented diet (+73 g/d; $P < 0{\cdot}01$).

Microbiota analysis

A total of eighteen Phyla, twenty-seven Classes, sixty Orders, ninety-five Families and 271 Genera were identified (online Supplementary Table S3). Only sixty-three OTU were affiliated to fifty-one different known species (BLAST identity > 99 %), and 26·5 % of the OTU remained unclassified at the genus level. The dominant bacterial phyla were *Firmicutes* (64·3 % ± 7·4 %) and *Bacteroidetes* (25·2 % ± 5·7 %), respectively, dominated by *Ruminococcaceae* (20·6 % ± 3·7 %) and *Prevotellaceae* (14·1 % ± 5·1 %) families, represented by 25·9 % and 16·2 % of the OTU (Fig. 3 and online Supplementary Fig. S1).

The richness was higher at the end of the third period when compared with periods 1 and 2; however, Shannon indices were stable over the three periods. Dietary LY supplementation had no effect on α-diversity (online Supplementary Fig. S2). Among the eight most abundant phyla, only *Spirochaetes* showed a significantly increased relative abundance when compared with thermoneutrality conditions ($P < 0{\cdot}05$), while LY dietary supplementation had no effect on its abundance. A LY supplementation only affected *Elusimicrobia* phylum abundance, which was increased in pigs receiving the supplemented diet (online Supplementary Table S4).

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Table 2. Effect of dietary live yeast supplementation (*Saccharomyces cerevisiae var. boulardii*; CNCM I-1079, 1×10^6 CFU/g of feed) on nitrogen and energy balance in finishing male pigs housed at thermoneutrality or high ambient temperature (*n* 10; LS-means and standard deviations)

	Period (ambient temperature)			Significance*			
	P1 (22°C)	P2 (28°C)	P3 (28°C)	rSD	Diet	Period	D × P
Energy balance							
ME intake (kJ/kg $BW^{0·60}$ per d)				182	<0·01	<0·01	0·18
Non-supplemented diet	2441	1962	1876				
Supplemented diet	2554	2286	2304				
Heat production (kJ/kg $BW^{0·60}$ per d)							
RHP				48	0·76	<0·01	0·24
Non-supplemented diet	985	843	812				
Supplemented diet	968	899	817				
FHP†				64	0·98	–	–
Non-supplemented diet			796				
Supplemented diet			795				
AHP				25	0·09	<0·01	0·37
Non-supplemented diet	184	238	209				
Supplemented diet	199	266	257				
TEF_s				29	0·03	<0·01	0·09
Non-supplemented diet	243	170	161				
Supplemented diet	265	191	236				
Total				57	0·11	<0·01	0·12
Non-supplemented diet	1411	1252	1183				
Supplemented diet	1432	1356	1310				
Energy retention (kJ/kg $BW^{0·60}$ per d)							
As protein				39	0·13	0·02	0·72
Non-supplemented diet	316	266	248				
Supplemented diet	343	307	304				
As fat				119	<0·01	<0·01	0·26
Non-supplemented diet	714	444	445				
Supplemented diet	779	623	691				
Total				141	<0·01	<0·01	0·28
Non-supplemented diet	1030	710	693				
Supplemented diet	1122	930	995				
Utilisation of dietary ME (%)							
As AHP				1·3	0·93	<0·01	0·84
Non-supplemented diet	7·5	12·0	10·9				
Supplemented diet	7·9	11·7	11·1				
As TEF_s				1·5	0·28	0·06	0·24
Non-supplemented diet	10·1	8·8	8·5				
Supplemented diet	10·4	8·4	10·4				
As energy retention				3·1	0·03	0·02	0·23
Non-supplemented diet	41·6	35·5	36·3				
Supplemented diet	43·2	40·5	42·8				
Respiratory quotient (L CO_2/L O_2)				0·015	0·02	<0·01	0·12
Non-supplemented diet	1·104	1·066	1·069				
Supplemented diet	1·112	1·092	1·106				
Nutrient deposition							
Protein (g/d)				28	0·10	0·23	0·61
Non-supplemented diet	213	186	181				
Supplemented diet	233	220	227				
Fat (g/d)				49	<0·01	<0·01	0·18
Non-supplemented diet	288	187	194				
Supplemented diet	316	265	308				

ME, metabolisable energy; RHP, resting heat production; FHP, fasting heat production; AHP, physical activity related heat production; TEFs, short-term component of thermic effect of feeding; rSD, residual standard deviation.
* Unless stated otherwise, data were analysed for the effects of diet, period and their interaction (D × P) with the pig as repeated unit between periods.
† Only one value of FHP was obtained for each pig at the end of period 3. The data were analysed for the effect of diet.

A supervised classification method (Sparse Partial Least Square Discriminant Analysis) was applied to decipher the period and diet effects on the faecal microbiota composition. This analysis allowed to discriminate between pigs fed the non-supplemented diet and pigs fed the LY-supplemented diet, keeping only twenty-seven and nineteen OTU to compute, respectively, components 1 and 2 (Fig. 4, online Supplementary Table S5). Interestingly, the separation between samples from the non-supplemented and supplemented diets pig was accentuated under HS condition, especially at the end of the third period (Fig. 4(a)). Among the forty-six selected OTU, twenty-two were affiliated to the predominant *Ruminococcaceae* family (Fig. 4(b)). Ten OTU were selected with a stability frequency across subsampling cross-validation higher than 0·5 (Fig. 4(c) and (d)). Those most stable OTU selected on the first component were affiliated to *Alloprevotella*, *Oxalobacter*, *Anaeroplasma*, *Ruminococcaceae*, *Christensenellaceae* and

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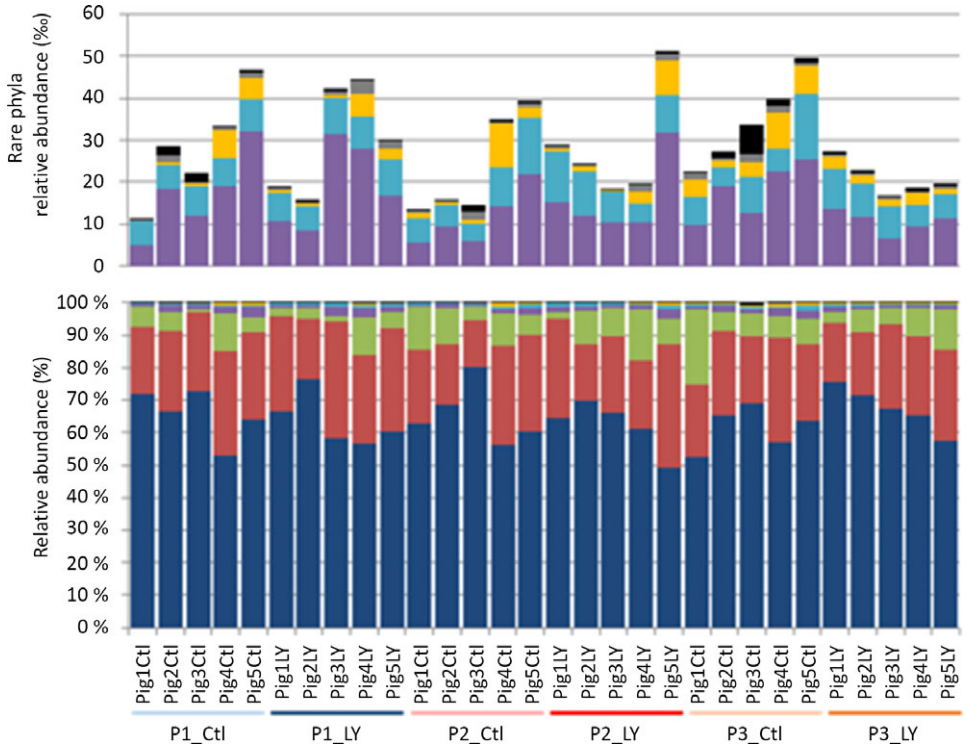

Fig. 3. Bar plot representation of relative bacterial composition at the phylum level, in faeces of pigs sampled at the end of the three experimental periods. P1_Ctl, P2_Ctl, P3_Ctl, Pigs fed with the standard control diet and, respectively, sampled at the end of the P1 (22°C), P2 (28°C) and P3 (28°C) period; P1_LY, P2_ LY, P3_ LY, Pigs fed with the live yeast-supplemented diet and, respectively, sampled at the end of the P1 (22°C), P2 (28°C) and P3 (28°C) period. ■, Firmicutes; ■, Bacteroidetes; ■, Spirochaetae; ■, Proteobacteria; ■, Actinobacteria; ■, Fibrobacteres; ■, Other; ■, Unknown.

Romboutsia and were more abundant in the non-supplemented groups under heat conditions. The most stable OTU selected on the second component belonged to *Coprostanoligenes group* and was more abundant at the end of the period P2, in pigs fed the LY-supplemented diet. A differential analysis was applied to the forty-six discriminative OTU to test the effect of the period and dietary LY supplementation (Fig. 5). The relative abundance of twenty-three OTU was significantly affected by the period or LY dietary supplementation: four OTU were only significantly affected under HS ($P < 0{\cdot}05$; Fig. 5(a)), while the abundance of fourteen OTU was changed when diet was supplemented with LY (Fig. 5(b)). Three OTU had their abundance affected by both the period and the diet (Fig. 5(c)). Among the twenty-three genera identified using the discriminant approach, seven genera were significantly increased under HS ($P < 0{\cdot}05$). Interestingly, *Romboutsia* and *Lactococcus* increases under HS were, respectively, attenuated and exacerbated with LY dietary supplementation. Moreover, LY supplementation decreased *Ruminoccocus 1*, *Coprococcus 1*, *Peptococcus* and *Oxalobacter* abundances (Fig. 6, online Supplementary Table S6).

Regularised canonical correlation analysis was applied to assess the relationship between the OTU composition at the end of the three periods and pig performances (DM intake, average daily gain and RE:ME ratio for the three periods). This multivariate approach highlighted strong correlations between the performance data set and microbiota composition at the end of the first period ($\rho 1 = 0{\cdot}919$), and eight correlated OTU were selected using a 0·79 threshold. Interestingly, the relative abundances of OTU411 (99 % with *Ruminococcus bromii*) and OTU141 (100 % identity with *Clostridium butyricum*), measured at the end of the first period, were, respectively, positively and negatively correlated with DM intake and RE:ME ratio measured during periods P2 and P3. No significant correlation could be pointed out between microbiota composition and performance during period P2. On the contrary, a strong correlation ($\rho 1 = 0{\cdot}915$) between the performance data set and microbiota composition at the end of period P3 was evidenced with twenty-nine correlated OTU selected with a 0·73 threshold (Fig. 7).

Taking into account the microbiota at the end of period P3, we could highlight three clusters of OTU strongly correlated with performance parameters measured during the three periods. The largest cluster (fourteen OTU) was mostly characterised by a negative correlation with DMI during period P1, while a second cluster (five OTU) positively correlated with DM intake and average daily gain during period P1 at thermoneutrality. Three OTU belonging to that cluster were closely related to *Clostridium chartatabidum*, *Lactobacillus reuteri* and *Anaerovibrio lipolyticus* species. A third cluster was positively correlated with DM intake and RE:ME ratio measured during HS periods (P2 and P3), except one OTU, closely related to the pathogenic *Campylobacter hyointestinalis* species, which was negatively correlated with RE:ME ratio during period P3.

Discussion

Pigs subjected to HS decrease their feed intake that causes important economic losses, and finishing boars are peculiarly

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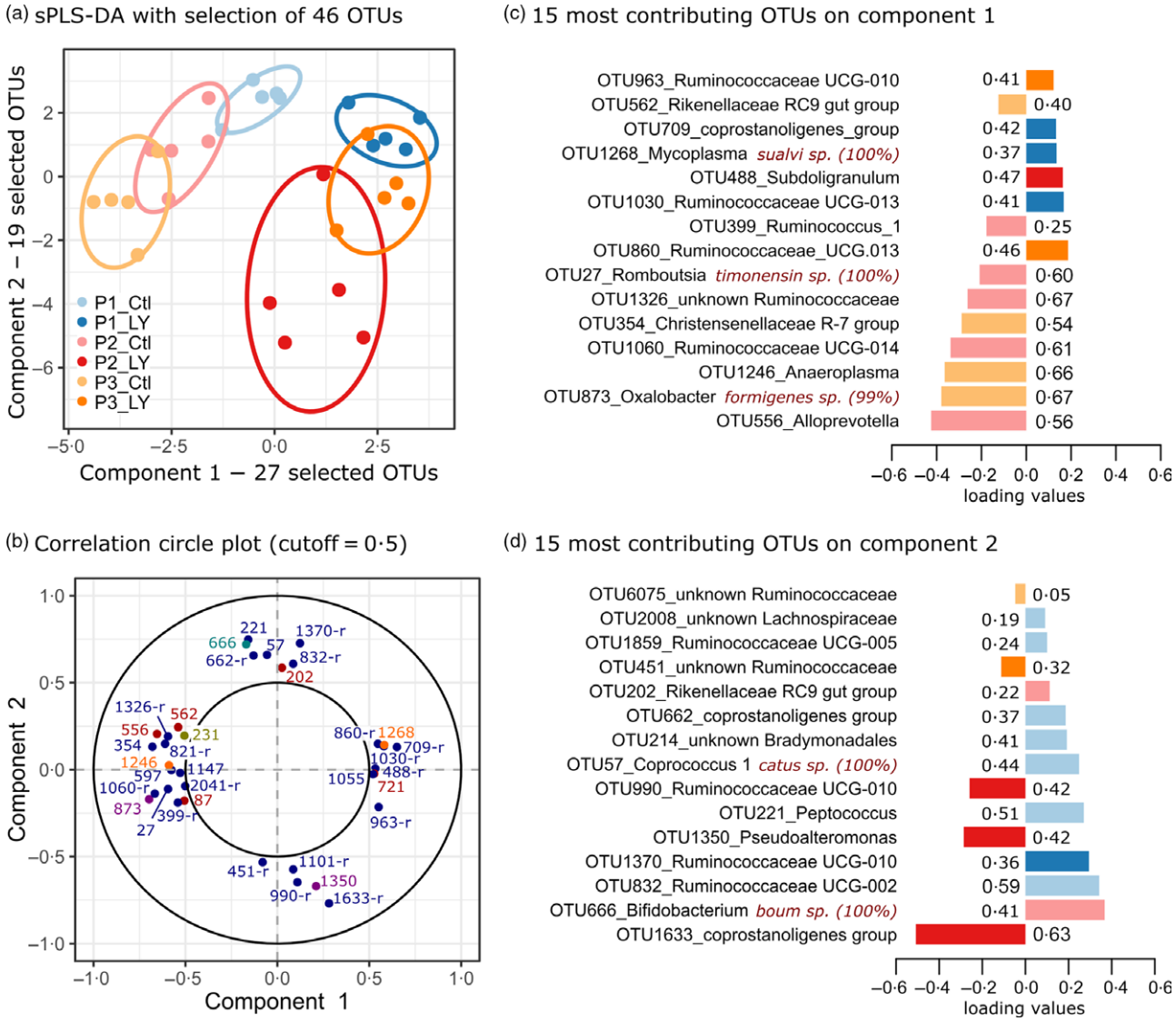

Fig. 4. Supervised analysis of the microbiota and Operational Taxonomic Unit (OTU)'s selection using a two-component Sparse Partial Least Square Discriminant Analysis (sPLS-DA) model. (a) Samples plot with 0·8 confidence ellipses represented. (b) Correlation circle plot highlights correlations between each selected OTU and its associated latent component. A 0·5 threshold (inner circle) was set, and OTU with a weaker association are not represented[38]. OTU's identification numbers are indicated, and colours are attributed according to the phylum affiliation (Dark blue, *Firmicutes;* Red, *Bacteroidetes*; Orange, *Tenericutes*; Turquoise blue, *Actinobacteria*; Purple, *Proteobacteria*; Green, *Spirochaetae*). -r, OTU belonging to *Ruminococcaceae* family. c-d) Loading plot of the most contributing OTU on component 1 (c) and component 2 (d) are coloured according to the group in which the median abundance is maximal. For each OTU, the frequency of selection is given (calculated during a 200 times repeated cross-validation process, dividing the data set into ten subsets and iteratively leaving one subset out to fit the model). Frequency higher than 0·5 is highlighted in bold. Species annotation is given when a known species with an identity percentage higher than 95 % was identified using BLAST (given between bracket). P1_Ctl, P2_Ctl, P3_Ctl, pigs fed with the standard control diet and sampled at the end of the P1 (22°C), P2 (28°C) and P3 (28°C) period; P1_LY, P2_LY, P3_ LY, pigs fed with the live yeast-supplemented diet and sampled at the end of the P1 (22°C), P2 (28°C) and P3 (28°C) period. ●, P1_Ctl; ●, P1_LY; ●, P2_Ctl; ●, P2_LY; ●, P3_Ctl; ●, P3_LY.

sensible to HS[1]. We hypothesised that (i) a dietary *S. cerevisiae boulardii* supplementation in finishing male pigs would help to cope with HS first maintaining feed intake and (ii) this action would be mediated through gut microbiota modulation.

Heat stress consequences on feeding behaviour and energy partitioning

According to previous estimates[1], increasing ambient temperature from 22 to 28°C resulted in a decreased feed intake (–410 in our study *v.* –430 g DM/d in literature) of pigs fed a non-supplemented diet. Deleterious effects of HS on performances were associated with changes in feeding behaviour and energy partitioning, as described previously in young pigs[42], growing pigs[1,22,43], gestating[44] and lactating sows[27]. At all physiological stages except in gestating sows which are under feed restriction, the duration of the meals decreased in HS condition, which is also associated with an increased feeding rate. It may be argued that the increased feeding rate limits the duration in standing position while eating, which is associated with an increased HP[7]. Under HS conditions, the major challenge for pigs

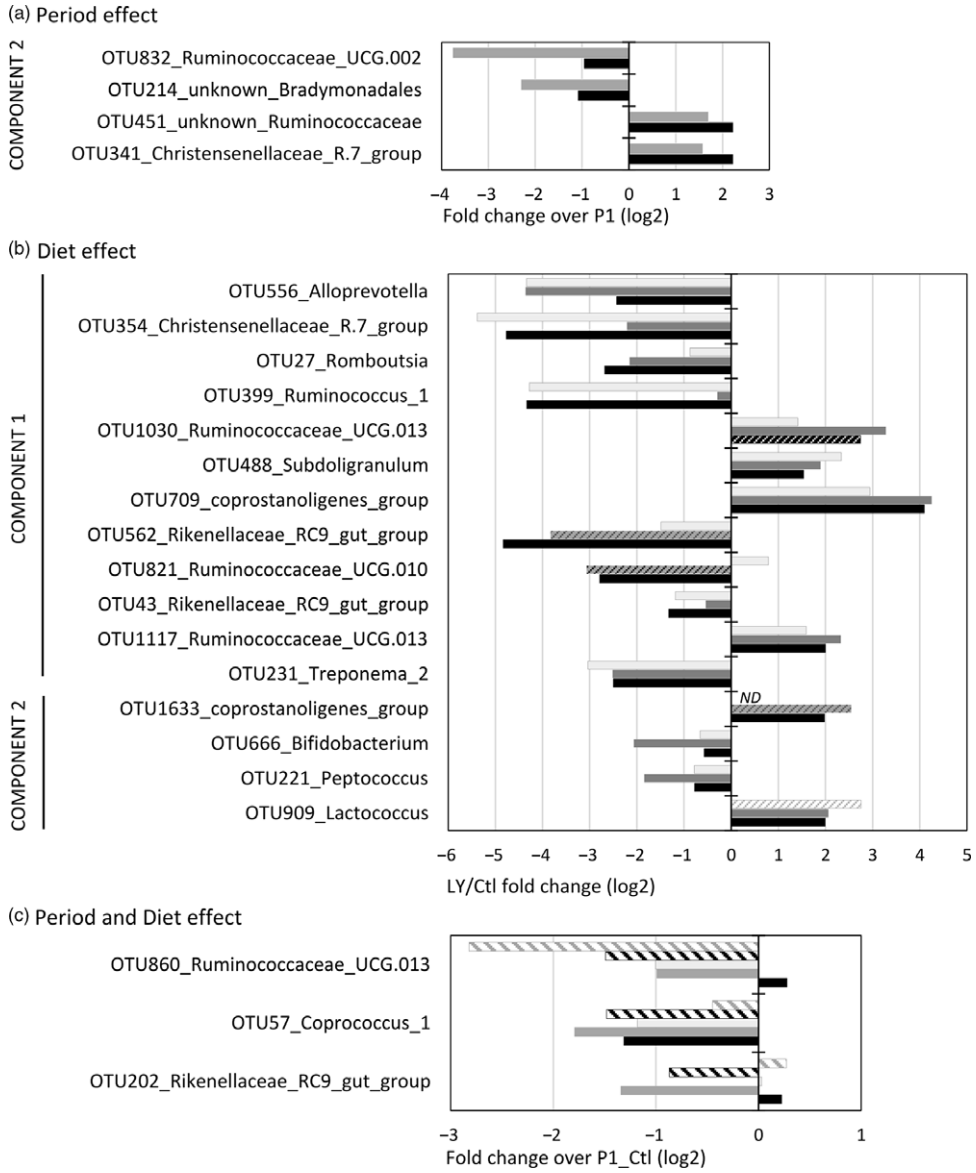

Fig. 5. Histogram plots of the Operational Taxonomic Units (OTUs) selected using Sparse Partial Least Square Discriminant Analysis (sPLS-DA) and significantly affected by the period and/or live yeast dietary supplementation. OTU are ordered according to their sPLS-DA component and to their loading value on the component (highest to lowest values from top to bottom). Log2 fold changes of mean relative abundances are represented. In panel b, bars are stripped when the OTU was not detectable in one of the compared group, null abundances were replaced by 0·01 % to calculate log2 ratio. P1, P2, P3, pigs sampled at the end of period 1 (22°C), period 2 (28°C) and period 3 (28°C); Ctl, Pigs fed with the standard control diet; LY, pigs fed with the live yeast-supplemented diet; P1_Ctl, P2_Ctl, P3_Ctl, Pigs fed with the standard control diet and sampled at the end of the P1, P2 and P3 period; P1_LY, P2_ LY, P3_ LY, pigs fed with the live yeast-supplemented diet and sampled at the end of the P1, P2 and P3 period. *ND*, not detected. □, P1; ■, P2; ■, P3; ▨, P2_Ctl; ▨, P3_Ctl; □, P1_LY; ■, P2_LY; ■, P3_LY

is their ability to maintain their body temperature, by an adequate equilibrium between HP and heat loss. During HS, HP associated with the utilisation of nutrients (TEFs) decreased because of the decreased feed intake[3,22], whereas the HP due to physical activity increased because of increased panting[22]. Despite the lower HP during HS than at thermoneutrality, skin temperature was higher during HS that may indicate that the pig develops physiological adaptations (increased peripheral blood flow, increased respiratory rate) to face the difficulties to maintain their body temperature at a constant level. The consequence of the decreased feed intake was a decreased intake of energy, of which a lower proportion was retained as protein and fat. The decreased protein retention (–16 %) was less pronounced than what was observed before with castrated males (–54 % in 60 kg BW pigs housed at 32°C[3]) but similar to previous measurements with piglets (–22 % in 25 kg BW piglets housed at 33°C[45]). These results suggest that finishing entire males tried to reach their potential for protein retention despite their limited feed intake. Nevertheless, protein deposition is also associated with a higher HP than lipid deposition because of the lower energetic efficiency associated with involved metabolic pathways[6]. The high level of protein deposition of entire male pigs during the finishing period evidences their high sensibility to HS, as suggested by the strong decrease in feed intake induced by a rather limited increase in ambient temperature.

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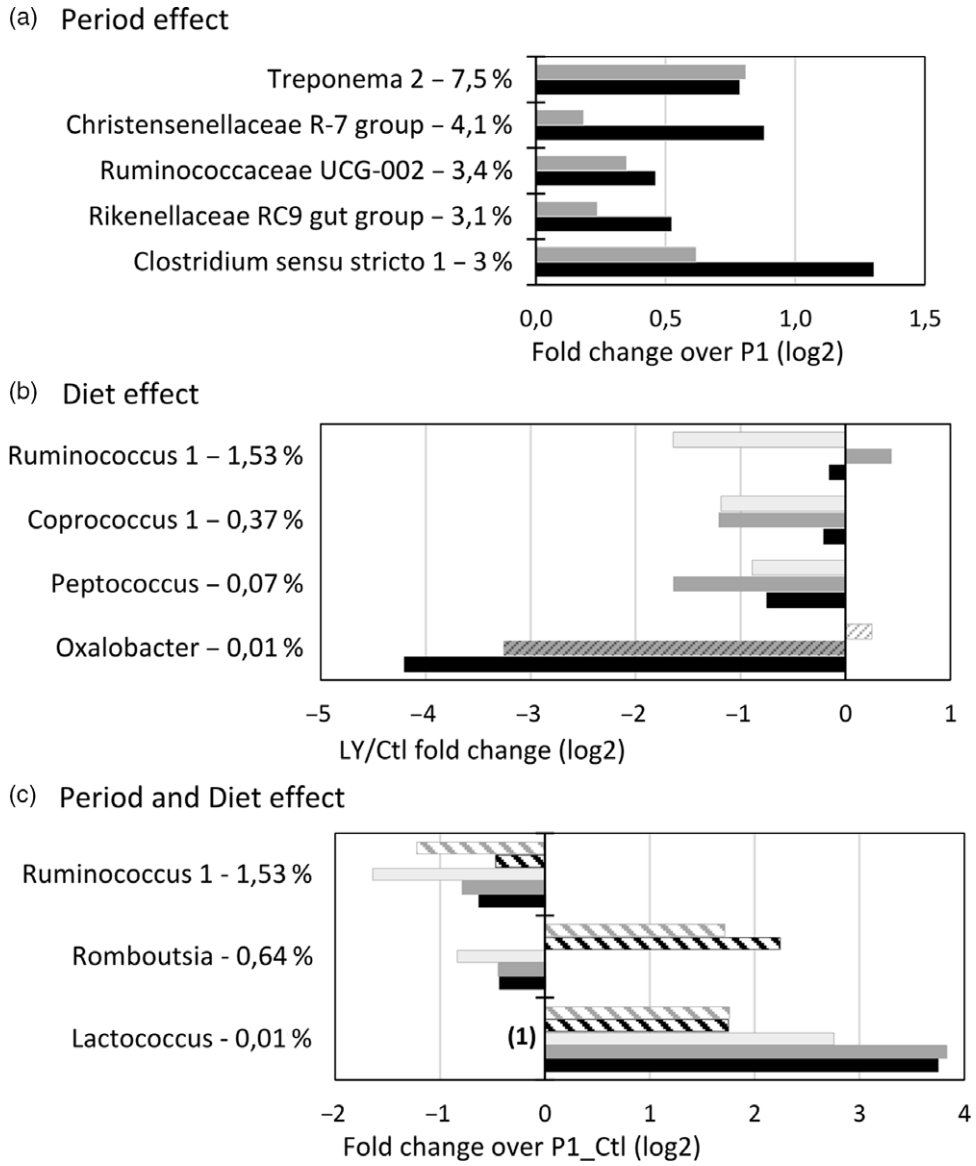

Fig. 6. Histogram plots of the genera selected using Sparse Partial Least Square Discriminant Analysis (sPLS-DA) and significantly affected by the period and/or live yeast supplementation. Overall mean relative abundances are given next to the genus name. Log2 fold changes of mean relative abundances are represented. In panel b, bar is stripped when the OTU was not detectable in one of the compared group, null abundances were replaced by 0·01 % to calculate log2 ratio. P1, P2, P3, Pigs sampled at the end of period 1 (22°C), period 2 (28°C) and period 3 (28°C); Ctl, Pigs fed with the standard control diet; LY, Pigs fed with the live yeast-supplemented diet; P1_Ctl, P2_Ctl, P3_Ctl, Pigs fed with the standard control diet and sampled at the end of the P1, P2 and P3 period; P1_LY, P2_ LY, P3_ LY, Pigs fed with the live yeast-supplemented diet and sampled at the end of the P1, P2 and P3 period. ND, not detected[1]. *Lactococcus* was not detectable in P1_Ctl group, P1_Ctl abundance was replaced by 0·01 % to calculate log2 ratio. ▫, P1; ▪, P2; ■, P3; ▫, P2_Ctl; ▪, P3_Ctl; ▫, P1_LY; ▪, P2_LY; ■, P3_LY.

Microbiota is involved in thermal heat acclimatisation

One of the important physiological functions played by the gut microbiota is its influence on the central nervous system and behaviour[46]. In humans and rodents, the gut microbiota–brain axis has been shown to influence the appetite–satiety balance[47]. In particular, bacterial peptides may act in the hypothalamus to regulate the appetite. A long-term regulation could explain different feeding behaviour in heat-stressed animals. We highlighted in the present study the impact of HS on the faecal microbiota of growing pigs. As previously described in broilers[18] and in growing pigs[20], a higher richness was evidenced under HS, while Shannon index was not changed, suggesting the development of minor species and uneven abundances. When considering the effects of HS on the microbiota composition, confounding effects such as ageing or level of feed intake have to be considered. The effect of feed restriction on the microbiota of pigs has been indeed demonstrated[48]. However, one can reasonably assume that the observed effects on microbiota composition were not simply due to confounding factors as opposite effects were observed with HS that the one expected with ageing. Indeed, decreased microbial diversity has been described during the finishing period, between 84 and 154[49] or between 93 and 147 d of age[50]. The latter then suggests

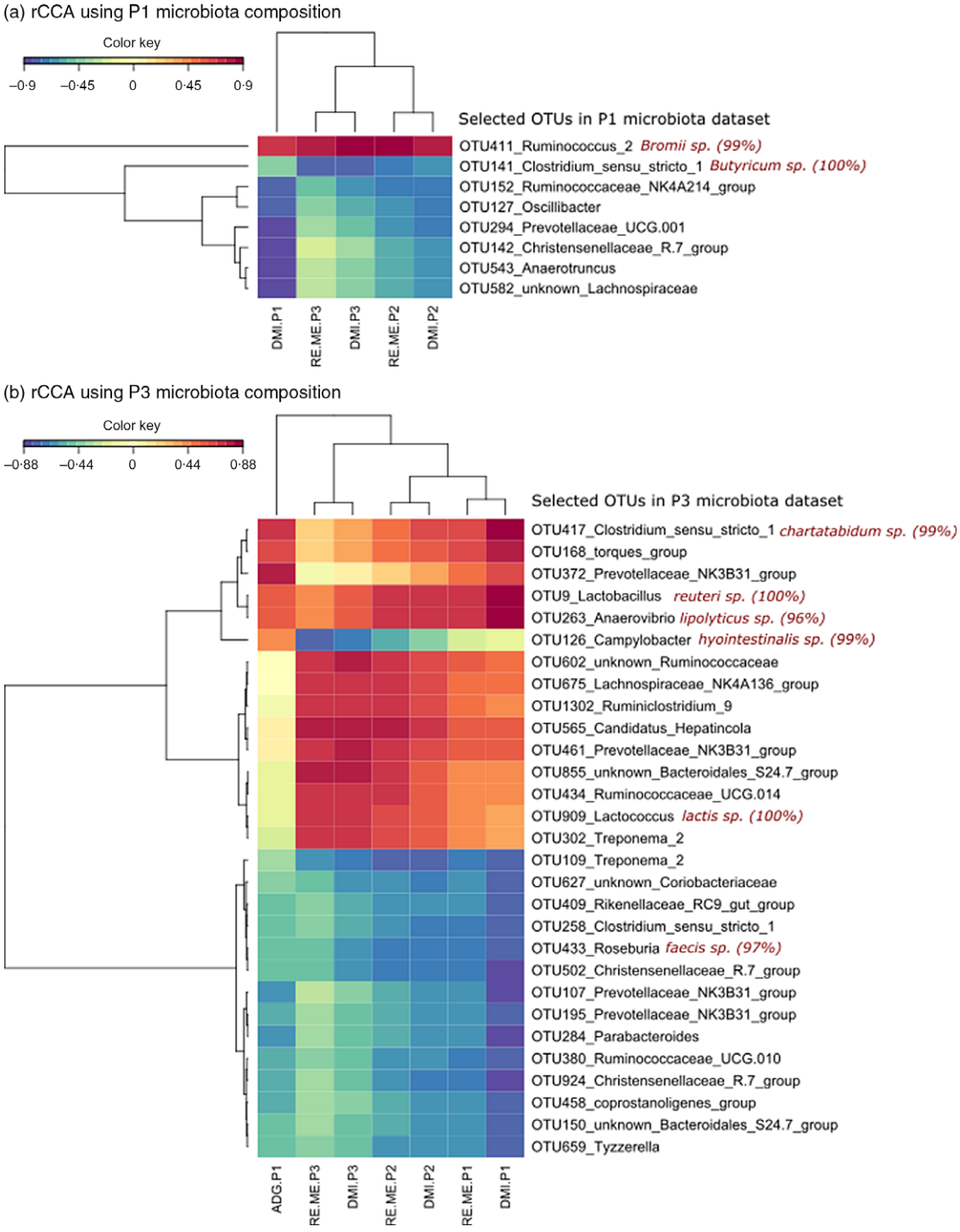

Fig. 7. Clustered Image Map representing the correlation between microbiota composition and growth performance (ADG, average daily gain, DMI, DM intake) and energy balance (RE:ME, ratio between retained energy and metabolisable energy) during periods 1, 2 or 3 (P1, P2, P3). Species annotation is given when a known species with an identity percentage higher than 95 % was identified using BLAST (given between brackets). Colour key.

that the effects of HS were predominant over the effects of ageing on microbiota diversity.

Treponema and *Clostridium sensu stricto* genera, as well as genera belonging to Christensenellaceae, Ruminococcaceae and Rikenellaceae families, had their relative abundance increased under HS. In particular, the level of several *Clostridiales* including of *Christensenellaceae R-7, Ruminococcaceae UCG-002, Clostridium sensu stricto 1* group and *Romboutsia* was increased under HS as described in sows[19]. Thus, some similar effects of HS on the gut microbiota of gestating sows and growing pigs were interestingly evidenced despite differences in feed composition, feeding behaviour between experiments and

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physiological stages. On the contrary, Le Sciellour *et al.* have shown that OTU affiliated to the Clostridiaceae 1 and Peptostreptococcaceae were less abundant in growing pigs when HS is applied for 3 weeks[20]. Alpha-diversity and discriminant analysis results suggested nevertheless an exacerbated effect on the microbiota composition after a longer exposure to HS. Difference in length of exposure to HS or housing conditions could thus explain some discrepancies between our results and others.

Treponema was one of the predominant genus in this study (7·5 % ± 4·7 %), with 98 % of the Treponema sequences belonging to the Spirochaetes phylum, and HS was associated with its increased abundance. This is in accordance with the effect of HS described in growing pigs[20] but in contradiction with what has been observed in gestating sows[19]. *Treponema* species may contain opportunistic pathogens, involved in shoulder ulcers and ear necrosis in pigs[51]. *Treponema* species are nevertheless found in the gut of healthy pigs and have the ability to degrade cellulose, lignin and xylan, as described not only in wood-feeding termites[52] but also in pigs[53]. Niu *et al.* have moreover shown that *Treponema* were positively correlated with fibre digestibility in growing pigs[53], but not in sows[54]. This suggest that different *Treponema* species, exhibiting different functions, could be present at different physiological stages in pigs. Interestingly, the abundance at the end of period P3 of two OTU belonging to the *Treponema* genus was correlated with DM intake levels however in different ways: one OTU was negatively associated with DM intake, whereas another OTU was positively correlated with DM intake, especially at the end of period P3. This illustrates the potentially different function of two species belonging to the same genus which is difficult to cultivate and is thus so far poorly described.

Live yeast alleviates heat stress effect on feeding behaviour and drives microbiota towards a new equilibrium

S. cerevisiae var. boulardii (S. boulardii) is a strain of *S. cerevisiae* exhibiting specific features[55]. Beneficial effects of *S. boulardii* have been well documented in humans, in particular to prevent the occurrence of several types of diarrhoea[56], preserving the integrity of the intestinal barrier[57]. *S. boulardii* is also known to positively modulate the immune system[56]. Modulation of the microbiota composition has been observed in weanling piglets and sows[58] fed a diet supplemented with *S. boulardii* and associated with improved performances.

We demonstrated for the first time the modulation of the microbiota composition in LY dietary-supplemented pigs, in a context of thermoneutrality and HS. Our results suggest that a modulation of the microbiota using LY supplementation could affect the regulation of the feeding behaviour in heat-stressed animals, through the gut–brain–axis. When the diet was supplemented with LY, DM intake significantly increased both at thermoneutrality and under HS. The increase in DM intake at thermoneutrality (+190 g DM/d) is consistent with previous findings in lactating sows (+200 g DM/d[10]), which may suggest an increased palatability of the supplemented diet. It is also associated with an increased number of meals resulting in similar feed intake per meal for the two groups of pigs (436 g DM/meal on average). Interestingly, the quantity of feed consumed per meal did not differ between the two treatments under HS, even if this amount decreased during the second period as a mark of rapid adaptation of pigs to their new environmental conditions (347 and 424 g DM/meal on average during periods P2 and P3, respectively)[3]. It can then be supposed that the amount of feed consumed at each meal is a rather constant characteristic of individual feeding behaviour, depending on age[43] and environmental conditions[3]. Under HS, the pigs fed the LY-supplemented diet exhibited an increased feed intake as a direct consequence of an increased daily number of meals. Moreover, LY supplementation modulated the microbiota composition of pigs, in interaction or not with the ambient temperature. In a mice model, *S. boulardii* has been shown to reverse intestinal dysmotility induced by a wire-mesh restraint stress, which illustrates the effect of *S. boulardii* on the gut–brain axis[59]. However, the mechanisms underlying the effect of the microbiota on behaviour, especially feeding behaviour, remain to be elucidated.

Because the thermic effect of feeding as percentage of ME intake did not differ between diets, the increase in HP consecutively to a meal should be similar for the two groups of pigs. Consequently, the instantaneous load on body temperature might not be higher for the pigs fed the LY-supplemented diet. Body temperature is an indicator of the balance between HP and heat loss. In order to avoid the increase in body temperature, ATP production associated with dietary nutrient utilisation leads to HP that should be balanced with heat loss. The lower skin temperature of pigs fed the diet supplemented with LY can be indicative of a limited sensible heat loss or a higher ability of these pigs to export the heat associated with the higher feed intake. In pigs submitted to HS, the sensible route of heat loss associated with conduction, radiation and convection is less important than the latent route of heat loss associated with evaporation of water, mainly at lung level (61 % of total heat loss from the latent route, among which 67 % originate from the respiratory tract in 30–60 kg BW pigs submitted at a 32–33°C ambient temperature[3,60]). Because pigs that were fed the diet supplemented with LY also have a lower skin temperature, their ability to export heat from the sensible route may be lower than that of pigs fed the non-supplemented diet. The technology used to measure physical activity enables also the measurement of breath movements as a component of the physical activity of the whole body. Therefore, the tendency for an increased AHP in pigs fed the supplemented diet can be indicative for an enhanced panting. Nevertheless, the increase in breath movements might not be the only route involved: the loss of extra heat in pigs fed the LY-supplemented diet would induce an increase of thirty-five breath movements per minute, considering the evaporation of 21 g of water per day for each extra breath[3]. Such a value is difficult to assume in pigs, where respiratory rate is already high under HS (up to ninety-nine movements per min in 60 kg BW pigs housed at 32°C[3]). The numerically higher water consumption of pigs fed the LY-supplemented diet suggests that cooling of the body via water consumption (up to 25 % of the extra HP might be used to heat the extra water intake at the level of body temperature) or evaporation of water at the level of the skin may

also be involved in the thermal acclimatisation of pigs. The high ability to export heat of pigs fed the LY-supplemented diet enables them to achieve significantly higher level of energy intake and nutrient deposition that were always higher than those of pigs fed the non-supplemented diet. Indeed, total energy retention during the second period was only reduced by 17 % for pigs fed the LY-supplemented diet, whereas pigs fed the non-supplemented diet or pigs in previous papers exhibit a decrease in energy retention ranging from 31 (present study) to 56 %[3,61]. We thus observed an improved energy retention when the diet was supplemented with LY irrespective of thermal condition, whereas the proportions of ME lost as AHP or TEF_s, two components owing to extra heat were not modified. During the last period, the FHP was also not modified by dietary the LY supplementation, whereas the increased feeding level in LY-supplemented pigs should have resulted in an enhanced BMR[62]. From previous findings, it can be calculated that the difference in feeding level between the two groups of pigs should have led to a 7·5 % increase in FHP (60 kJ/kg $BW^{0·60}$ per d). Because fasting HP was not modified, it seems that LY-supplemented pigs were able to save energy that can be devoted to production purposes. It can also be considered that inflammation and immune system activation are responsible for an increased HP in growing pigs[61]. Any improvement in the inflammatory status of the pigs may then lead to a better energy retention. *Lactococcus* genus, and more specifically an OTU affiliated to *Lactococcus lactis* species (100 % identity), was markedly increased with LY supplementation, especially under HS condition. *Lactococcus lactis* is known to produce nisin, a bacteriocin and has been approved as a food preservative[63]. The production of bacteriocin could beneficially interact with the host immune system, thus allowing some energy saving by the host. In accordance with this hypothesis, *Lactococcus lactis* was positively correlated with RE:ME ratio and DM intake.

The results of the canonical correlation analysis suggest that the composition of the microbiota at the end of the first period can influence energy balance and DM intake during HS periods. Although correlation does not necessarily mean causation, our results suggest that early dietary supplementation could help to better cope with HS through a beneficial modulation of the microbiota. Indeed, higher levels of the beneficial species *Ruminococcus Bromii*, described as a keystone species promoting the growth of other organisms able to degrade resistant starch[64], at the end of the period P1 (thermoneutrality) are positively correlated with a higher RE:ME ratio during the HS periods P2 and P3. Similarly, correlations were evidenced between DM intake during period P1 and the microbiota composition at the end of period P3 suggesting that the level of ingestion can shape the microbiota. Interestingly, at the end of the HS periods, another OTU closely related to *C. chartatabidum*, a key species involved in fibre digestion was positively correlated with initial DM intake level. This illustrates an interesting link between DM intake and species involved in fibre digestion and butyrate production[65]. Moreover, higher DM intake under normal conditions could promote the development of *L. reuteri* when pigs were submitted to HS. *L. reuteri* is a well-studied probiotic that has been associated with several beneficial effects[66]. It is worth noticing that OTU affiliated to genera associated with high feed efficiency (low residual feed intake) in previous studies[67] (*Oscillibacter, Prevotellaceae, Christensenellaceae*) were negatively correlated with initial DM intake level. This could suggest that species associated with high feed efficiency in pigs are more a consequence of low feed intake levels than a factor explaining them.

To conclude, finishing pigs are highly susceptible to HS because of their high BMR and their high level of protein deposition. The dietary LY supplementation results in microbiota modulation that may influence feeding behaviour and metabolism of pigs. Modulation of feeding behaviour by LY in thermoneutral conditions would help the animal to cope with HS: an increased daily number of meals helps the pigs to cope with HS through an enhanced partitioning of energy intake and instantaneous load on body temperature regulation. Gut microbiota modification by dietary LY supplementation at thermoneutrality is associated with beneficial consequences on pig performances when they are submitted to HS.

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E. L. and members from Lallemand SAS designed the experiments. E. L. and S. D. conducted the experiments, performed heat production measurements and E. L. was responsible for N and energy balance calculations. S.C. was responsible for microbiota analyses. E. L and C. A. developed the statistical models. E. L. and C. A. wrote the manuscript. All authors read and contributed to the finalisation of the manuscript.

Caroline Achard and Mathieu Castex are employed by Lallemand SAS.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114521001756

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