

# Thermoregulatory responses during thermal acclimation in pigs divergently selected for residual feed intake

Paulo Henrique Reis Furtado Campos · Jean Noblet · Yolande Jaguelin-Peyraud ·  
Hélène Gilbert · Pierre Mormède · Rita Flavia Miranda de Oliveira Donzele ·  
Juarez Lopes Donzele · David Renaudeau

Received: 1 August 2013 / Revised: 24 September 2013 / Accepted: 23 October 2013  
© ISB 2014

**Abstract** The objective of this study was to evaluate the performance and thermoregulatory responses during acclimation to high ambient temperature ( $T_a$ ) of pigs from two lines selected for high ( $RFI^+$ ) or low ( $RFI^-$ ) residual feed intake with the hypothesis that  $RFI^-$  pigs producing less heat would better tolerate high  $T_a$ . Pigs (50 kg initial body weight; 17 per line among which 10 of them were catheterized) were individually housed in a climatic-controlled room where  $T_a$  was maintained at  $24.2 \pm 0.4$  °C during 7 days and thereafter at  $30.4 \pm 0.7$  °C during 14 days. Irrespective of  $T_a$ ,  $RFI^-$  pigs had lower feed intake (ADFI) and similar average daily gain (ADG) than  $RFI^+$  pigs. Whatever the line, ADFI, ADG, and feed efficiency decreased with increased  $T_a$ . Overall, the  $T_a$  increase resulted in an increase in rectal temperature (RT), skin temperature (ST), and respiratory rate (RR) within the first 24–48 h and, subsequently, in a decrease followed by stabilization. The RT decrease during acclimation occurred 24 h earlier in  $RFI^-$  pigs than in  $RFI^+$ . Thyroid hormones and cortisol decreased at high  $T_a$  and it was similar in both lines.

Based on performance and RT, ST, and RR responses, it seems that selection for low RFI tends to ameliorate pigs' tolerance to high  $T_a$ . Nevertheless, this selection does not induce significant differences between lines in endocrine and metabolite responses during thermal stress.

**Keywords** Adaptation · Growing pigs · Heat stress · Hormones · Residual feed intake

## Introduction

Heat stress has been suggested as a major threat to the viability and sustainability of livestock production systems (Gaughan et al. 2009), particularly in the current context of global warming (Bernabucci et al. 2010) and the increase of animal production in tropical and subtropical areas (Renaudeau et al. 2012). Under hot conditions, behavioral, physiological, and metabolic adjustments allow maintaining homeostasis with negative consequences on animal productivity and health (Renaudeau et al. 2012). Pig production is particularly concerned by the negative effects of heat stress because pigs have a decreased capacity to dissipate their heat by evaporative mechanisms compared to most livestock species due to their limited number of functional sweat glands (Curtis 1983). In addition, modern genotypes seem to be more susceptible to heat stress (Renaudeau et al. 2010) because of their increased heat production (Brown-Brandl et al. 2003). According to these authors, the fasting heat production in pigs increased 19 % from 1984 to 2002. Currently, selection for heat-tolerant animals has been suggested as a promising strategy for mitigation of negative effects of heat stress on livestock production systems (Gaughan et al. 2009). Animal's thermotolerance and resilience are determined by its capacity to maintain homeostasis in thermal challenging environments with minimal performance losses (e.g., growth, egg and milk

P. H. R. F. Campos · J. Noblet · Y. Jaguelin-Peyraud · D. Renaudeau  
INRA, UMR 1348 PEGASE, 35590 Saint-Gilles, France

P. H. R. F. Campos · R. F. M. de Oliveira Donzele · J. L. Donzele  
Departamento de Zootecnia, Universidade Federal de Viçosa/UFV,  
Viçosa, MG, Brazil

J. Noblet · Y. Jaguelin-Peyraud · D. Renaudeau  
Agrocampus Ouest, UMR 1348 PEGASE, 35000 Rennes, France

H. Gilbert · P. Mormède  
INRA, UMR 444 LGC, 31326 Toulouse, France

H. Gilbert  
INRA, UMR 1313 GABI, 78352 Jouy-en-Josas, France

D. Renaudeau (✉)  
INRA, UR 143 URZ, 97170 Petit Bourg, France  
e-mail: david.renaudeau@rennes.inra.fr

production, and reproduction). In hot conditions, this capacity is driven by the animal's ability to dissipate heat loss or/and reduce metabolic heat production (Renaudeau et al. 2010). Studies have demonstrated that selection for low residual feed intake (RFI), besides selecting for more efficient animals (Gilbert et al. 2007), reduces metabolic heat production in beef cattle (Herd and Bishop 2000), laying hens (Luiting et al. 1991), and pigs (Barea et al. 2010). Thus, it is hypothesized that selection for RFI could modify animal's adaptability to heat stress. On the other hand, it has been recently shown that exposure to high ambient temperature had rather comparable effects on energy metabolism and components of heat production in two lines of pigs divergently selected for RFI (Renaudeau et al. 2013), which suggests that selection for low RFI does not improve significantly the ability of the pig to cope with thermal stress. The objective of the present study was to validate this hypothesis by comparing the thermoregulatory responses and blood parameters in pigs from low and high RFI divergent lines during short and medium acclimation to heat.

## Materials and methods

The experiment was conducted in accordance with the French legislation on animal experimentation and ethics.

### Animals and experimental design

This study was designed to evaluate the effects of selection for RFI on thermoregulatory responses in growing pigs. Animals originated from a divergent selection experiment for low (RFI<sup>-</sup>) and high (RFI<sup>+</sup>) RFI conducted at the Institut National de la Recherche Agronomique (INRA), France since 2000. Details of the selection process have been described by Gilbert et al. (2007). Briefly, from an initial purebred Large White population composed of 30 sires and 30 dams, two divergent lines were produced in two INRA experimental farms (INRA Génétique, Expérimentation et Systèmes Innovants-GenESI, Le Magneraud, Charente-Maritime, France) by selecting sires with the lowest and highest RFI values at each generation; no selection was performed in females, each dam being replaced by one daughter in the next generation (Gilbert et al. 2012a). The RFI selection index was calculated from 35 to 95 kg of body weight (BW) using the following formula:  $RFI = DFI - (1.06 \times ADG) - (37 \times UBT)$ ; in which DFI is daily feed intake (in gram per day), ADG is the average daily gain (in gram per day), and UBT is ultrasonic back-fat thickness (in millimeter). Residual feed intake, defined as the fraction of total feed intake that is unexplained by maintenance and production requirements, has been considered as a

suitable selection trait to improve feed efficiency in pig production (Gilbert et al. 2007). In particular, selection for low RFI reduces feed intake and improves feed efficiency with no correlated effects on growth (Gilbert et al. 2007; Cai et al. 2008), contrary to selection based on the feed conversion ratio (Hoque and Suzuki 2009).

In the present study, pigs from the seventh generation of selection were used. In this generation, the lines difference amounted to 3.1 genetic standard deviation (SD) of the selection criterion calculated from 35 to 95 kg BW, corresponding to 148 g/day of feed of RFI measured on castrated males and females from 70 days of age to slaughter (110 kg; 2.9, genetic SD). Our experiment included a total of 34 Large White castrated males and was carried out in two successive replicates of 16 (eight RFI<sup>-</sup> from five different litters and eight RFI<sup>+</sup> from four different litters) and 18 (nine RFI<sup>-</sup> and nine RFI<sup>+</sup> from five different litters/line) animals, respectively. At weaning (i.e., at 4 weeks of age), piglets were transported from the selection herd facilities (INRA-GenESI, Le Magneraud, France) to the experimental facilities of INRA in Saint-Gilles (INRA Physiologie, Environnement, Génétique Animal et Système d'Élevage - PEGASE, France), where the experiment was carried out. Animals were initially group-housed in pens (1.85×2.60 m each; four or five pigs per pen according to their line of origin) with metal slatted floors equipped with semi-automatic feeders and nipple drinkers. Pigs had free access to water and were fed ad libitum with commercial weaner and starter diets until their transfer to an experimental climatic-controlled room (i.e., at approximately 80 days of age and 40 kg of BW).

Pigs remained in the climatic-controlled room during 35 days, which consisted in a 14-day adaptation period and a subsequent 21-day experimental period. Pigs were housed in individual metal-slatted pens (0.70×2.30 m) and each pen (18 in total) was equipped with a feed dispenser and a nipple drinker designed to avoid water and feed spillage. Photoperiod was fixed to 12 h of artificial light (0800–2000 hours) and 12 h of darkness. Room temperature and humidity were recorded every 5 min using a data logger (EL-USB-2+, DATAQ Instruments, Inc., Akron, OH, USA) located in the center of the room. Relative humidity was not controlled. Throughout the adaptation period, the room ambient temperature (Ta) was maintained at 24 °C.

The experimental period was divided in two successive periods: period 0 (P0) in which pigs were kept at 24 °C for 7 days (from days -7 to -1) and period 1 (P1) in which pigs were kept at 30 °C for 14 days (P1, from days 0 to 13). Between P0 and P1 (on day 0), Ta was gradually changed from 24 to 30 °C at a constant rate of 2 °C/h beginning at 1000 hours. Pigs had free access to water and feed. Diet

was formulated to meet the nutritional requirements of this animal category according to standard recommendations (Table 1).

**Table 1** Composition, nutritional levels and energy values of the experimental diet

Ingredients (%)	
Corn	25.39
Wheat	23.00
Barley	23.00
Soybean meal	18.70
Molasses	2.00
Wheat bran	5.00
Dicalcium phosphate	1.10
Calcium carbonate	0.70
Salt	0.40
L-lysine HCL	0.18
L-threonine	0.03
Vitamins and trace minerals mixture <sup>a</sup>	0.50
Analyzed chemical composition <sup>b</sup> (%)	
Dry matter	87.0
Ash	5.6
Crude protein	18.3
Fat	2.3
Starch	51.3
Neutral detergent fiber	14.2
Acid detergent fiber	4.85
Acid detergent lignin	0.70
Gross energy (MJ/kg)	18.2
Nutritional levels <sup>b,c</sup>	
Digestible energy (MJ/kg)	15.2
Metabolizable energy (MJ/kg)	14.6
Net energy (MJ/kg)	10.9
Digestible amino acids (%)	
Lysine	0.81
Threonine	0.52
Methionine + cystine	0.50
Tryptophan	0.17
Threonine/lysine	64
Methionine + cystine/lysine	62
Tryptophan/lysine	21

<sup>a</sup> Vitamins and minerals mixture supplied per kilogram of diet: 5,000 IU of vitamin A, 1,000 IU of vitamin D<sub>3</sub>, 20 mg of vitamin E, 2 mg of vitamin B<sub>1</sub>, 4 mg of vitamin B<sub>2</sub>, 10.85 mg of calcium pantothenate, 15 mg of niacin, 0.02 mg of vitamin B<sub>12</sub>, 1 mg of vitamin B<sub>6</sub>, 2 mg of vitamin K<sub>3</sub>, 1 mg of folic acid, 0.2 mg of biotin, 500 mg of choline chloride, 56 mg of Fe<sub>(sulfate)</sub>, 24 mg of Fe<sub>(carbonate)</sub>, 10 mg of Cu<sub>(sulfate)</sub>, 100 mg of Zn<sub>(oxide)</sub>, 40 mg of Mn<sub>(oxide)</sub>, 0.2 mg of I<sub>(iodate)</sub>, 0.1 mg of Co<sub>(carbonate)</sub>, 0.15 mg of Se<sub>(selenite)</sub>

<sup>b</sup> Values expressed on a DM basis

<sup>c</sup> Values calculated according to Sauvant et al. (2002)

## Surgery

Two weeks before the beginning of the experimental period, four and six pigs/line in the first and the second replicate, respectively, were randomly selected and surgically fitted with a jugular catheter according to the protocol previously described by Melchior et al. (2004). Briefly, after 24 h of fasting, an indwelling silicone catheter (id 1.02 mm, od 2.16 mm; VWR International S.A.S, Fontenay-sous-Bois, France; catalog ref. 228-0656) was implanted through a collateral vein in the right external jugular vein. The catheter was tunneled under the skin and externalized on the back of the animal. The surgical operation did not exceed 20 min/pig. Except during blood samplings, the catheter was stored in a strengthened purse sewed on the back of the pig. Every 2–3 days, the catheters were flushed with 5 ml of normal saline solution containing 5 % of heparin.

## Measurements and sampling procedures

Pigs were individually weighed at the beginning and at the end of the experimental period, and on day 0 at 0800 hours, before the temperature change. Every morning, between 0800 and 0830 hours, feed refusals were collected and fresh feed was immediately distributed, except on days with blood samplings in which animals were submitted to 2 h of fast in order to standardize the blood sampling procedure and conditions. Every day, one sample of feed refusals of each animal was collected. The samples were pooled per period (P0 or P1), and then DM content was measured. Samples of the allowed feed were also taken every day and similarly pooled per period for DM determination and further chemical analyses.

Respiratory rate (RR), heart rate (HR), rectal temperature (RT), and skin temperature (ST) were measured in all pigs twice daily (0830 and 1500 hours) on days -6, -4, -1, 1, 2, 3, 6, 8, and 10 of the experiment. On day 0 (day of Ta transition from 24 to 30 °C), the same measurements were performed each hour, from 0900 till 1300 hours, only in pigs without catheter. These physiological measurements were performed according to the following protocol previously described by Renaudeau et al. (2008): first, RR was visually determined by counting flank movements over a period of 1 min, only in resting pigs. Then, HR was measured using a heart rate monitor (polar monitoring system, CW Kalenji 100, Oxylane, France). Finally, RT was measured using a digital thermometer (Microlife Corp., Paris, France), and ST was measured on the back and flank using a skin surface thermocouple probe (type K, model 88002K-IEC, Omega Engineering Inc., Stamford, CT, USA) connected to a microprocessor-based handheld thermometer (model HH-21, Omega Engineering Inc.). The RT and CT measurements were performed in not restrained animals and with the minimum of stress. For that,

during the adaptation period, animals were adapted to the presence of the experimenter in the pen, to the instruments and measurements conditions to avoid any influence of the procedures and measurements by themselves in the observations.

Blood samples were collected between 1030 and 1100 hours, via the jugular catheter, on days -5, -1, 1, 2, 3, 7, and 13. The same sampling protocol was done in each sampling: firstly, 2 mL of blood was taken and thrown away in order to eliminate dilution from the heparin block. Afterwards, 20 mL of blood were collected and put in heparinized collection tubes kept on ice for 10 min until centrifugation. Finally, 5 mL of saline solution with heparin were injected to prevent blood clot development. The collection tubes were centrifuged for 10 min at 3,000 rpm (1,620×g) at 4 °C (refrigerated centrifuge) and plasma were immediately subdivided in aliquots and stored at -20 or -80 °C depending of the type of analysis to be performed.

### Chemical analyses

Feed samples of each period were analyzed for DM, ash, fat contents, and crude protein ( $N \times 6.25$ ) according to AOAC (1990) methods. Gross energy content was measured using an adiabatic bomb calorimeter (IKA, Staufen, Germany). Crude fiber content and cell wall components (neutral and acid detergent fiber, and acid detergent lignin) were determined according to Van Soest and Wine methods (1967).

Blood samples were analyzed for hematocrit levels and insulin, leptin, glucose, lactate, glycerol,  $\alpha$ -amino acids, thyroxin ( $T_3$ ), triiodothyronine ( $T_4$ ), insulin-like growth factor I (IGF-I), and cortisol concentrations. Plasma glucose, lactate, glycerol, and  $\alpha$ -amino acids were analyzed on a multianalyzer Konelab 20 apparatus (Thermo Electron Corporation, Cergy Pontoise, France). Intra-assay coefficients of variation (CV) for these metabolites were less than 2 %. To perform  $\alpha$ -amino acids analysis, plasma samples were previously deproteinized with trichloroacetic acid and centrifuged. Plasma IGF-I concentrations were determined using a validated radioimmunoassay (Louveau and Bonneau 1996) that used recombinant IGF-I (GroPep, Adelaide, Australia). Intra-assay CV was less than 3.1 %. Thyroid hormones were determined using a  $T_3$  solid phase component system kit and a  $T_4$  monoclonal solid phase RIA kit (MP Biomedicals, Orangeburg, SC, USA). The CV was less than 1.25 and 7.9 %, respectively, for  $T_3$  and  $T_4$  intra-assay. Commercial RIA kits were used to measure plasma concentrations of insulin (CIS Bio International, Gif-sur-Yvette, France), leptin (Millipore Corp., Billerica, MA, USA), and cortisol (DiaSorin, Antony, France). The CV was less than 7.6 and 7.2 %, respectively, for insulin and leptin intra-assay. For the cortisol, intra and inter-assay CV were 7.3 and 12.3 %, respectively.

### Calculations and statistical analyses

Feed intake of each pig was determined from daily weighing of proposals and refusals. Then, daily feed intake (ADFI in gram of DM/day or in gram of DM/kg metabolic BW/day ( $BW^{0.60}$ ; Noblet et al. 1999), average daily gain (ADG in gram per day) and gain/feed ratio (G:F in gram of gain per gram of feed) were calculated for each period (P0 and P1) and for each day for daily feed intake. These data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA) including the fixed effects of line, period (or day for daily feed intake), replicate, and their interactions.

The ST was calculated as the average of measurements on back and flank locations. Short-term RR, HR, RT, and ST responses to heat stress were evaluated using data collected on day 0 from 0900 to 1300 hours (period of  $T_a$  transition from 24 to 30 °C). These data were analyzed with a MIXED procedure (SAS Inst., Inc.) including the fixed effects of line, time of measurement (0900, 1000, 1100, 1200, or 1300 hours), replicate, and their interactions. Medium-term responses (i.e., RR, HR, RT, and ST; from days 0 to 10) were analyzed using the MIXED procedure (SAS Inst.) including the effects of line, day, replicate, and their interaction. Daily diurnal changes of thermoregulatory responses were also calculated as the difference between values measured in the afternoon (1500 hours) and in the morning (0830 hours), and analyzed with the MIXED model used for the thermoregulatory responses.

When an interaction between line and day was found for the thermoregulatory responses, data were analyzed using a nonlinear mixed model (NLMM) previously described by Renaudeau et al. (2010):

$$Y_{ij} = y_{0i} + v_{1i}d_{ij} - r_1(v_{1i} - v_{2i}) \ln \left\{ 1 + \exp \left[ \frac{(d_{ij} - td_{1i})}{r_1} \right] \right\} - r_2(v_{2i} - v_{3i}) \ln \left\{ 1 + \exp \left[ \frac{(d_{ij} - td_{2i})}{r_2} \right] \right\} + \varepsilon_{ij},$$

where  $Y$  is the response between days -1 and 10;  $i$  is 1 to  $n$  pigs;  $j$  is the low or high RFI line;  $y_0$  is the value of  $Y$  at day 0;  $d$  is the experimental day;  $td_1$  and  $td_2$  (day of exposure) are the threshold days; and  $v_1$ ,  $v_2$ , and  $v_3$  are the linear variations of  $Y$  before and after  $td_1$  and after  $td_2$ , respectively. The  $r_1$  and  $r_2$  coefficients determine the smoothness of the transition around  $td_1$  and  $td_2$ , respectively. In our study,  $r_1$  and  $r_2$  values were fixed for each variable and corresponded, respectively, to 0.1 and 0.5 for RT and 0.25 and 0.50 for RR and ST. In this model, the first threshold day ( $td_1$ ) marks the transition from the short-term heat acclimation (STHA) phase to the medium-term heat acclimation (MTHA) phase. The second threshold day ( $td_2$ ) divides the MTHA in two phases: the first one characterized by a rapid decline of the response variable ( $Y$ ); and the second one characterized by a stabilization or slight change of  $Y$ .

The NLMM was fitted using the NLMIXED procedure of SAS (SAS Inst., Inc.), and the adjusted  $R^2$  values were estimated using the following formula (Robbins et al. 2006):

$$\text{adjusted } R^2 = 1 - \left[ \text{SSE} / (n - p - q - 1) \right] / \left[ \text{CTSS} / (n - 1) \right],$$

where SSE is the sum of squares for error (calculated from the estimation of residual values), CTSS is the corrected total sum of squares,  $n$  is the number of observations,  $p$  is the number of parameters, and  $q$  is the number of random effects.

Blood parameters were analyzed using the MIXED procedure of SAS (SAS Inst., Inc.) including the fixed effects of line, day, replicate, and their interactions. In all the statistical analyses using the MIXED procedure of SAS, the repeated measurements option was used with a compound symmetry covariance structure to account for animal effect over time.

## Results

### Climatic measurements

During P0, Ta and relative humidity averaged  $24.2 \pm 0.4$  °C and  $49.6 \pm 9.6$  %, respectively, the corresponding values during P1 were  $30.4 \pm 0.7$  °C and  $36.6 \pm 9.4$  %. These values are in accordance with the objectives of the experiment.

### Growth performance

Because of excessive feed spillage, performance data of one RFI<sup>-</sup> pig during the second replicate was not considered in the analyses. No interaction ( $P > 0.05$ ) between line and period was found for any performance trait (Table 2). Irrespective of period, RFI<sup>+</sup> pigs had greater ADFI (2,423 vs. 2,138 g/day;  $P < 0.01$ ; 209 vs. 188 g/kg BW<sup>0.60</sup>/day;  $P < 0.01$ ) than RFI<sup>-</sup>

pigs. Nevertheless, similar ADG was observed in both lines (967 g/day on average;  $P > 0.05$ ). Consequently, RFI<sup>+</sup> pigs were less efficient than RFI<sup>-</sup> pigs (0.40 vs. 0.45 for G:F;  $P < 0.01$ ). The high Ta affected negatively the overall growth performance. Whatever the line, the Ta rise from 24 to 30 °C resulted in a reduction in ADFI ( $-49$  g/day/°C and  $-7$  g/kg BW<sup>0.60</sup>/day/°C;  $P < 0.01$ ) and ADG ( $-53$  g/day/°C;  $P < 0.01$ ). The G:F ratio was also decreased at 30 °C (0.38 vs. 0.47;  $P < 0.01$ ). Although no interactions ( $P > 0.05$ ) between line and ambient temperature were found, when exposed to high Ta, RFI<sup>+</sup> pigs had a numerically higher reduction in ADG ( $-365$  vs.  $-268$  g/day;  $P = 0.44$ ) and ADFI ( $-382$  vs.  $-200$  g/day;  $P = 0.17$ ) than RFI<sup>-</sup> pigs. Daily feed intake (in gram per kilogram BW<sup>0.60</sup> per day) during the experimental period is presented in Fig. 1. Except on days -6, -5, and -4, ADFI/kg BW<sup>0.60</sup> did not differ between lines ( $P > 0.05$ ).

### Thermoregulatory responses

*Short-term responses (day 0)* Average Ta at 900, 1000, 1100, 1200, and 1300 hours were 24.2, 24.7, 26.3, 27.9, and 29.2 °C, respectively. Whatever the thermoregulatory response (RT, ST, RR, or HR), the interaction between line and time was not significant ( $P > 0.05$ ) and only least square means per time are presented (Fig. 2). As expected, all thermoregulatory responses were influenced by the temperature increase. However, these responses were not influenced by line ( $P > 0.05$ ). The Ta increase resulted primary (i.e., at about 1100 hours) in a gradual increase in ST and a decrease in HR, respectively; and subsequently (i.e., at about 1200 hours) in a gradual increase of RT and RR.

*Medium-term responses* Preliminary analyses indicated that thermoregulatory responses did not differ ( $P > 0.05$ ) on days -6, -4, and -1 (i.e., during P0). Thus, in a second approach,

**Table 2** Effects of line and exposure to high ambient temperature on growth performance in pigs (least squares means of 16 pigs RFI<sup>-</sup> and 17 pigs RFI<sup>+</sup>)

Item	Line		Period <sup>a</sup>		RSD	Significant effects <sup>b</sup>
	RFI <sup>-</sup>	RFI <sup>+</sup>	0	1		
Mean BW (kg)	58.2	60.2	54.4	64.0	0.8	P**, R*
ADFI (g/d) <sup>c</sup>	2,138	2,423	2,426	2,135	262	L**, P**, R*
ADFI (g/kg BW <sup>0.60</sup> /d) <sup>c</sup>	188	209	220	176	22	L**, P**
ADG (g/d)	970	965	1,126	810	250	P**
G:F (g of gain/g of feed) <sup>c</sup>	0.45	0.40	0.47	0.38	0.08	L**, P**, R**

RSD residual standard deviation

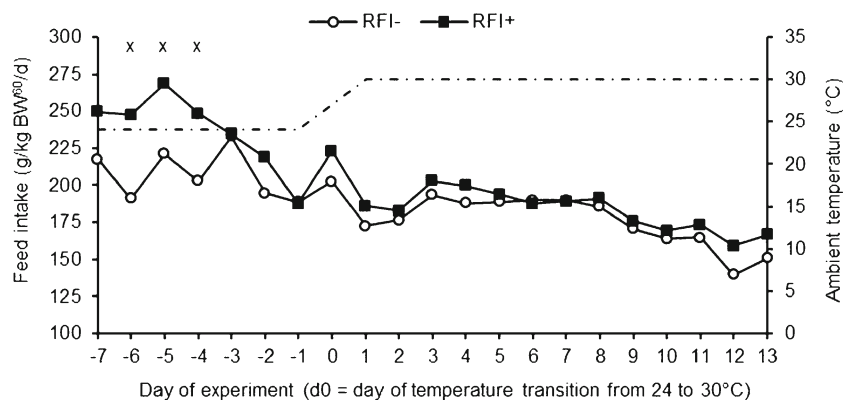
<sup>a</sup> Period 0: 24 °C, 7 days; period 1: 30 °C, 14 days

<sup>b</sup> Data were analyzed using a general linear mixed model including the effects of period (P), line (L), replicate (R), and the P and L interaction as fixed effects

<sup>c</sup> For an average DM of 86.6 %

\* $P < 0.05$ , statistically significant; \*\* $P < 0.01$ , statistically significant

**Fig. 1** Effect of exposure to high ambient temperature on ADFI (in gram per kilogram BW<sup>0.60</sup> per day) in RFI<sup>-</sup> and RFI<sup>+</sup> pigs. Each point is the least squares mean of 16 pigs RFI<sup>-</sup> and 17 pigs RFI<sup>+</sup>. Dotted line ambient temperature, × daily feed intake significantly different between lines ( $P < 0.05$ )

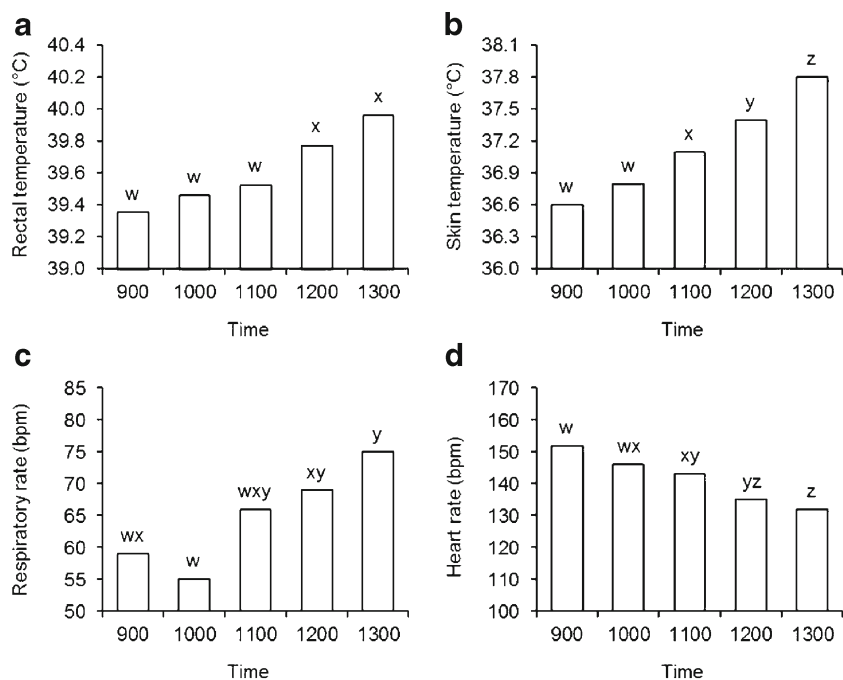


daily mean values of traits measured at 30 °C were compared to the mean value in P0 using a contrast (Table 3). On average, RFI<sup>+</sup> pigs had higher ST (37.8 vs. 37.5 °C;  $P < 0.05$ ) and HR (142 vs. 135 bpm;  $P < 0.05$ ) than RFI<sup>-</sup> pigs. However, RT and RR were not affected by line. All thermoregulatory traits were affected by the day of experiment. Whatever the day of exposure, the RT values on P1 were higher ( $P < 0.05$ ) than in P0, except on day 6 ( $P = 0.06$ ). Within P1, RT was greater on days 1 and 2 (40.1 °C on average;  $P < 0.01$ ) and thereafter it decreased to a constant value of about 39.8 °C. Irrespective of day of exposure, ST and RR values over P1 were higher than in P0 (37.9 vs. 37.0 °C and 81 vs. 55 bpm on average for ST and RR, respectively;  $P < 0.05$ ). During P1, ST was higher on day 1 (38.3 °C;  $P < 0.01$ ). From days 2 to 8, ST

remained at a constant value of 38.0 °C. Similarly to RT and ST, RR was greater on day 1 (96 bpm;  $P < 0.01$ ) than on day 0. Thereafter, it decreased from days 2 and 3 and remained constant at 82 bpm from days 3 to 10. With regard to HR, it did not differ within P1 but it was lower in P1 than in P0 (135 vs. 146 bpm;  $P < 0.05$ ).

Interaction between line and day of experiment was found only for RT, ST, and RR. The patterns of these changes and their profiles during exposure to high Ta, studied using a modeling approach, are presented in Table 4 and Fig. 3, respectively. The magnitude of the linear increase of RT before  $td_1$  (i.e.,  $v_1$ ), 0.30 °C/day on average, did not differ between lines. However,  $td_1$  was 24 h earlier in RFI<sup>-</sup> pigs than in RFI<sup>+</sup> (0.85 vs. 1.88 day;  $P < 0.01$ ).

**Fig. 2** Effect of temperature transition from 24 to 30 °C (2 °C per hour) on rectal temperature (a), skin temperature (b), respiratory rate (c), and heart rate (d). Average ambient temperature at 900, 1000, 1100, 1200, and 1300 hours were 24.2, 24.7, 26.3, 27.9, and 29.2 °C, respectively. The interaction between line and time was not significant for any trait ( $P > 0.05$ ). Each vertical bar is the least square mean of seven pigs RFI<sup>-</sup> and eight pigs RFI<sup>+</sup>. Within each graphic, least square means with different letters differ ( $P < 0.05$ ) according to the time



**Table 3** Effects of line and day of exposure to high ambient temperature on thermoregulatory responses in pigs (least squares means of 17 pigs RFI<sup>-</sup> and 17 pigs RFI<sup>+</sup>)

Item	Line		Day of exposure										RSD	Significant effects <sup>b</sup>
	RFI <sup>-</sup>	RFI <sup>+</sup>	-6	-4	-1	0 <sup>a</sup>	1	2	3	6	8	10		
Ambient temperature (°C)	-	-	24.1	24.2	24.2	27.1	30.6	30.3	30.4	30.5	30.5	30.5		
Rectal temperature (°C)														
Daily mean	39.7	39.8	39.5	39.5	39.7	39.8 <sup>w</sup> d	40.1 <sup>x</sup> d	40.0 <sup>x</sup> d	39.8 <sup>w</sup> d	39.7 <sup>w</sup>	39.8 <sup>w</sup> d	39.8 <sup>w</sup> d	0.2	D**, DL*
Diurnal change <sup>c</sup>	0.3	0.4	0.1	0.1	0.2	-	0.4 <sup>wx</sup> d	0.3 <sup>w</sup> d	0.6 <sup>x</sup> d	0.4 <sup>w</sup> d	0.4 <sup>wx</sup> d	0.4 <sup>wx</sup> d	0.3	D**, LR*
Skin temperature (°C)														
Daily mean	37.5	37.8	37.0	37.0	37.0	37.4 <sup>w</sup> d	38.3 <sup>z</sup> d	38.1 <sup>yz</sup> d	38.0 <sup>xy</sup> d	37.9 <sup>xy</sup> d	38.0 <sup>xy</sup> d	37.8 <sup>x</sup> d	0.3	L*, D**, DL**
Diurnal change <sup>c</sup>	0.3	0.4	0.4	0.1	0.1	-	0.3	0.2	0.6 d	0.4	0.3	0.5 d	0.5	D*, DL*, DLR*
Respiratory rate (bpm)														
Daily mean	73	74	58	52	54	65 <sup>w</sup> d	96 <sup>y</sup> d	87 <sup>xy</sup> d	80 <sup>x</sup> d	79 <sup>x</sup> d	81 <sup>x</sup> d	81 <sup>x</sup> d	11	D**, R**, DL**
Diurnal change <sup>c</sup>	1	4	-1	-3	-3	-	2	7	7	5	6	3	19	DL*
Heart rate (bpm)														
Daily mean	135	142	147	149	142	140 d	135 d	139 d	134 d	136 d	136 d	128 d	10	L*, D**, R*
Diurnal change <sup>c</sup>	-11	-1	-6	-4	4	-	-7	-13	-8	-8	-9	-1	16	L*

RSD residual standard deviation

<sup>a</sup> Day 0: day of ambient temperature transition from 24 to 30 °C; mean values of measurements performed at 0900 and 1300 hours in 10 pigs RFI<sup>-</sup> and 8 pigs RFI<sup>+</sup>

<sup>b</sup> Data were analyzed using a general linear mixed model including the effects of day of experiment (D), line (L), replicate (R), and their interactions as fixed effects

Statistical significance: \* $P < 0.05$ ; \*\* $P < 0.01$

<sup>c</sup> Differences between mean values measured in the afternoon (1500 hours) and in the morning (0830 hours)

<sup>d</sup> Mean value statistically different ( $P < 0.05$ ) from the mean of period 0 (mean of days -6, -4 and -1); values were compared using the contrast statement of the MIXED procedure (SAS Inst., Inc.)

<sup>w, x, y, z</sup> Within period 1 (days 0–10), means in the same row with different superscripts letters are statistically different ( $P < 0.05$ )

Although not significant,  $td_2$  was 36 h earlier in RFI<sup>-</sup> pigs compared to RFI<sup>+</sup> (1.52 vs. 2.94 days;  $P = 0.42$ ). This absence of significance could be related to the high within line variation of this parameter, especially in RFI<sup>-</sup> pigs. The  $v_2$  and  $v_3$  estimates did not differ between lines ( $P > 0.05$ ). Concerning ST, the MTHA period in RFI<sup>+</sup> pigs could not be modeled in two distinct phases, thus, ST response in RFI<sup>+</sup> was adjusted using the following model, previously described by Renaudeau et al. (2010):

$$Y_{ij} = y_{0i} + v_{1i}d_{ij} - r_1(v_{1i} - v_{2i}) \ln\{1 + \exp[(d_{ij} - td_{1i})/r_1]\} + \varepsilon_{ij}$$

The  $r_1$  was fixed to 0.25. The  $v_1$  value for ST increased similarly in both lines (0.60 °C/day on average) but  $td_1$  and then STHA period was shorter in RFI<sup>+</sup> than RFI<sup>-</sup> pigs (0.98 vs. 1.64 days;  $P < 0.05$ ). The  $v_2$  estimate did not differ between lines ( $P > 0.05$ ); however, unlike what was observed in RFI<sup>-</sup> pigs, the ST in RFI<sup>+</sup> pigs did not fall immediately after  $td_1$ . Although interaction between line and day of experiment was found for RR, when data were analyzed using the NLMM, no differences were found for any parameter ( $P >$

0.05). That absence of significance may be due to the high residual variance of the model (i.e., 140 bpm) due to a high inter individual variation. Despite this nonsignificance, the decrease in RR after  $td_1$  was twice as fast in RFI<sup>-</sup> pigs than RFI<sup>+</sup> pigs (-21.0 vs. -9.5 bpm/day;  $P = 0.38$ ), and  $td_2$  was approximately 40 h earlier in RFI<sup>-</sup> pigs compared to RFI<sup>+</sup> pigs (2.31 vs. 3.98 days;  $P = 0.19$ ).

#### Blood parameters

The effects of line and exposure to high ambient temperatures on blood parameters are presented in Table 5. Because of blood clots, five intravenous catheters (three in RFI<sup>-</sup> and two in RFI<sup>+</sup> pigs) stopped working during the first replicate. Therefore, blood measurements were obtained only from seven RFI<sup>-</sup> pigs and eight RFI<sup>+</sup> pigs. Since blood parameters during P0 did not differ, mean values of each day in P1 were compared to the mean value in P0. No effect of line was found for any blood parameter ( $P > 0.05$ ), whereas most of them were affected by ambient temperature. As expected, plasma concentrations of T<sub>3</sub> during P1 were lower than during P0 (81

**Table 4** Effect of exposure to high ambient temperatures on thermoregulatory responses in RFI<sup>-</sup> and RFI<sup>+</sup> pigs (estimated mean  $\pm$  SE)

Item	Parameter	Line		$\sigma_e^2$	Adjusted R <sup>2</sup>
		RFI <sup>-</sup>	RFI <sup>+</sup>		
Rectal temperature	$y_0$	39.82 $\pm$ 0.14	39.86 $\pm$ 0.07	0.061	0.69
	$v_1$	0.33 $\pm$ 0.13	0.26 $\pm$ 0.04		
	$v_2$	-0.63 $\pm$ 1.02	-0.67 $\pm$ 0.43		
	$v_3$	-0.003 $\pm$ 0.014	0.033 $\pm$ 0.023		
	$td_1^d$	0.85 $\pm$ 0.26	1.88 $\pm$ 0.16		
	$td_2$	1.52 $\pm$ 1.58	2.94 $\pm$ 0.78		
Skin temperature	$y_0$	37.46 $\pm$ 0.14	37.72 $\pm$ 0.09	0.096	0.76
	$v_1$	0.48 $\pm$ 0.13	0.72 $\pm$ 0.07		
	$v_2$	-1.85 $\pm$ 2.25	-0.04 $\pm$ 0.01		
	$v_3$	0.040 $\pm$ 0.019	-		
	$td_1^d$	1.64 $\pm$ 0.16	0.98 $\pm$ 0.16		
	$td_2$	2.05 $\pm$ 0.80	-		
Respiratory rate	$y_0$	77.4 $\pm$ 4.2	72.0 $\pm$ 3.6	140	0.71
	$v_1$	21.9 $\pm$ 3.5	20.8 $\pm$ 2.3		
	$v_2$	-21.0 $\pm$ 11.6	-9.5 $\pm$ 5.8		
	$v_3$	1.13 $\pm$ 0.66	1.58 $\pm$ 1.11		
	$td_1$	1.01 $\pm$ 0.24	1.37 $\pm$ 0.24		
	$td_2$	2.31 $\pm$ 0.52	3.98 $\pm$ 1.13		

Rectal temperature, skin temperature, and respiratory rate responses were subjected to a nonlinear mixed model:  $Y=y_0+v_1d-r_1(v_1-v_2)\ln\{1+\exp[(d-td_1)/r_1]\}-r_2(v_2-v_3)\ln\{1+\exp[(d-td_2)/r_2]\}+\varepsilon$  or  $Y=y_0+v_1d-r_1(v_1-v_2)\ln\{1+\exp[(d-td_1)/r_1]\}+\varepsilon$ ; where  $Y$  is the response variable;  $y_0$  is the value of  $Y$  at  $d=0$  (in degree Celsius for rectal and skin temperature and breaths per min (bpm) for respiratory rate);  $td_1$  and  $td_2$  (day of exposure) are the threshold days; and  $v_1$ ,  $v_2$ , and  $v_3$  are the linear variations of  $Y$  before and after  $td_1$  and  $td_2$ , respectively; and  $\sigma_e^2$  is the residual variance of the model

<sup>d</sup> Estimated means significantly different ( $P < 0.05$ ). Estimated means were compared using the contrast statement of the NLMIXED procedure

vs. 107 ng/dL on average;  $P < 0.05$ ). Within P1,  $T_3$  concentration was lower on day 1 (70 ng/dL;  $P < 0.05$ ) and then it increased, remaining steady at about 84 ng/dL between days 2 and 13. The  $T_4$  concentration at 30 °C was lower than at 24 °C only on days 1, 2, and 3 (-18 %, on average;  $P < 0.05$ ). Cortisol was significantly lower at 30 °C than at 24 °C (-44 % on average;  $P < 0.05$ ), whereas it remained constant within P1 (16.5 ng/mL on average;  $P < 0.05$ ). Blood concentration of IGF-I was similar throughout the experiment (239 ng/mL on average;  $P > 0.05$ ), except on day 13 when it decreased by about 20 %. Immediately after Ta increase (on day 1), plasma levels of  $\alpha$ -amino-acids N rose compared to at thermoneutrality (672 vs. 625 mg/L;  $P < 0.05$ ) and then decreased over the next days, averaging 582 mg/L on day 13. Glycerol concentrations over P1 differed from P0 only on day 13 (4.64 vs. 3.86 on day 13 and in P0, respectively;  $P > 0.05$ ). Insulin, leptin, glucose, and lactate concentrations were not affected by Ta ( $P > 0.05$ ) and averaged 25.0  $\mu$ UI/mL, 2.63 ng/mL, 1,047 mg/L, and 93 mg/L, respectively.

Except for hematocrit values and  $T_3$  concentrations, the interaction between line and day of experiment was not significant ( $P > 0.05$ ) for any blood parameter. Except on days 2 and 3, hematocrit values tended to be slightly higher in RFI<sup>+</sup> pigs than in RFI<sup>-</sup>. With regard to  $T_3$ , RFI<sup>-</sup> pigs tended to have higher plasmatic concentrations than RFI<sup>+</sup> pigs except on days -5 and 7.

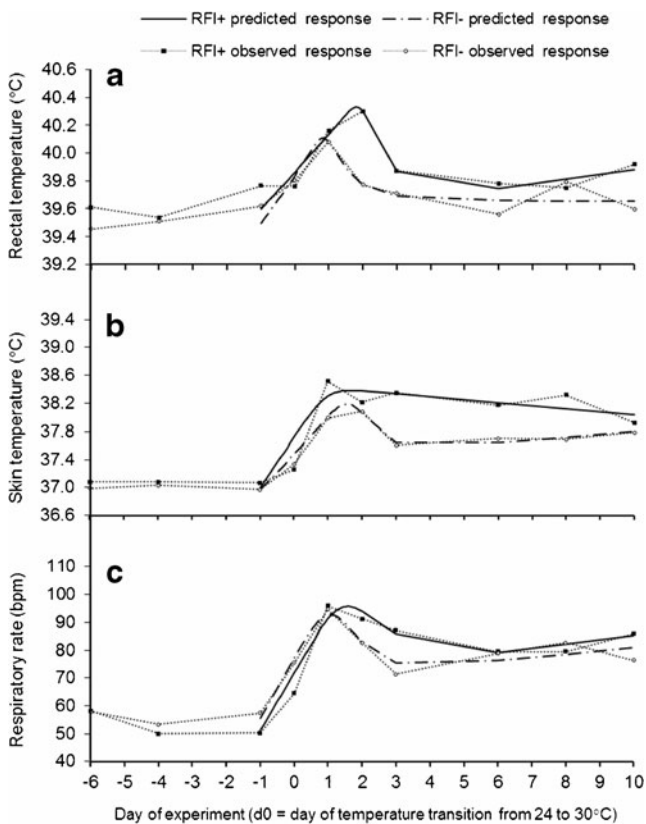
## Discussion

### Effect of high ambient temperature on performance in growing pigs

In a recent meta-analysis, Renaudeau et al. (2011) estimated that each degree increase in Ta between 20 and 30 °C results in a decrease of 32 and 18 g/day in ADFI and ADG, respectively, in 50 kg BW pigs. The present study is in agreement with these findings and shows that, whatever the line, the Ta rise from 24 to 30 °C resulted in an associated ADFI reduction of 49 g/day/°C. Nevertheless, this ADFI reduction was much lower than that reported by Renaudeau et al. (2013) in comparable pigs (i.e., same origin) for a 24 to 32 °C temperature range (i.e., -100 g/day/°C). This discrepancy could be explained by the more stressful experimental conditions applied to the pigs in this study compared to the present study. First, their animals were housed in respiration chambers in which the temperatures of the floor and walls were similar to the Ta. Second, pigs were submitted to a higher Ta increase (+8 vs. +6 °C), the decrease in ADFI (in gram per degree Celsius) being as high as Ta is high (Renaudeau et al. 2010). Third, the relative humidity was higher (75 vs. 37 %) which may have decreased pig's ability to dissipate heat by evaporation.

The lower ADFI in hot conditions is an adaptive response to minimize heat production associated to digestive and





**Fig. 3** Effect of exposure to high ambient temperature on rectal temperature (a), skin temperature (b), and respiratory rate (c) profiles in RFI<sup>-</sup> and RFI<sup>+</sup> pigs. Each point is the least squares mean of 17 pigs RFI<sup>-</sup> or 17 pigs RFI<sup>+</sup>. From days -1 to 10, predicted responses were estimated using a nonlinear model (see Table 4 for parameters values)

metabolic processes; however, it results in a lower amount of nutrients available for growth which then impairs growth performance. In our study, the Ta rise resulted in an average reduction of 53 g/day/°C in ADG. A similar reduction (i.e., 50 g/day/°C) was reported by Renaudeau et al. (2008) in 50 kg growing pigs in a Ta range of 24–32 °C. Nevertheless, the ADG reduction in the present study was higher than that estimated in the meta-analysis of Renaudeau et al. (2011), which could be related to the fact that most of the published results on the effects of high Ta on pigs performance were obtained in pigs previously acclimated to elevated temperatures, which was not the case in our study. According to our results, the reduction in growth rate in P1 was more than twice higher than the reduction in feed consumption (-28 vs. -12 %). This is consistent with the fact that the reduction in feed intake will mainly impact the amount of feed energy available for production purposes, as it represents about 60 % of the total requirements. This situation might even be emphasized by the increased energy requirements for maintenance in hot conditions, in connection with increased physical activity due to intense panting to maintain homeothermy (Quiniou et al. 2001; Renaudeau et al. 2013). Lower feed efficiency in heat-stressed pigs was also reported by Tavares

et al. (1999) and Kiefer et al. (2009), whereas Le Bellego et al. (2002) and Collin et al. (2002) observed no difference. This discrepancy in the results could be related to differences in breed, BW, diet composition, pre-experimental conditions, and, also, temperature range.

#### Thermoregulatory responses and blood parameters during thermal acclimation

**Short-term acclimation** In our study, the STHA responses were evaluated in a 4-h period in which Ta linearly increased from 24 to 30 °C. This Ta rise resulted primarily (i.e., at about 26 °C of Ta) in a ST increase and HR decrease, and posteriorly (i.e., at about 28 °C of Ta) in a RR and RT increases. The rise in ST is the consequence of an increased blood flow from the core to the skin associated with a cutaneous vasodilation to enhance the heat exchange between the skin and the environment (Collin et al. 2002). Within this STHA period, ST increased by 0.22 °C per extra degree of Ta. Similar ST increase was reported by Renaudeau et al. (2007; 0.22 °C/°C for a Ta range of 24–31 °C). Little is known about the effects of Ta on HR response in pigs. In our study, HR started to decrease within the first hours of heat exposure. In contrast, a short-term increase in HR was reported in rats (Horowitz and Meiri 1993) and humans (Crandall et al. 2008) exposed to high Ta. Although pigs' behavior was not recorded in the present study, pigs became visually quieter when Ta increased. Since pigs' heart rate tends to decrease with the decrease in physical activity (D'Allaire and DeRoth 1986), it can be suggested that the short-term reduction in HR in our study was mainly a direct consequence of a decreased physical activity with thermal stress.

As Ta increases, pigs' ability to lose heat by sensible pathways (radiation, convection, and conduction) decreases due to a reduction in the gradient of temperature between the skin and the environment (Quiniou et al. 2000). Thus, above 28 °C, almost all heat dissipation in growing pigs is accomplished through evaporative heat losses (Renaudeau et al. 2008). As pigs have a limited number of sweat glands, their major evaporative pathway to dissipate heat is by respiratory evaporation (Huyhn et al. 2005). Our result agrees with this statement, and RR started to rise from 28 °C. This threshold value also called evaporative critical temperature is similar to those reported by Renaudeau et al. (2007) in individually housed growing pigs. According to our results, RT also increased from about 28 °C on, in agreement with previous results (Renaudeau et al. 2007). In fact, a rise in RT under hot conditions indicates a relative inability of the animals to adequately dissipate the excess heat load (Nienaber et al. 1999).

**Medium-term acclimation** Regarding pigs' acclimation during P1, the exposure to 30 °C resulted initially in an increase in

**Table 5** Effects of line and day of exposure to high ambient temperature on blood parameters in pigs (least squares means of seven pigs RFI<sup>-</sup> and eight pigs RFI<sup>+</sup>)

Item	Line		Day of experiment							RSD	Significant effects <sup>a</sup>
	RFI <sup>-</sup>	RFI <sup>+</sup>	-5	-1	1	2	3	7	13		
Ambient temperature (°C)	–	–	24.2	24.2	30.6	30.3	30.4	30.5	30.5	–	–
Hematocrit (%)	31	31	32	32	31 <sup>wx</sup>	32 <sup>x</sup>	29 <sup>w</sup> d	32 <sup>x</sup>	31 <sup>wx</sup>	2	D*, DL*
Hormones											
T <sub>3</sub> (ng/dL)	92	85	105	110	70 <sup>w</sup> d	81 <sup>wx</sup> d	84 <sup>x</sup> d	89 <sup>x</sup> d	80 <sup>wx</sup> d	12	D**, DL*, LR*
T <sub>4</sub> (µg/dL)	2.99	2.96	3.36	3.22	2.55 <sup>w</sup> d	2.78 <sup>wx</sup> d	2.74 <sup>wx</sup> d	3.19 <sup>x</sup>	3.00 <sup>wx</sup>	0.43	D**, R*
Cortisol (ng/mL)	22.4	18.2	30.8	28.6	18.7 d	15.6 d	12.8 d	17.1 d	18.3 d	11.2	D**
IGF-I (ng/mL)	225	239	239	251	240	222	238	243	192 d	43	D*, R*
Insulin (µUI/mL)	23.6	26.4	18.6	23.7	27.4	25.9	25.4	27.9	26.0	7.9	R*
Leptin (ng/mL)	2.79	2.47	2.22	2.62	2.64	2.66	2.81	2.92	2.54	0.55	R*
Metabolites											
Glycerol (mg/L)	3.98	3.95	3.94	3.78	4.18 <sup>wx</sup>	3.60 <sup>wx</sup>	3.49 <sup>w</sup>	4.14 <sup>wx</sup>	4.64 <sup>x</sup> d	0.87	D*
α-amino-acids N (mg/L)	600	646	611	638	672 <sup>x</sup> d	642 <sup>wx</sup>	599 <sup>w</sup>	617 <sup>wx</sup>	582 <sup>w</sup> d	57	D*
Glucose (mg/L)	1,050	1,044	1,022	1,058	1,088	1,041	1,039	1,046	1,037	65	
Lactate (mg/L)	88	98	99	89	91	98	89	93	90	18	

RSD residual standard deviation

<sup>a</sup> Data were analyzed using a general linear mixed model including the effects of day of experiment (D), line (L), replicate (R), and their interactions as fixed effects. Statistical significance: \* $P < 0.05$ ; \*\* $P < 0.01$

<sup>d</sup> Mean value statistically different ( $P < 0.05$ ) from the mean of period 0 (mean of days -5 and -1); values were compared using the contrast statement of the MIXED procedure (SAS Inst. Inc.)

<sup>w, x</sup> Within period 1 (days 0–13), means in the same row with different letters are statistically different ( $P < 0.05$ )

ST, RR, and RT within the first 24–48 h and, subsequently, in a decrease followed by stabilization. This pattern of response was previously described by Giles et al. (1991) and Renaudeau et al. (2010) in pigs and by Bianca (1959) in calves; it consists in a biphasic profile of acclimation to heat stress characterized by an initial phase of rapid activation of the thermoregulatory responses and hyperthermia and a subsequent recovery phase characterized by a gradual decrease of the thermoregulatory responses. According to Renaudeau et al. (2013), the decrease in thermoregulatory responses during acclimation can be explained by a reduction in endogenous heat production that decreases body demand for heat dissipation. In this study, the decrease in heat production occurred mainly within the first 24 h of heat exposure and was principally related to a decrease in the thermic effect of feed due to a decrease in feed consumption. In our study, besides a drop in ADFI observed within the first 24 h of heat exposure, the decline in ST, RR, and RT also occurred within the first days of heat exposure. That coincides chronologically with the drop in the thermic effect of feed described by Renaudeau et al. (2013). Therefore, it can be suggested that the medium-term decrease in RT observed in our study is mainly associated to a decrease in heat production caused by a decrease in feed consumption. As suggested by Giles et al. (1991), a decline in ST and RR over the acclimation period at

30 °C is interpreted as a reduction of the cooling demand. In contrast to RT, RR, and CT, HR did not show a biphasic response to heat stress. This parameter decreased in hot conditions but remained relatively constant during P1, despite a numerically lower value was observed on day 10. A medium-to long-term decrease in HR during thermal acclimation was also observed in rats (Horowitz and Meiri 1993). It can be hypothesized that the HR decrease is a consequence of a decreased metabolism in hot conditions; however further studies should be undertaken to confirm this hypothesis and the results obtained in our study.

Besides affecting the thermoregulatory responses discussed above, heat stress is also thought to modify metabolic and hormonal profiles in homoeothermic animals. One of the primary modifications during heat acclimation is a decrease in the endogenous levels of thyroid hormones (i.e., T<sub>3</sub> and T<sub>4</sub>; Macari et al. 1986; Collin et al. 2002) to decrease the metabolic rate and consequently reduce the amount of heat produced by cells (Bernabucci et al. 2010). In our study, the exposure to heat stress resulted in a decrease in these hormones followed by a gradual increase over time. This suggests that thyroid hormones are also involved in the acclimation of pigs to a sustained heat load by a direct or an indirect effect on metabolic rate. Whether T<sub>3</sub> and T<sub>4</sub> decline over duration of exposure to 30 °C occurs in direct response to thermal

inhibition of hypothalamus or as an indirect effect of lower feed intake and metabolism is still unclear. In our study, plasma cortisol concentrations decreased under hot conditions, which is in agreement with the studies of Marple et al. (1972), Heo et al. (2005), and Kim et al. (2009). Although not significant, insulin concentrations were numerically higher in P1 than in P0. This result corroborates recent studies demonstrating an increase in insulin concentration in hot conditions (Pearce et al. 2013; Rhoads et al. 2013). The causes for this increase are not well known, however, it has been associated to the role of insulin in activating and up-regulating heat shock proteins (Li et al. 2006; Pearce et al. 2013). More generally, these latter authors suggest that insulin plays a critical role in the ability of animals to respond and acclimate to a thermal challenge. Since IGF-I is mainly implicated in cell proliferation and protein deposition (Florini et al. 1996) and pigs growth rate decreased during heat exposure, a decrease in IGF-I levels was expected; however, it was not observed in our study. The lack of influence of high Ta on IGF-I concentrations was also observed in growing pigs (Becker et al. 1993) and dairy cows (Titto et al. 2012). In contrast, Collin et al. (2002) reported a decrease in plasma IGF-I concentrations in heat stressed piglets. The present results indicate that glucose and leptin concentrations were not affected by heat stress. In the study by Pearce et al. (2013), heat stressed pigs had lower levels of glucose compared to those at thermoneutrality; however, these authors suggested that it was mainly associated to a decrease in feed intake rather than a direct effect of heat stress once it was not observed in pigs under pair feeding conditions. In the study by Marple et al. (1972), both glucose and NEFA concentrations were increased, but to a variable extent depending upon humidity level. In addition, we hypothesize that our experimental design could partially account for this absence of effect since animals were fasted for 2 h prior to blood collection. Regarding leptin concentrations, we were expecting a decrease of this hormone in hot conditions as a consequence of feed intake reduction. Nevertheless, differences in leptin plasma concentration have been mainly associated with differences in body composition (Lefaucheur et al. 2011) and not with nutritional status, which may explain our results.

#### Effects of RFI selection on pig's acclimation to high ambient temperature

Selection for low RFI reduces feed intake without correlated effects on growth rate and body composition; therefore, feed efficiency is improved (Gilbert et al. 2007; Hoque and Suzuki 2009) and heat production is reduced (Barea et al. 2010; Renaudeau et al. 2013). The results of the present study, even obtained over a short period and with a reduced number of pigs per line are consistent with this assumption and show

that, irrespective of ambient temperature, RFI<sup>-</sup> pigs had a lower ADFI (-12 %) and greater G:F ratio (+13 %) than RFI<sup>+</sup> pigs, without difference in ADG. It was also hypothesized that the negative effects of heat stress on performance are accentuated in RFI<sup>+</sup> pigs. In our study, although not significant, RFI<sup>+</sup> pigs had a numerically higher reduction in ADG (-365 vs. -268 g/day) and ADFI (-382 vs. -200 g/d) than RFI<sup>-</sup> pigs when exposed to hot conditions. Nevertheless, Renaudeau et al. (2013) reported that the effects of heat stress on performance did not differ between RFI<sup>-</sup> and RFI<sup>+</sup> lines. On the other hand, Gilbert et al. (2012b) suggested that RFI<sup>-</sup> pigs have lower ADG than RFI<sup>+</sup> pigs when raised in a tropical environment, which reduced their advantage in terms of feed efficiency compared to RFI<sup>+</sup> pigs. In fact, it seems that experimental conditions (Ta range, humidity, housing conditions, diet) have a major influence in the between line responses to heat stress, since studies performed on animals from the same genetic program but in different environmental conditions exhibited different responses.

In general, RFI<sup>+</sup> pigs had higher ST and HR than RFI<sup>-</sup> pigs, which can be associated to their greater metabolic rate and increased physical activity, respectively (Barea et al. 2010). This higher ST is in agreement with the greater sensible heat losses in RFI<sup>+</sup> line reported by Renaudeau et al. (2013). In our study, RR was not influenced by the line. In contrast, Renaudeau et al. (2013) reported greater RR in RFI<sup>+</sup> pigs irrespective of Ta. It suggests that, in our experimental conditions, RFI<sup>+</sup> pigs had a greater capacity to dissipate heat by the sensible pathways and then depended less on the evaporative losses to dissipate their extra heat, which can be related to the Ta and relative humidity differences between the two studies.

Concerning the acclimation process, STHA responses were similar in RFI<sup>-</sup> and RFI<sup>+</sup> pigs. Similarly, Renaudeau et al. (2008; experiment 2) reported no differences in STHA between Large White and Caribbean Creole growing pigs. From these results, it could be suggested that the immediate responses to thermal heat stress are essentially similar within a given species since they correspond to the primary survival-oriented responses that are less influenced by inter-individual differences. According to the present study and based on RT measurements, the onset of the MTHA response (i.e., td<sub>1</sub>) was delayed in RFI<sup>+</sup> pigs. This line effect could be related to the high metabolic heat production and high demand for heat dissipation of RFI<sup>+</sup> pigs compared to RFI<sup>-</sup> pigs. Similar results were obtained when two breeds with different metabolic heat production were compared using the same thermal challenge (Renaudeau et al. 2007). After td<sub>1</sub>, the pattern of thermoregulatory responses was not affected by RFI selection, suggesting that the physiological mechanisms are similar in both lines during this period. Overall, metabolic and endocrine profiles during acclimation were similar in both lines. Our results are in agreement with those of Le Naou et al. (2012) that reported no differences in plasma concentration of

nutrients (i.e., glucose and lactate) and hormones (i.e., IGF-I, leptin, and insulin) between RFI<sup>-</sup> and RFI<sup>+</sup> pigs from the sixth and seventh generation of the same selection program as in the present study. Our hypothesis was that at least thyroid hormones profiles during acclimation would be different between lines. However, only slight and isolated differences between lines were observed for T<sub>3</sub> concentrations, which do not allow to do draw definitive conclusions about the effects of RFI selection on thyroid hormones. In fact, it seems that the between-lines differences (e.g., heat production, feed efficiency, and physical activity) are not sufficient to induce significant changes in endocrine and metabolite responses during thermal stress.

## Conclusion

Based on performance data and thermoregulatory responses, our study suggests that selection for low RFI tends to slightly ameliorate pigs' tolerance to heat stress. This higher thermotolerance of low RFI animals would be related to their lower total heat production rather than to metabolic or hormonal differences since blood parameters during acclimation in our study were similar in both lines. Therefore, experiments in commercial conditions in tropical and subtropical areas are still needed in order to evaluate the effectiveness of RFI selection as a genetic strategy to mitigate the effects of heat stress.

**Acknowledgments** The authors gratefully acknowledge Loïc Gaillard, Francis Le Gouevic, Alain Chauvin, Régis Janvier, Serge Dubois, and the GENESI staff for animal care and technical assistance. Aline Foury, Laure Gress, Anne Pasquier, and Christine Tréfeu for the laboratory analysis. Bruno Silva is thanked for the critical evaluation of the study. This experiment was supported by the French National Research Agency (ANR), program "PIG\_FEED" (ANR-08-GENM-038). Financial support from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) is also acknowledged.

## References

- AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington, DC
- Barea R, Dubois S, Gilbert H et al (2010) Energy utilization in pigs selected for high and low residual feed intake. *J Anim Sci* 88(6):2062–2072
- Becker BA, Knight CD, Veenhuizen JJ et al (1993) Performance, carcass composition, and blood hormones and metabolites of finishing pigs treated with porcine somatotropin in hot and cold environments. *J Anim Sci* 71(9):2375–2387
- Bernabucci U, Lacetera N, Baumgard LH et al (2010) Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal* 4(7):1167–1183
- Bianca W (1959) Acclimatization of calves to hot dry environment. *J Agric Sci* 52:296–304
- Brown-Brandl TM, Nienaber JA, Xin H, Gates RS (2003) A literature review of swine heat and moisture production. *Swine Housing II Proceedings*. Amer Soc Agr Engineers, North Carolina, pp 031–040
- Cai W, Casey DS, Dekkers JC (2008) Selection response and genetic parameters for residual feed intake in Yorkshire swine. *J Anim Sci* 86(2):287–298
- Collin A, Vaz MJ, Le Dividich J (2002) Effects of high temperature on body temperature and hormonal adjustments in piglets. *Reprod Nutr Dev* 42(1):45–53
- Crandall CG, Wilson TE, Marving J et al (2008) Effects of passive heating on central blood volume and ventricular dimensions in humans. *J Physiol* 586(1):293–301
- Curtis SE (1983) Environmental management in animal agriculture. Iowa State University Press, Ames
- D'Allaire S, DeRoth L (1986) Physiological responses to treadmill exercise and ambient temperature in normal and malignant hyperthermia susceptible pigs. *Can J Vet Res* 50(1):78–83
- Florini JR, Ewton DZ, Coolican SA (1996) Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev* 17(5):481–517
- Gaughan J, Lacetera N, Valtorta S et al (2009) Response of domestic animals to climate challenges. In: Ebi KL, Burton I, McGregor G (eds) *Biometeorology for adaptation to climate variability and change*, vol 1. Springer, Heidelberg, pp 131–170
- Gilbert H, Bidanel JP, Gruand J et al (2007) Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. *J Anim Sci* 85(12):3182–3188
- Gilbert H, Bidanel JP, Billon Y et al (2012a) Correlated responses in sow appetite, residual feed intake, body composition, and reproduction after divergent selection for residual feed intake in the growing pig. *J Anim Sci* 90(4):1097–1108
- Gilbert H, Billon Y, Fleury J et al (2012b) Are responses to selection in lines divergently selected for residual feed intake in growing pigs affected by GxE interactions when bred in a tropical environment? *Proceedings of the AnGR-NordicNET Workshop*, Tuusula, Finland, pp 26–27
- Giles LR, Black JL, Gooden JM, Annison EF (1991) Energy expenditure of growing pigs maintained at high ambient temperature. In: Batterham ES (ed) *Manipulating pig production*. Australian Pig Science Association, Albury, pp 52–55
- Heo J, Kattesh HG, Roberts MP et al (2005) Hepatic corticosteroid-binding globulin (CBG) messenger RNA expression and plasma CBG concentrations in young pigs in response to heat and social stress. *J Anim Sci* 83(1):208–215
- Herd RM, Bishop SC (2000) Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. *Livest Prod Sci* 63(2):111–119
- Hoque MA, Suzuki K (2009) Genetics of residual feed intake in cattle and pigs: a review. *Asian-Aust J Anim Sci* 22(5):747–755
- Horowitz M, Meiri U (1993) Central and peripheral contributions to control of heart rate during heat acclimation. *Pflugers Arch* 422(4):386–392
- Huynh TT, Aamink AJ, Verstegen MW et al (2005) Effects of increasing temperatures on physiological changes in pigs at different relative humidities. *J Anim Sci* 83(6):1385–1396
- Kiefer C, Meignen BCG, Sanches JF, Carrijo EAS (2009) Response of growing swine maintained in different thermal environments. *Arch Zootec* 58(221):55–64
- Kim BG, Lindemann MD, Cromwell GL (2009) The effects of dietary chromium (III) picolinate on growth performance, blood measurements, and respiratory rate in pigs kept in high and low ambient temperature. *J Anim Sci* 87(5):1695–1704
- Le Bellego L, van Milgen J, Noblet J (2002) Effect of high temperature and low-protein diets on the performance of growing-finishing pigs. *J Anim Sci* 80(3):691–701

- Le Naou T, Le Flo'h N, Louveau I et al (2012) Metabolic changes and tissue responses to selection on residual feed intake in growing pigs. *J Anim Sci* 90(13):4771–4780
- Lefaucheur L, Lebret B, Ecolan P et al (2011) Muscle characteristics and meat quality traits are affected by divergent selection on residual feed intake in pigs. *J Anim Sci* 89(4):996–1010
- Li G, Ali IS, Currie RW (2006) Insulin induces myocardial protection and Hsp70 localization to plasma membranes in rat hearts. *Am J Physiol Heart Circ Physiol* 291(4):H1709–H1721
- Louveau I, Bonneau M (1996) Effect of a growth hormone infusion on plasma insulin-like growth factor-I in Meishan and Large White pigs. *Reprod Nutr Dev* 36(3):301–310
- Luiting P, Schrama JW, van der Hel W, Urff EM (1991) Metabolic differences between White Leghorns selected for high and low residual food consumption. *Br Poult Sci* 32(4):763–782
- Macari M, Zuim SM, Secato ER, Guerreiro JR (1986) Effects of ambient temperature and thyroid hormones on food intake by pigs. *Physiol Behav* 36(6):1035–1039
- Marple DN, Aberle ED, Forrest JC et al (1972) Effects of humidity and temperature on porcine plasma adrenal corticoids, ACTH and growth hormone levels. *J Anim Sci* 34(5):809–812
- Melchior D, Sève B, Le Flo'h N (2004) Chronic lung inflammation affects plasma amino acid concentrations in pigs. *J Anim Sci* 82(4):1091–1099
- Nienaber JA, Hahn GL, Eigenberg RA (1999) Quantifying livestock responses for heat stress management: a review. *Int J Biometeorol* 42(4):183–188
- Noblet J, Karege C, Dubois S, van Milgen J (1999) Metabolic utilization of energy and maintenance requirements in growing pigs: effects of sex and genotype. *J Anim Sci* 77(5):1208–1216
- Pearce SC, Gabler NK, Ross JW et al (2013) The effects of heat stress and plane of nutrition on metabolism in growing pigs. *J Anim Sci* 91(5):2108–2118
- Quiniou N, Dubois S, Noblet J (2000) Voluntary feed intake and feeding behaviour of group-housed growing pigs are affected by ambient temperature and body weight. *Livest Prod Sci* 63(3):245–253
- Quiniou N, Noblet J, van Milgen J, Dubois S (2001) Modelling heat production and energy balance in group-housed growing pigs exposed to low or high ambient temperatures. *Br J Nutr* 85(1):97–106
- Renaudeau D, Huc E, Noblet J (2007) Acclimation to high ambient temperature in Large White and Caribbean Creole growing pigs. *J Anim Sci* 85(3):779–790
- Renaudeau D, Kerdoncuff M, Anais C, Gourdine JL (2008) Effect of temperature level on thermal acclimation in Large White growing pigs. *Animal* 2(11):1619–1626
- Renaudeau D, Anais C, Tel L, Gourdine JL (2010) Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function. *J Anim Sci* 88(11):3715–3724
- Renaudeau D, Gourdine JL, St-Pierre NR (2011) Meta-analysis of the effects of high ambient temperature on growth performance of growing-finishing pigs. *J Anim Sci* 89(7):2220–2230
- Renaudeau D, Collin A, Yahav S et al (2012) Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* 6(5):707–728
- Renaudeau D, Frances G, Dubois S et al (2013) Effect of thermal heat stress on energy utilization in two lines of pigs divergently selected for residual feed intake. *J Anim Sci* 91(3):1162–1175
- Rhoads RP, Baumgard LH, Suagee JK (2013) Metabolic priorities during heat stress with an emphasis on skeletal muscle. *J Anim Sci* 91(6):2492–2503
- Robbins KR, Saxton AM, Southern LL (2006) Estimation of nutrient requirements using broken-line regression analysis. *J Anim Sci* 84(suppl):E155–E165
- Sauvant D, Perez JM, Tran G (2002) Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. INRA, Versailles
- Tavares SLS, Oliveira RFM, Donzele JL, Ferreira AS (1999) Influence of the environmental temperature on the performance and on the physiological parameters of female piglets from 30 to 60 kg. *R Bras Zootec* 28(4):791–798
- Titto CG, Negrão JA, Titto EA et al (2012) Effects of an evaporative cooling system on plasma cortisol, IGF-I, and milk production in dairy cows in a tropical environment. *Int J Biometeorol* 57(2):299–306
- Van Soest PJ, Wine RH (1967) Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J Assoc Off Anal Chem* 50:50–55